PHYTOCHEMICALS, NUTRITIONAL AND PHARMACOLOGICAL CHARACTERIZATION OF DIOSCOREA BULBIFERA

A Dissertation Submitted to the Sambalpur University in Partial Fulfilment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY
IN
FOOD SCIENCE AND TECHNOLOGY

by

SADHNI INDUAR
Regd. No. 234/2016/Food Sc. & Tech.

Food Science and Technology
Department of Home Science, Sambalpur University
Jyoti Vihar, Burla-768019, Odisha, INDIA

February, 2022

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Under the Supervision of

Dr. Pradeep K. Naik, Professor, Department of Biotechnology and Bioinformatics, Sambalpur University

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DECLARATION

I hereby declare that the work reported in the Ph.D. thesis entitled "PHYTOCHEMICALS, NUTRITIONAL AND PHARMACOLOGICAL CHARACTERIZATION OF DIOSCOREA BULBIFERA" submitted at ***Sambalpur University*** *is the original report of my research, under the guidance of* ***Prof. (Dr.) Pradeep Kumar Naik,*** *Department of Biotechnology and Bioinformatics, Sambalpur University.*

I have not submitted this work previously to any other organization for any degree or professional qualification. I have confirmed the norms and guidelines given in the ethical code of conduct of the University. Whenever I have used materials (data, theoretical analysis and text) from other sources, I have given due credit to them by citing them in the text of the thesis and giving their details in the references.

Date: **(Sadhni Induar)**

COURSE WORK AND COMPREHENSIVE EXAMINATION COMPLETION CERTIFICATE

This is to certify that ***Miss. Sadhni Induar****, bearing Registration No.* ***234/2016/Food Sc & Tech.****, a bonafide Ph.D. scholar of Sambalpur University, Sambalpur, who has successfully completed her course work and comprehensive examination, which is a part of her Ph.D. Programme.*

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*This is to certify that the research work entitled, "**PHYTOCHEMICALS, NUTRITIONAL AND PHARMACOLOGICAL CHARACTERIZATION OF DIOSCOREA BULBIFERA**" submitted by **Sadhni Induar** at **Sambalpur University**, **Odisha, India** is a bonafide record of her original work carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for any degree or diploma. I recommend this thesis in fulfillment of the award of the degree of **Doctor of Philosophy** in **Food Science and Technology.***

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Date:

"Dedicated To My Parents"

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(Sadhni Induar)

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ABBREVIATIONS

%	Percentage
°C	Degree Celcius
AOAC	Association of Analytical chemistry
ANOVA	Analysis of Variance
et al	And others
FSTN	Food Science Technology and Nutrition
IMS	Institute of medical Science
FW	Fresh weight
GAE	Gallic acid equivalent
QE	Quercetin equivalent
g	Gram
h	Hours
kg	Kilogram
L	Litre
M	Molarity
N	Normality
Min	Minute
mg	Mili gram
ml	Mililitre
µl	Microlitre
nm	Nanometer
mm	Mili meter
SD	Standard deviation
NaOH	Sodium Hydroxide
R^2	Coefficient of determination
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
Conc.	Concentration
DPPH	2,2-diphenyl-1-picrylhydrazyl
BHT	Butylated hydroxy toluene
Abs	Absorption
ND	Not detected
W/W	Weight/Weight
OECD	Organisation for Economic Co-operation and Development
GC-MS	Gas chromatography-mass spectrometry
NIST	National Institute of Standards and Technology
R.time	Retention time
MW	Molecular weight
MF	Molecular formula
HPLC	High performance Liquid Chromatography
DNS	3,5-Dinitrosalicyclic acid
V	Volume

S	Second
VS	Verses
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
DMSO	Dimethylsulfoxide
CFU	Colony forming Unit
AO	Acridine Orange
Etbr	Ethidium bromide
PBS	Phosphate buffered saline
DAPI	4’,6-diamidino-2-phenylindole
IC_{50}	50% inhibition concentration
HNO_3	Nitric acid
HCl	Hydrochloric acid
Na_2CO_3	Sodium carbonate
CO_2	Carbon dioxide
Rpm	Revolutions per minute
LD	Lethal dose
AST	Asparate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
LBD	Loose bulk density
PBD	Packed bulk density
WAC	Water absorption capacity
OAC	Oil absorption capacity
ES	Emulsion stability
Dbf	Dioscorea bulbifera flour
Wf	Wheat flour
OTG	Oven Toaster Grill
CO0	Cookies made from 100% wheat
CO1	Cookies made from (25%+75%wf)
CO2	Cookies made from (50%Dbf+75%wf)
CO3	Cookies made from (75%Dbf+25%wf)
C0	Cake made from (100% wheat)
C1	Cake made from (25%Dbf+75%wf)
C2	Cake made from (50%Dbf+50%wf)
C3	Cake made from (75%Dbf+25%wf)
P0	Papad made from 100% sagu
P1	Papad made from (25%sagu+75%wf)
P2	Papad made from (50% sagu+50%wf)
P3	Papad made from (75%sagu+25%wf)

Abstract

Wild foods make an important contribution to tribal's diet during the food scarcity period. It not only enriches the food baskets of tribals but also used as remedies for various diseases due to the presence of potential phytochemicals. Tubers are the storage form of starchy material in subterranean stems, roots, rhizomes and corms which grow beneath of soil's surface. *Dioscorea bulbifera* is one of the important wild species, the tuber of which is consumed by the tribal population of central India as food, particularly in Madhya Pradesh, Chhattisgarh, Jharkhand and Orissa mainly because of its high content of starch. The tuber in raw form is bitter in taste and thus has been soaked overnight in water or left overnight in a stream and subjected to successive boiling to remove the bitterness. The tuber is also known for its several pharmacological activities. It is good for intestinal colic, relieving dysmenorrhoea, reducing acidity, rheumatoid arthritis, relieving intense inflammation in the acute phase, spasmodic asthma, menopausal problems, labor pain, prevention of early miscarriage, hernia, relieving the pain of childbirth, etc. In a quest to utilize the tuber of *Dioscorea bulbifera* as foods and medicines, an attempt has been taken in this study to investigate the nutritional profile, phytochemical composition, pharmacological activity such as antioxidant, anticancer and antibacterial activity, toxicity evaluation, development of value added food products and their nutritional characterization.

Nutritional analysis of both raw and boiled tuber was performed following the standard methods. Total carbohydrate and starch content were estimated using the anthrone reagent method. Reducing sugar content of both the raw and boiled tu0ber samples was estimated by dinitrosalicyclic acid method. The protein content was estimated by Lowery's method. The total free amino acid was determined using the ninhydrin reagent method. The amino acid and vitamins (B1, B2, B3 and B6) profiling of both the raw and boiled tuber samples were performed using HPLC. Minerals composition of tuber samples was done using an atomic absorption spectrometer. The ascorbic acid content was estimated as the standard method. Total phenolics and flavonoid content, as well as antioxidant activity, were determined using six solvents extracts of tuber (methanol, acetone, water, ethylacetate, chloroform and petroleum ether) using the standard methods. Other secondary metabolites such as tannins, saponins and diosgenin were determined following standard methods. Functional parameters (bulk density, water and oil absorption capacity, emulsion stability) were studied using standard methods. Cake, cookies and papad were prepared from composite flours of *D.*

bulbifera and wheat with different proportion and their nutritional characterization was performed. Sensory parameters of cake, cookies and papad were evaluated by nine-point hedonic scale. Acute and subacute toxicity of flours were studied according to the OECD guidelines. Antimicrobial activity was analyzed by agar well diffusion methods. Anticancer activity of the tuber extract was studied using two human breast cancer cell lines, MCF-7 and MDAMB-231. Phytochemical analysis was carried out by GC-MS.

Proximate analysis of the raw and boiled tuber was determined and compared. The moisture content of the raw tuber was found to be quite low (74.89±0.54%) compared to the boiled tuber (80.48±1.18%). The ash content of the raw tuber was found to be high than the boiled tuber (1.66±0.34%). The fat content was low in both raw tuber (0.19±0.01%) and boiled tuber (0.14±0.012%). Total carbohydrate and reducing sugar content of raw tuber was found to be quite high (31.62±0.46% and 0.018±0.012%, respectively) compared to the boiled tuber (23.94±0.50% and 0.012±0.008%). In contrast, it was found that boiled tuber contained an albeit high amount of starch (11.67±0.65%) as compared to the raw tuber (8.6±0.54%). The protein content of the raw and boiled tuber was found to be 3.48±0.92% and 2.25±0.16%, respectively. The total free amino acid content was found to be slightly high in the raw tuber (1.45±0.05%) compared to the boiled tuber (0.59±0.13%). Phenylalanine was present in the highest amount in both raw and boiled tuber followed by valine. Histidine, methionine and cysteine amino acids were found in minimum quantity in the raw tuber and were not detected in the boiled samples. Potassium was present in the highest amount in both raw and boiled tuber followed by sodium and calcium. The ascorbic acid content of the raw and boiled tuber was found to be 99.5±0.94 mg/100g dry mass and 70.7±1.19 mg/100g dry mass, respectively. The content of vitamin B1, vitamin B2, vitamin B3 and vitamin B6 for the raw tuber was found to be 0.007±0.0004, 0.027±0.007, 27.38±1.42 and 0.128±0.028 mg/100g dry mass, respectively. Overall it was seen that the tuber of *D. bulbifera* is very rich in nutritional value and could be utilized as food.

The GC-MS spectrum confirmed the presence of 24 peaks of different compounds which have been identified by searching with the NIST library. Further, the analysis showed that raw and boiled tuber contained 160.2±0.84 mg/100gm and 12.5±0.11 mg/100gm of diosgenin. Tannin content was found to be 180.11±0.32 mg/100gm and 12.09±0.12 mg/100gm for the raw and boiled tuber, respectively. Similarly, the saponin content of raw and boiled tuber was found to be 150.34±0.67 mg/100gm and 21.26±0.89 mg/100gm respectively. Out of six solvent extracts, methanolic extracts contained the highest amount of total phenol, flavonoid and possessed excellent DPPH inhibition potential. Hence DPPH inhibition activity of methanolic extracts compared with standard ascorbic acid and BHT. It was found that the methanolic extract of the raw tuber has significantly higher antioxidant activity (IC_{50} value is 46.11 µg/ml) compared to the ascorbic acid (IC_{50} value is 92.86 µg/ml) and BHT (IC_{50} value is 54.35 µg/ml).

Aqueous and methanolic extract of tubers were tested to demonstrate inhibition of proliferation of cancer cells (MCF-7 and MDAMB-231) at dosages ranging from12.25 µg/ml to 200 µg/ml. The methanolic extract has the lowest IC_{50} value of 55 µg/ml and 75 µg/ml, respectively using MCF-7 and MDAMB-231 cancer cell lines. The methanolic extract effectively induced apoptosis to cancer cells treated at IC_{50} concentration. The treated cells appeared condensed chromatin, membrane blebs, and many shattered nuclei, all of which suggested induction of apoptosis to cancer cells. MIC and MBC values of three tuber extracts such as ethyl acetate, methanol and water have revealed maximum antibacterial activities. The MIC value of 1.56 mg/ml of ethyl acetate extract was registered against *S. aureus*, *E. faecalis*, *C. freundii* and *P. mirabilis*, while the value of 3.125 mg/ml against *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* were recorded. Similarly, MIC value of 1.56 mg/ml for methanol extract was registered against *S. aureus*, *E. faecalis*, *C. freundii* and *P. mirabilis*, while the value of 3.125 mg/ml against *A. baumannii, K. pneumoniae* and *P. aeruginosa* were recorded. Likewise, the MIC value of 1.56 mg/ml of water extract was registered against *S. aureus*, *A.baumannii, K. pneumoniae* and *P. mirabilis,* while the value of 3.125 mg/ml against *E.faecalis, C.freundii, K.pneumonniae* and *P. aeruginosa* were recorded. The MBC value of 6.25 mg/ml of the ethyl acetate extract was found against *S. aureus, E. faecalis, C. freundii, K.pneumoniae* and *P. mirabilis* while the value of 12.5 mg/ml against *A. baumannii* and *P. aeruginosa* was recorded. Similarly, the MBC value of 6.25 mg/ml of the methanol was registered against *S.aureus*, *E. faecalis*, *C. freundii*, *K. pneumoniae* and *P. mirabilis*, while the values of 12.5 mg/ml and 25 mg/ml against *A.baumannii* and *P. aeruginosa* were registered. Further, the MBC value of 6.25 mg/ml of the water extract was recorded against *S. aureus, A. baumannii, K. pneumoniae* and *P. aeruginosa,* while the value of 12.5 mg/ml against *E. faecalis* and *C. freundii* were registered. The value of 3.125 mg/ml was registered against *P. mirabilis*. Toxicity evaluation of aqueous extracts of raw tubers with increasing oral doses of 200 mg/kg, 400 mg/kg and 800 mg/kg body weight, as well as boiled tubers with oral doses of 2000 mg/kg, 4000 mg/kg and 8000 mg/ kg body weight neither caused any death nor produced any significant toxicity to animals. Therefore, LD_{50} of raw *D. bulbifera* aqueous extract and boiled *D. bulbifera* aqueous extract may be considered to be greater than 800 mg/kg and 8000 mg/kg body weight, respectively.

Functional properties including loose bulk density (LBD), packed bulk density (PBD), water absorption capacity (WAC), oil absorption capacity (OAC) and emulsion stability (ES) are compared in between wheat flour (Wf) and three composite flours having the ratio of 25%, 50% and 75% of *Dioscorea bulbifera* flour (Dbf) with wheat flour. LBD and PBD found lowest in wheat flour (0.38 g/ml and 0.66 g/ml) while highest in *D.bulbifera* flour (0.51 g/ml and 0.78 g/ml) respectively. There was no significant difference in PBD between composite flours of 25%Dbf+75%Wf, 50%Dbf+50%Wf and 75%Dbf+ 25%Wf. Similarly, LBD of 100%Wf, 25%Dbf+75%Wf, 50%Dbf+50%Wf, 75%Dbf+25%Wf and 100%Dbf were found to be 0.38, 0.44, 0.46, 0.49 and 0.51, respectively. Furthermore, 100%Wf and 25%Dbf+75%Wf received similar OAC value (0.92 ml/g). Dbf had lowest ES value (42.10%) while 75%Dbf+25%Wf obtained highest ES value (57.37%). There was no significant difference between the ES value of 100%Wf and 50%Dbf+50%Wf.

The inclusion of *D.bulbifera* flour to wheat flour significantly increased the moisture content of cookies made from composite flours. Cookies made from 75%Dbf+25%Wf composite flour contained highest moisture content (18.95%) followed by 50%dbf+50%Wf (15.35%), 25%Dbf+75% Wf (11.21%) and 100%Wf (8.05%). Ash content was slightly higher in cookies with an increase in the percentage of Dbf in composite flour. Generally, cookies are poor sources of fiber but the addition of Dbf to wheat flour improves the fiber content of cookies. The highest amount of fat content was found in 100% wheat flour cookies which was gradually declined with 25%, 50% and 75% Dbf substituted cookies. This is maybe because Dbf is a poor source of fat. In contrast, the carbohydrate and protein content of cookies decreased gradually with an increased level of Dbf in cookies. Carbohydrate and protein content were ranged between 51.08%-55.15% and 6.78%-9.12%, respectively.

Wheat flour cake contained the lowest moisture, ash and fiber content than composite flour made cakes. Moisture content increased significantly with an increase in the level of Dbf in cake formulation. The fiber content of cakes considerably improved with an increased percentage of Dbf in the formulation. The addition of Dbf with wheat flour showed an appreciable enhancement of ash in cakes. In contrast, the substitution of

Dbf (25%, 50% and 75%) to wheat flour leads to a gradual decrease in carbohydrate and protein content in the cakes. Wheat flour cake contained a significantly higher amount of fat than composite flour made cakes. The moisture and fiber content increased due to the replacement of sagu with Dbf in the formulation of papad. Protein and carbohydrate content gradually decreased with an increased level of Dbf to sagu in papads. The Overall acceptability of sensory score represents that the combination of 50%Dbf+50%Wf was found to be best among all the samples of cake and cookies. Whereas 25%Dbf+75%sagu was most suitable in case of papad preparation.

The nutritional composition of raw tuber was found to be high as compared to the boiled tuber. The phytochemical components like tannin, saponin and diosgenin were found to be lower in the case of boiled tubers, which makes it more suitable for human consumption because of de-bittering conditions. The pharmacological assay implies that the tubers are a good source of antioxidants. It has anti-microbial activity against seven pathogenic bacteria including *S.aureus, E.fecalis, A.baumannii, K.pneumonniae, P.mirabils* and *P.aeruginosa.* The tuber extracts were also found to have anti-cancer activity. Oral administration of the aqueous raw *D. bulbifera* tuber extract in doses from 200 to 800 mg/kg per body weight and aqueous boiled *D. bulbifera* tuber extract in doses from 2000 to 8000 mg/kg per body weight neither caused any death nor produced any severe toxicity to animals. The LD_{50} of the extracts from raw and boiled tuber was found to be greater than 800 mg/kg and 8000 mg/kg, respectively. The formulated cookies and cakes showed the overall acceptability of sensory score as well as nutritional properties in which 50%Dbf+50%Wf was found to be best among all the samples for making cookies and cakes, whereas 25% Dbf+75% sagu was found to be best for making papads.

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

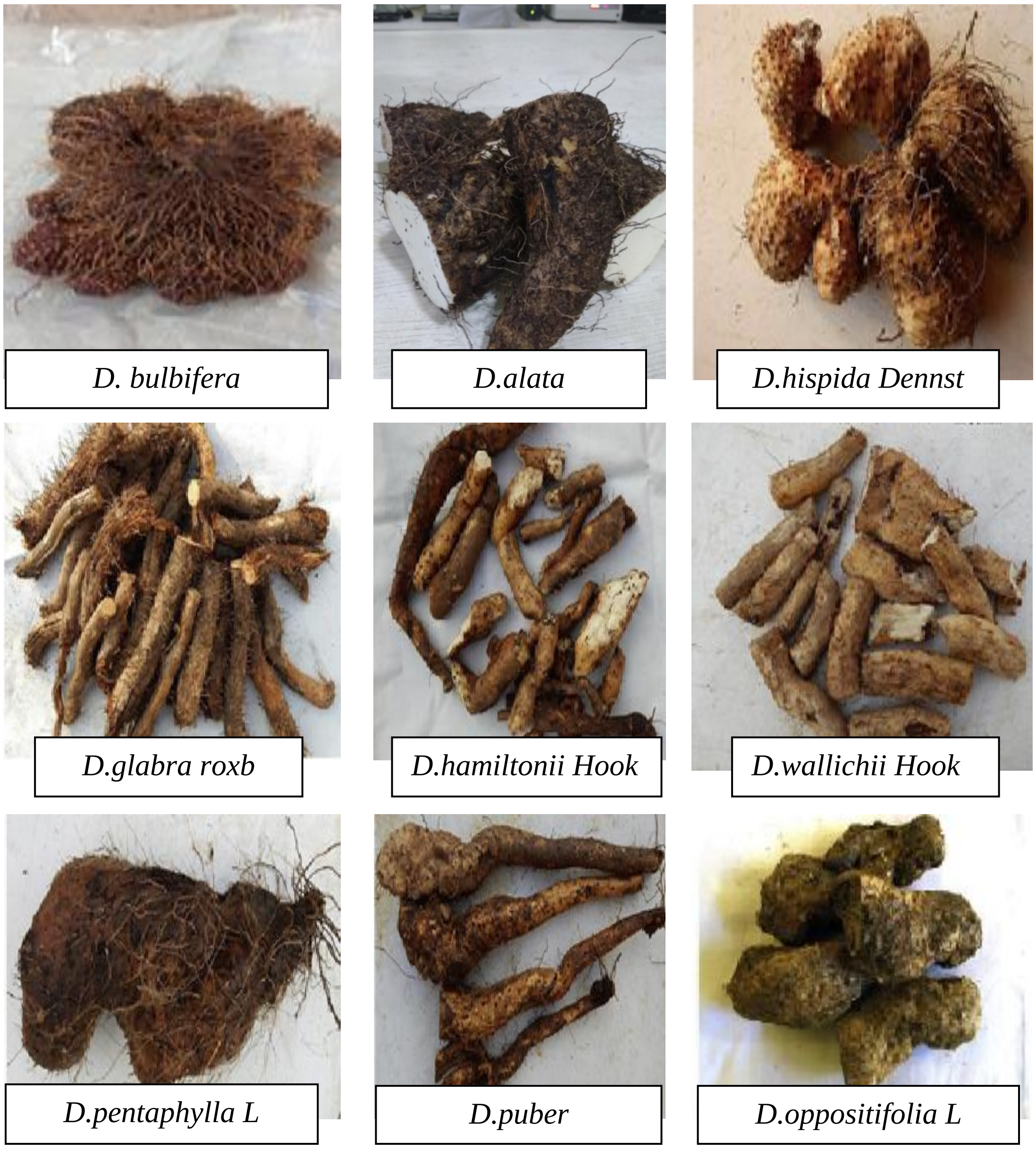
1.1 Introduction

Forest has been served as an important source of livelihood for rural and tribal people from time immemorial. Wild flora and fauna collected from the forest for foods are called wild foods. It includes leaves, flowers, fruits, stems, roots, rhizomes, bulbils, etc. of wild plants. Wild foods make an important contribution to tribes' diet during the food scarcity period. It not only enriches the food baskets of tribes but is also used as remedies for various diseases due to the presence of potential phytochemicals. Wild tubers particularly are the storage form of starchy material in subterranean stems, roots, rhizomes, and corms that grow beneath of soil's surface (Chandrasekara and Kumar, 2016). Roots and tuber crops occupy the second position after cereals as a source of carbohydrate. Wild tubers used by tribes mainly belong to the family Malvaceae, Araceae, Zingiberaceae, Asparagaceae, Cyperaceae, Costaceae, Hypoxidaceae, Dioscoreaceae, Liliaceae, Asclepiadaceae, Convulvulaceae, Vitaceae, Euphorbiaceae, Marantaceae, Cucurbitaceae, Fabaceae, Nymphaeaceae, Smilacaceae and Taccaceae (Padhan and Panda, 2016). Out of these the family Dioscoreaceae is widely spread and is distributed throughout the tropical and temperate regions. It is commonly known as the 'yam family' and is represented by a single genus *Dioscorea* (Hutchinson, 1959). The genus *Dioscorea* L. is one of the largest groups among monocotyledons (Ayensu, 1972)**.** The different species of the genus *Dioscorea* are commonly known as "Yam" (Goswami *et al*., 2013). It is named after the ancient Greek physician and botanist Dioscorides. The genus *Dioscorea* includes 600 species that are of considerable economic importance **(**Das *et al*., 2013). It is also a part of the lineage that is closely related to the group containing the grasses. Therefore, it represents an important biological link between eudicots and grasses, which contain all the model flowering plants (Goswami *et al*., 2013). In India, there are 32 species of *Dioscorea*, out of which 21 are distributed in the Western Ghats. About 5 million people are directly and indirectly depend on this crop for their food, feed and medicine (Arackal *et al*., 2015). Many wild *Dioscorea* species are an important source of secondary metabolites used in the pharmaceutical industry and medicine. As an example, several *Dioscorea* species are the source for different compounds used in the synthesis of sex hormones and corticosteroids **(**Das *et al*., 2013**).** The different species of *Dioscorea* are commonly known by their local name (Table 1.1). The images of some wild tubers of species of genus *Dioscorea* available in Odisha are included in Figure 1.1.

Table 1.1:- Some important species of genus *Dioscorea* and their local name.

Scientific name	Local name
D.alata L.	Khombo alu
D.belophylla voigt.ex Haines	Kunda alu
D.bulbifera	**Pita kanda**
D.glabra roxb	Konta alu
D.hamiltonii Hook	Suta alu
D.hispida Dennst	Banya alu
D.oppositifolia L.	Pani alu
D.pentaphylla L.	Mundi alu
D.puber Bl.Enum	Kosa alu
D.wallichii Hook. f.	Suta alu.

(Source:-Padhan and panda, 2016)



Source:-Padhan *et al*., 2020

Figure 1.1. The wild tubers of different species of *Dioscorea*.

1.2. *Dioscorea bulbifera*

Dioscorea bulbifera is one of the important wild species commonly called air potato, potato yam, bitter yam or air yam (IKewuchi *et al*., 2017). It is called air potato because it produces potato-like aerial bulbs in the leaf axils of the twining stems (Celestine and David, 2015). The edible species of *Dioscorea bulbifera* are grown and distributed extensively in West Africa, the Carribean Islands, South East Asia, South Pacific and West Indies. The wild forms occur in both Africa and Asia (Abara, 2015). Its synonyms include *Dioscorea anthropophagum* Chev, D. Hoffa Cordemy, *D. sativa* Thunb, and *D. sylvestris* de Wild (IKewuchi *et al*., 2017). *Dioscorea bulbifera* is a glabrous, twining, vine with alternate heart-shaped leaves (Figure 1.2). The vines may reach 20 m in length during a growing season. As an aggressive high-climbing vine, it grows often over the tops of low-lying vegetation and into tree canopies. It is dioecious and propagated by both sexual and vegetative reproduction through the production of bulbils. It is a bulb like growth produced in the leaf axils. Scientific classifications and vernacular names of *Dioscorea bulbifera* were included in Table 1.2.

Figure 1.2. ***Dioscorea bulbifera*** **plant**

Table 1.2.:- Classification and Vernacular names of *Dioscorea bulbifera*.

Taxonomical classification	Vernacular names
Super division: Spermatophyta	English:-Air potato, Potato yam
Division: Magnoliophyta	Sanskrit:-Varahikanda, Aluka, Shukara
Class: Liliopsida	Hindi name:-Varahi kand, Kadu kanda, Ratalu
Subclass: Lilidae,	Gujarati name:-Dukkarkanda
Order: Liliales	Bengali-Ratalu, Ban Alu
Family Dioscoreaceae	Tamil:-Kodikilanga, Kaattu-k-kaay-valli
Genus: *Dioscorea*	Marathi:-Manakund, Kadu-karanda, Varahi
Species: *bulbifera*	Kannada name:-Kuntagenasu
	Malayalam name:-Pannikizhangu, Kattukachil
	Oriya-Pita Alu
	Telugu:-Adavi Dumpa

(Sources: Galani Varsha J., 2017)

1.2.1 Distribution and ecology

Dioscorea spp. is native to tropical, temperate, and montane regions of numerous countries such as Africa, Asia (Asia-Temperate and Asia-Tropical) and Australia. All the species of *Dioscorea* are documented to exist in this region of the world, whereas, *D. bulbifera* is the only species believed to be native to both Asia and Africa (Martin, 1974; Wilkin, 2001). The native range of *D. bulbifera* in Africa includes the east tropical African countries of Tanzania and Uganda; the southern African countries of Zambia, Zimbabwe, Malawi, Mozambique and Namibia; Cameroon in west-central tropical Africa; and, the west tropical Africa countries of Benin, Burkina Faso, Ivory Coast, Ghana, Guinea, Liberia, Nigeria, Senegal and Sierra Leone (Coursey, 1967; Wilkin, 2001). In Asia, *D. bulbifera* exists as a native species in two distinct regions, Asia-Temperate (namely, China) and Asia-Tropical which is composed of the Indian subcontinent, Indo-China and Malesia. Countries of the Indian subcontinent in which *D. bulbifera* is native include: Bhutan, India, Nepal and Sri Lanka. *D. bulbifera* is native to the Indo-China countries of Cambodia, Laos, Myanmar, Thailand and Vietnam. The Malesia countries where *D. bulbifera* is native include Indonesia, Malaysia, Papua New Guinea and the Philippines. *D. bulbifera* is also indigenous to portions of the northern coastline of Australia: Queensland, the Northern Territory, and Western Australia.

Around 50 species were found all over India except in the dry north-western regions (Subasini *et al*., 2013). Only 13 species are reported from Odisha (Behera *et al.*, 2010). In India, it is used as a food by the tribal population of Madhya Pradesh, Chhattisgarh, Jharkhand, Tamilnadu, Arunachal Pradesh, and Orissa (Subasini *et al*., 2013; Shajeela *et al*., 2011; Shanthakumari *et al*., 2008; Okon and Ofeni, 2013) (Figure 1.3). Mainly the tribals of the central mountain area and Western hilly region of the Odisha used *Dioscorea bulbifera* as food as well as medicine. Similipal Biosphere Reserve forest of Mayurbhanj district, Odisha is one of the suitable habitats for *Dioscorea bulbifera* and ancestral people of this area utilize this tuber as both cultural and medicinal food (Kumar *et al*., 2013).

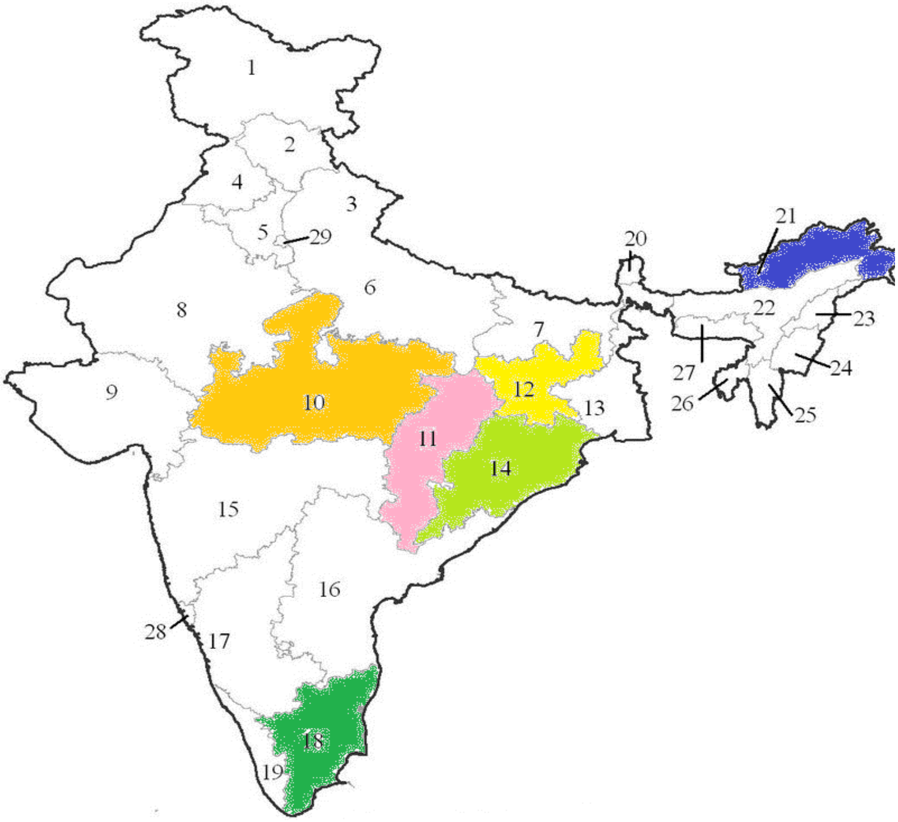


Figure 1.3. Highly consumption area of *Dioscorea bulbifera* that are painted in different colour patches.

1.2.2 Plant Description

Dioscorea bulbifera produces two types of tuber one aerial tuber (Figure 1.4.a) and another is an underground tuber (Figure 1.4. b). Tubers are roughly spherical, having a potato appearance. At the beginning of the growing season, as of mid-April, the previous year's tubers and new bulbils produce thick spaghetti-like roots from the rhizomatous (or head) end of a given tuber (Figure 1.4.c). Leaves are cordate-shaped with elongated tips (Figure 1.4.d), thin and glabrous, and range from 10-20 cm in length and 5-15 cm in width. Leaves are long-petioled, often ≥8 cm on mature and between 2-3 cm on newer leaves nearest the terminal bud. The leaves are arranged in an alternate arrangement. Leaf venation is parallel and converges at the leaf base. Petioles are distinctly flattened along the upper surface and, at the point of attachment to individual leaves; flare out to create small wing-like structures which are ruffled in appearance. The petioles often have a reddish-purple color. Stem is also reddish-purple (Figure 1.4.e). Flowers are green to white and fragrant (Figure 1.4.f).

a. Areal Tuber

b. Underground

c.Thick roots from head

d. Leaf

e Stem

f. Flower

Figure 1.4. Different parts of *Dioscorea bulbifera* plant

1.2.3 Traditional uses of *Dioscorea bulbifera*

As per source of dietary nutrients, *Dioscorea* species rank as the world's fourth most important root and tuber crops after potatoes, cassava and sweet potatoes (Lev and Shriver, 1998; Kumar *et al*., 2017**).** *Dioscorea bulbifera* yam is used as food and it is a good source of calories and minerals such as iron, calcium, and phosphorous (Abara, 2015). The raw tuber of *Dioscorea bulbifera* is bitter. Therefore, before consumption tubers are soaked overnight in water or left overnight in a water stream and may get boiled to reduce the bitterness in the tuber (Bhandari and Kawabata, 2005; Kumar *et al*., 2012; Mishra *et al*., 2013)**.** The wild tuber is consumed by the tribal population of central India as food particularly in Madhya Pradesh, Chhattisgarh, Jharkhand and Orissa (Subasini *et al*., 2015). The raw tuber has been considered to enhance appetite (Mishra, R.K. *et al*., 2008). The tuber has goodness in balancing intestinal colic, reducing acidity as well as against rheumatoid arthritis, to relieve intense inflammation, relieving dysmenorrhoea, in the acute phase of spasmodic asthma. It has several benefits for menopausal problems, for labor pain and for the prevention of early miscarriage, relieving the pain of childbirth, etc. **(**Nayak *et al*., 2004; Patil and Patil, 2005; Bhogaonkar and Kadam, 2006; Mehta and Bhatt, 2007)**.** About 10 gm of tuber powder is given once a day for 5–6 days after menses as a contraceptive (Kamble *et al*., 2010). Tuber bulbils are also used to reduce throat pain (Mbiantcha *et al*., 2011). Boiled tubers with reduced bitterness are taken orally to reduce body heat (Singh *et al*., 2009). The tuber is used against boils, dysentery (Nag, 1999) and diarrhea (Jadhav *et al*., 2011). Further, it is used to treat skin infection, bronchial cough, ulcers, piles, syphilis, etc. (Tiwari and Pande, 2006; Bhatt and Negi, 2006; Dutta, 2015; Abhyankar and Upadhyay, 2011)**.**

1.2.4 Nutritional importance

Several studies have been conducted to evaluate the food potential of *Dioscorea bulbifera*. Data obtained from these studies indicated that *Dioscorea bulbifera* is a good source of various nutrients.

1.2.4.1. Carbohydrates

Carbohydrates are major constituents of the *Dioscorea bulbifera (Db)* tuber. Ifeanacho *et al.* (2017) reported a soaring amount of carbohydrate both in fresh and dry states of Db tuber (60.83% and 68.41%) respectively. Abara (2011) found a good quantity of carbohydrates in the flesh of the tuber (34.60%-82.50%). Adeosun *et al*.,

(2016) and Achy *et al*., (2016) revealed that bulbils of Db contain 78.31-79.86% of carbohydrates. Ogubagu (2008) showed cooked Db contained a higher amount of carbohydrate (83.21%) than uncooked Db (79.15%). Polycarb *et al*. (2012) estimated carbohydrate content of Db (81.76%-82.52%) and compared with *D.cayenesis* (consists of 80.01%-80.07%), *D.alta* (consists of 81.10-80.47%), *and D.esculenta* (consists of 80. 05%). Celestine and David, (2015) determined carbohydrate content of both underground tuber (consists of 77.49%) and bulbils (consists of 76.68%) of Db.

1.2.4.2. Starch

The tuber of *Dioscorea bulbifera* is mainly known for its starch content**.** Due to the high content of starch tubers are served as an energy source after cereals. Shanthakumari *et al*. (2008), Arinathan *et al*. **(**2009) and Shajella *et al*. (2011) reported starch content of *D. bulbifera* of 42.86%, 18.10% and 38.10%, respectively. In contrast, *D. esculenta* (60-62.4%), *D. oppositifolia* (40.37-64.29%), *D. pentaphylla* (42.58-61.26%), *D. tomentosa* (49.86%-56.26%), *D. willichi* (56.25-59.30%) contained a higher amount of starch than *D. bulbifera.* But starch content of D. *trilobus* (32.66%) and *D. spicata* (41.33%) can be compared with the starch content of *D. bulbifera* (Shanthakumari *et al*., 2008; Arinathan *et al*., 2009 and Shajella *et al*., 2011).

1.2.4.3. Protein

Abara (2011) reported the protein content of *D. bulbifera* tuber flesh and peel in both wet (2.55%-3%) and dry matter (6.35%-7.47), respectively. Ogbuagu, (2008) found higher protein content in the uncooked *D. bulbifera* (9.28%) compared to cooked *D. bulbifera* (5.56%). Whereas Arinathan ***et*** *al*., (2009) found the lowest protein content for *D. bulbifera* (5.16%) compared to *D. oppositifolia* Var dukhumensis (6.33%), *D. oppositifolia* Var. oppositifolia (6.31%), *D. pentaphylla* var pentaphylla (5.38%), *D. tomentosa* (8.51%) and *D. trilobus* (7.08%). In contrast, Bhandari *et al*., (2003) found the highest amount of protein content for *D. bulbifera* (3.1%) compared to *D. deltoidea* (1.6%), *D. versicolor* (1.7%) and *D. triphylla* (2.3%). Polycarb *et al*., (2012) analyzed crude protein content of two varieties of *D. bulbifera* tuber (light grey consists of 5.38% and deep grey consists of 5.30%) and compared with the light yellow variety of *D. cayensis* (5.78%), purple *D. cayensis* (5.30%), *D. alta* (5.91%), *D. esculenta* (5.60%) as well as white and yellow varieties of *D. dumentorum* (consists of 6.21% and 6.52%). Celestine and David, (2015) reported protein content of both underground (4.36%) and aerial bulbils (6.18%) of *D. bulbifera.* Achy *et al*., (2016) reported a high value of protein content (8.12%) for *Dioscorea bulbifera* tuber. All the previous reports indicate tuber of *Dioscorea bulbifera* is a good source of protein.

1.2.4.4. Amino acid

There is very limited data exist on the amino acid composition of *D. bulbifera* tuber. Bhandari *et al*. (2003) reported 17 amino acids from four Nepali yams, namely *Dioscorea bulbifera*, *Dioscorea detoida*, *Dioscorea versicolor*, and *Dioscorea triphylla*. Specifically, all yam tubers contained large amounts of aspartic acid and glutamic acid. It was reported that the amino acid composition of *D. bulbifera* was superior in comparison to other species. *Dioscorea bulbifera* contained 8.7g/100g of Leucine, 3.6g/100g of Lysine, 1.6g/100g of Methionine, 0.2% of Cystine, 5.6% of Phenylalanine, 4.6% of Threonine, 4% of Tyrosine, 5.7% of Valine, 4.3% of Isolysine, 1.9% of Histidine, 5.1% of Alanine, 5.1% of Glycine, 4.4% of Proline, 6.1% of Serine, 11.7% of Aspartic acid, 13.5% of Glutamic acid and 4.9% of Arginine (Bhandari *et al*., 2003).

1.2.4.5. Dietary fiber

The major constitution of dietary fiber is cellulose, hemicelluloses, lignin, and pectins; while minor components are cutin, gums, some proteins, and oligosaccharides. (Kelsay, 1978; Van soest, 1978; Adamson, 1985). Generally, our diet should comprise dietary fiber because it enhances the water holding capacity and bulk of the stool. It helps to reduce the blood low-density lipoprotein (LDL) cholesterol levels, bowel cancer as well as heart disease (Abara *et al*., 2011). Abara *et al*., (2011) reported a good amount of fiber in *Dioscorea bulbifera.* It contains neutral detergent fibre (2.57% & 2.59%), acid detergent fiber (2.03% & 2.31%), hemicelluloses (0.27% & 0.28%), lignin (0.10% & 0.09%), and cellulose (1.13 and 0.97%) for the raw and cooked tubers, respectively. They also found the highest amount of neutral detergent fibre, acid detergent fiber, and hemicelluloses in *Dioscorea bulbifera* compared to *Dioscorea alta, Dioscorea cayenensis,* and *Dioscorea rotundata*. Ogubagu (2008) found 1.20% and 0.90% of crude fiber in the uncooked and cooked corm of *D. bulbifera*. Sanful *et al*. (2013) reported (0.73%-0.97%) crude fiber for five varieties of *Dioscorea bulbifera*.

1.2.4.6. Lipid

Shanthakumari *et al*. (2008), Arinanthan *et al*. (2009) and Shajeela *et al*. (2011) have found the highest amount of lipids (9.13%, 8.13% and 6.14% respectively) in *D. bulbifera* compared to other species such as *D. pentaphylla* (4-6.01%), *D. alata* (2.50%), *D. esculenta* (2.58-4.68%), *D. oppositifolia* (6.33-7.42%), *D. tomentosa* (2.86-6.04), *D.*

wallichi (1.18-3.34%). Polycarb *et al*., 2012 also compared fat content of *Dioscorea bulbifera* (0.53%-0.55%) with other yam tubers (0.41%-0.82%) namely *D. rotundata, D. cayenensis, D. praehensalis, D. dumentorum, D. alta* and *D. esculenta.* Underground tuber and aerial bulbil of Nigerian *Dioscorea bulbifera* contained 8.70% and 7.97% of fats (Celestine and David**,** 2015). Ogbonna *et al*., (2015) reported 0.37%**,** 4.15% and 2.21% of crude fats, respectively for green, yellow and red cultivars of *Dioscorea bulbifera*. However, Ogbuagu, (2008) and Abara, (2011) found a very less amount of lipid (0.20%-0.70%) in *D. bulbifera.*

1.2.4.7. Mineral content

Minerals are micronutrients of food components that are required in very small amounts but essential for humans. It was reported that the tuber of *D. bulbifera* is an important source of various minerals.

1.2.4.7.1. Sodium

The raw and boiled tubers of *Dioscorea bulbifera* were found to have 0.15% and 0.14% of sodium (Ogbuagu, 2008). An amount of 220 mg/100g and 550 mg/100g of sodium from wet and dry tubers was reported by Abara (2011). Arinathan *et al*., (2009) has found the lowest amount of sodium among *Dioscorea tomentosa* (123 mg/100g), *D. oppositifolia* (110.18 mg/100g) and *D. pentaphylla* (85.24 mg/100g). In contrast, Bhandari *et al*., (2002) reported the highest content of sodium for the raw tuber of *D. bulbifera* (560 mg/100g) compared to *D. deltoid* (340 mg/100g), *D. versicolor* (250 mg/100g) and *D. triphylla* (317 mg/100g). Light grey and deep grey cultivar of *D. bulbifera* contained 70 mg/100g and 92.5 mg/100g of sodium, respectively (Polycarb *et al*., 2012). While red, green and yellow cultivars of *D. bulbifera* contained 2403mg/100g, 575 mg/100g and 87.25 mg/100g of sodium, respectively **(**Ogbonna *et al*., 2015)**.** Underground tubers and aerial bulbils of *D. bulbifera* contained 39.80 mg/100g and 38.52 mg/100g of sodium (Celestine and David, 2015). Shajeela *et al*., (2011) reported 44.56%, 78.24%, 86.40%, 168.24%, 124%, 96.20%, 66.34%, 46.14% and 63.01% of sodium for *D. alta, D. bulbifera, D. esculenta, D. oppositifolia, D. pentaphylla, D. spicata, D. tomentosa and D. wallichi,* respectively.

1.2.4.7.2. Potassium

Potassium content in the tuber of *D. bulbifera* was reported to be 176 mg/100g and 440 mg/100g based on wet and dry weight (Abara, 2011). Celestine and David (2015) reported 334.71 mg/100g and 316.72 mg/100g of potassium content for both underground tubers and aerial bulbils, respectively. The flour of *Dioscorea bulbifera* bulbils contained 37.8 mg/100g of potassium (Achy, J.Y., *et al*., 2016). Ogbonna *et al*. (2015) reported potassium content of 387.73 mg/100g in the green cultivar, 667.53 mg/100g in the yellow cultivar and 467.53 mg/100g in the red cultivar. Light grey and deep grey cultivars of *D. bulbifera* contained 1475 mg/100g and 1250 mg/100g of potassium **(**Polycarb *et al.*, 2012). Arinathan *et al*., (2009)**,** Shajeela *et al*., (2011) and Shanthakumari *et al*., (2008) reported 1600 mg/100g, 1554.36 mg/100g and 1548 mg/100g of potassium, respectively for the wild tubers of *D. bulbifera* from Tamilnadu region of India.

1.2.4.7.3. Phosphorus

Phosphorus content in the tuber of *D. bulbifera* was estimated to be 64.40 and 150 mg/100g fresh weight and dry weight of tissue (Abara, 2011). The light grey and deep grey cultivars of *D. bulbifera* contained 223.5 and 224.5 mg/100g of tuber (Polycarb *et al*., 2012). Celestine and David (2015) reported 156.09 and 149.93 mg/100g phosphorus content for underground tuber and aerial bulbils, respectively**.** Phosphorous content of different species of *Dioscorea* was found to be 140.14 mg/100g (*D .alata*), 154.42 mg/100g (*D. bulbifera),* 138.10 mg/100g (*D. esculenta*), 114.10 & 124.12 mg/100g (*D. oppositifolia*), 158.18 mg/100g (*D. pentaphylla*), 166.30 mg/100g (*D. spicata,*), 104.06 mg/100g (*D. tomentosa*) and 106.40 mg/100g (*D. wallichi*) by Shajeela *et al*., (2011).

1.2.4.7.4. Calcium

The calcium content of *Dioscorea bulbifera* has been reported to be 228.15 mg/100g, 0.82 mg/100g, 77.1 mg/100g, 7.59 mg/100g, 238.15 mg/100g and 338.15 mg/100g, respectively by Shanthakumari *et al.* (2008), Chandra *et al*. (2012), Achy, J.Y. *et al*. (2016), Seal (2015), Arninathan *et al*. (2009) and Shajeela *et al*. (2011). Abara (2011) reported 35.30 mg/100g and 205.60 mg/100g of calcium from the tuber of *D. bulbifera* concerning fresh and dry weight basis. Celestine and David (2015) reported 280.25 and 174.44 mg/100g for underground tuber and aerial bulbils of *Dioscorea bulbifera*. Polycarb *et al*. (2012) reported 103 and 116.50 mg/100gm of calcium for light grey and deep grey cultivar of *D.bulbifera*. Sanful *et al.(*2013) found calcium content in the range of 78-240.2 mg/100g for different processed *Dioscorea bulbifera*.

1.24.7.5. Magnesium

Light grey and deep grey varieties of *Dioscorea bulbifera* contained 83.5 and 76.5 mg/100g of magnesium (Polycarb *et al*., 2012). Similarly, the magnesium content of 24.30 and 139 mg/100g was reported on a fresh weight and dry weight basis for the tuber of *Dioscorea bulbifera* by Abara (2011). Celestine and David (2015) reported 764.52 and 758.94 mg/100g of magnesium for underground tuber and aerial bulbils of *D. bulbifera*. In contrast, Achy, JY, *et al.* (2016) reported 86.5 mg/100g of magnesium for bulbils of *D. bulbifera*.

1.2.4.7.6. Iron

Different levels of the iron content of *D. bulbifera* tubers were reported by different workers among different varieties. Abara (2011) reported 2.36 mg/100g and 5.90 mg/100g of iron content on fresh weight and dry weight basis. Bhandari *et al*. (2003) reported 2.92 mg/100g of iron for Nepalin *Dioscorea bulbifera*. Polycarb *et al*. (2012) reported iron content of two varieties of *D. bulbifera* like light grey (6 mg/100g) and deep grey (6.50 mg/100g). While Ogbonna et al. (2015) estimated the iron content of three varieties of *D. bulbifera* such as green (137.53 mg/100g), yellow (1.10 mg/100g) and red (3.75 mg/100g).

1.2.4.7.7. Zinc

Zinc content of the *D. bulbifera* tuber also varies with respect to different species. *D. bulbifera* contained 0.53 mg/100g (wet tissue) and 1.52 mg/100g (dry tissue) of zinc (Abara, 2011). The zinc content of *D. bulbifera* is lowest (1.30 mg/100g) in comparison to *D. oppositifolia* var dukhumensis *(*1.40 mg/100g*), D. oppositifolia* var oppositifolia *(*5.24 mg/100g*), D. pentaphylla* var pentaphylla *(*3.22 mg/100g*), D. tomentosa (*6.20 mg/100g*)* (Arninathan *et al*., 2009). In contrast, Bhandari *et al*. (2003) estimated zinc content of four yam tubers and found that *D. bulbifera* contained the highest amount (0.53 mg/100g) of zinc compared to other species such as *D. deltoidea* (0.22 mg/100g*), D. versicolor* (0.30 mg/100g*)* and *D. pentaphylla* (0.39 mg/100g*).* Celestine and David (2015) estimated 0.36 mg/100g of zinc in the underground and 0.17 mg/100g in the bulbils of *D. bulbifera.* Polycarb *et al*., (2012) reported zinc content of the light grey (1.30 mg/100g) and deep grey (1.35 mg/100g) varieties of *Dioscorea bulbifera*.

Arninathan *et al*. (2009) also estimated the zinc content of different species of *Dioscorea* and found it to be 1.30 mg/100g (*D. bulbifera*), 1.40 mg/100g (*D. oppositifolia var dukhumensis*), 5.24 mg/100g (*D. oppositifolia var. oppositifolia*), 3.22 mg/100g (*D. pentaphylla var. pentaphylla*) and 6.20 mg/100g (*D. tomentosa*).

1.2.4.7.8. Manganese (Mn)

Manganese content was found to be in trace amounts compared to other minerals. As an example, 1.60 ppm and 4 ppm of Mn were found in fresh and dry weight basis of *Dioscorea bulbifera*, respectively (Abara, 2011). It also varied with respect to different species. Shanthakumari *et al*. (2008) reported Mn content of 1.26 mg/100g in *D. alata*, 1.30 mg/100g in *D. bulbifera*, 1.48 mg/100g in *D. esculenta*, 1.20 mg/100g in *D. oppositifolia*, 3.32 mg/100g in *D. pentaphylla*, 5.20 mg/100g in *D. tomentosa* and 4.44 mg/100g in *D. wallichi.* Polycarb et al. (2012) estimated 1.30 and 1.35 mg/100g of Mn for the light grey and deep grey variety of *D. bulbifera*. Bhandari *et al*. (2003) reported Mn content of *D. deltoid* (0.31 mg/100g*)*, *D. versicolor* (0.14 mg/100g) and *D. triphylla* (0.25 mg/100g). Celestine and David (2015) found a similar amount of Mn 0.46 mg/100g for both underground tuber and aerial bulbils of *Dioscorea bulbifera*. Green, yellow and red cultivars of *D. bulbifera* contained 25.03, 75.02 and 100.02 mg/100g of Mn, respectively (Ogbonna *et al*., 2015). Shajeela *et al.* (2008) found good amount of Mn for yam tubers namely *D. alata* (6.36 mg/100g*), D. bulbifera* (9.40 mg/100g*), D. esculenta* (5.46 mg/100g*), D. oppositifolia* var dukhumensis (7.42 mg/100g*), D. oppositifolia* var oppositifolia (9.04 mg/100g*), D. pentaphylla* var pentaphylla (3.46 mg/100g*), D. spicata* and *D. tomentosa* 16.70 mg/100g) as well as *D. wallichi* (2.10 mg/100g) (Shajeela *et al*.,2011). In other hand Shanthakumari *et al.* (2008) found Mn 5.36 mg/100g (*D. alata*), 10.60 mg/100g (*D. bulbifera*), 4.54 mg/100g (*D. esculenta*), 8.84 mg/100g (*D. oppositifolia*), 2.22 mg/100g (*D. pentaphylla*), 1.10 mg/100g (*D. tomentosa*) and 2.58 mg/100g (*D. wallichi*). Overall, it is indicated that *D. bulbifera* has superior quality of Mn than other yam tubers.

1.2.4.7.9. Copper

Available literature showed that yam tubers were poor sources of copper. A similar amount of copper was found in the light grey and deep grey cultivar of *D. bulbifera* (0.20 mg/100g) by Polycarb *et al*. (2012)**.** Copper contains of *D. bulbifera (*2.74 mg/100gm*), D. oppositifolia* var dukhumensis (11.50 mg/100gm*), D. oppositifolia* var. oppositifolia (2.78 mg/100gm*), D. pentaphylla* var pentaphylla (16.60 mg/100gm*),* *D. tomentosa* (1.44 mg/100gm) reported by Arninathan *et al*. (2009). Abara (2011) reported 2 ppm and 5 ppm for fresh and dry tissue of *D. bulbifera*. While Celestine and David (2015) reported 0.22 mg/100g and 0.10 mg/100g of copper for underground tubers and aerial bulbils, respectively.

1.2.4.7.10. Vitamins

The tuber of *D. bulbifera* was also found to be a good source of various vitamins. Arninathan *et al.* (2009) reported the presence of niacin and ascorbic acid in *D. bulbifera, D. oppositifolia* var. dukhumensis, *D. oppositifolia* var. oppositifolia, *D. pentaphylla* var. pentaphylla and *D. tomentosa.* Among the different species *D. tomentosa* possessed the highest amount of niacin, while *D. oppositifolia* var. dukhumensis contained the lowest amount of niacin (88.36 mg/100g). In contrast, *D. bulbifera* contained 23.69 mg/100g of niacin and 106.52 mg/100g of ascorbic acid. Ogbonna *et al*. (2015) reported vitamin A and Vitamin C content of three cultivars (red, green and yellow) of *Dioscorea bulbifera*. Vitamin A content of three cultivars was found to be much higher (443.91, 137.24 and 700.88 mg/100g) compared to vitamin C content (0.13, 0.26 and 0.04 mg/100g). Shajeela *et al*. (2011) reported 33.74 mg/100g and 91.04 mg/100g of niacin and ascorbic acid for *D. bulbifera* var vera. The vitamins composition of tubers of *Dioscorea bulbifera* is collated in Table 1.3.

1.2.5. Antinutritional components

Secondary metabolites are sometimes known as antinutrients because they interfere in the digestion and availability of nutritional components. Tubers of *Dioscorea bulbifera* consist of good amounts of various antinutritional components (Table 1.4.).

1.2.5.1. Phenols

Total phenolic content (TPC) of *Dioscorea bulbifera* was found to be in the highest amount compared to other yam tubers (Arninathan *et al*., 2009; Shajeela *et al*., 2011 and Shanthakumari *et al*., 2008). An amount of 3.37 g/100g, 2.20 g/100g and 1.40 g/100g of TPC was estimated by Arninathan *et al*. (2009), Shajeela *et al*. (2011) and Shanthakumari *et al*. (2008), respectively. Shajeela *et al*. (2011) showed the effect of soaking, cooking and autoclaving on the level of total free phenolics. It is reduced mostly by autoclaving (0.35 g/100g) followed by cooking (0.38 g/100g), soaking in sodium bicarbonate solution (1.28 g/100g) and soaking in distilled water (1.33 g/100g). Achy *et al*. (2016) reported 558 mg/100g of phenols for bulbils of *Dioscorea bulbifera*. Ogbonna *et al*. (2015) reported TPC for green (1.10 mg/100gm), red (1.22 mg/100gm) and yellow (1.64 mg/100g) cultivars of *Dioscorea bulbifera.*

Table 1.3. Vitamins composition of the tuber of *Dioscorea bulbifera* as reported by various workers. Vitamins composition varies with respect to different processed samples.

Name of vitamins	Amount	Sample type	Region	Reference
Niacin (mg/100g)	23.69±0.35	Raw tuber	Tamilnadu, India	Arinathan *et al*, 2009
Ascorbic acid (mg/100g)	106.52±0.11			
Niacin (mg/100g)	33.74±0.21	Raw tuber	Tamilnadu, India	Shajeela *et al*., 2011
Ascorbic acid (mg100g)	91.04±0.86			
Vitamins A (mg/100g)	443.91	Green	Enugu state, Nigeria	Ogbonna *et al*., 2015
	137.24	Yellow		
	700.88	Red		
Vitamins C (mg/100g)	0.13	Green	Enugu state, Nigeria	Ogbonna *et al*., 2015
	0.26	Yellow		
	0.04	Red		
Ascorbic acid (mg/100gm)	1.67±0.22	Sun dried for four days	Abia state, Nigeria	Okwu and Ndu, 2006
Niacin (mg/100gm)	0.01±0.2			
Ascorbic acid (mg/100gm)	138.67±0.034	Grated solar dried	Ghana	Sanful *et al*.,2013
	79.33±0.012	Boiled solar dried		
	77.78±0.009	Steamed solar dried		
	93.55±0.010	Fresh solar dried		
	122.44±0.024	Grated oven drie		
	105.78±0.008	Boiled oven dried		
	79.44±0.008	Steamed oven dried		
	53.33±0.010	Fresh oven dried		

1.2.5.2. Tannin

Raw tubers of *Dioscorea bulbifera* contained 1.59 g/100g of tannin, but after soaking in distilled water for nine hours, tannin content was reduced to 1.38 g/100g. Similarly, when tubers were soaked in sodium bicarbonate solution for nine hours tannin content was reduced to 1.45 g/100g. After cooking for 90 minutes the tannin content was reduced to 0.11 g/100g **(**Shanthakumari *et al*., 2008). According to Arinathan *et al*. (2009) and Shajeela *et al*. (2011) *D.bulbifera* was the richest source of tannin (1.48-2.55 g/100g) in comparison to *D. esculenta* (0.20 g/100gm*), D. oppositifolia* var dukhumensis (0.09-0.24 g/100gm*), D. oppositifolia* var. oppositifolia (0.02-0.36 g/100gm*), D. pentaphylla* var pentaphyll (0.06-0.09 g/100gm*)*, *D. spicata* (0.10 g/100gm) and *D.*

tomentosa (0.06-0.20 g/100g). Polycarb *et al*. (2012) reported 10.27 mg/100g and 10.98 mg/100g of tannins for light grey and deep grey variety of *Dioscorea bulbifera*, respectively. Celestine and David (2015) reported 118.47 mg/100g and 37.41 mg/100g of tannins for underground tuber and aerial bulbil of *Dioscorea bulbifera,* respectively. Ifeanacho *et al*. (2017) reported 4.11 mg/100g and 4.621 mg/100g of tannins on a fresh and dry weight basis. Achy et al. (2016) estimated 66 mg/100g of tannins for bulbils of *Dioscorea bulbifera*. Green, red and yellow cultivars of *Dioscorea bulbifera* contained 0.22, 0.19 and 0.18 mg/100g of tannins, respectively **(**Ogbonna *et al*., 2015**).**

Table 1.4. Different antinutritional components were reported from the tubers of *Dioscorea bulbifera*.

Antinutritional components	Amount	Sample type	Region	Reference
Total free phenolics (g/100g)	1.40±0.012	Raw tuber	Tamilnadu, India	Shanthakumari *et al*.,2008
	1.33±0.003	Soaking in distilled water		
	1.28±0.003	Soaking in sodium bicarbonate solution for 9 hours		
	0.38±0.001	Cooking 90 min		
	0.35±0.001	Autoclaving 45 min		
Tannins (g/100g)	1.59±0.014	Raw tuber	Tamilnadu, India	Shanthakumari *et al*.,2008
	1.38±0.006	Soaking in distilled water		
	1.45±0.004	Soaking in sodium bicarbonate solution for 9 hours		
	1.42±0.012	Cooking 90 min		
	1.39±0.001	Autoclaving 45 min		
Hydrogen cyanide (mg/100g	0.12±0.003	Raw tuber	Tamilnadu, India	Shanthakumari *et al*.,2008
	0.08±0.001	Soaking in distilled water		
	0.09±0.003	Soaking in sodium bicarbonate solution for 9 hours		
	0.03±0.007	Cooking 90 min		
	0.008±0.001	Autoclaving 45 min		
Total oxalate (g/gm)	0.98±0.001	Raw tuber	Tamilnadu, India	Shanthakumari *et al*.,2008
	0.86±0.001	Soaking in distilled water		
	0.87±0.001	Soaking in sodium bicarbonate solution for 9 hours		
	0.82±0.001	Cooking 90 min		
	0.80±0.003	Autoclaving 45 min		

Antinutritional components	Amount	Sample type	Region	Reference
Amylase inhibitor (AIU)	1.01	Raw tuber	Tamilnadu, India	Shanthakumari *et al*.,2008
	0.91	Soaking in distilled water		
	0.95	Soaking in sodium bicarbonate solution for 9 hours		
	0.81	Cooking 90 min		
	0.80	Autoclaving 45 min		
Trypsin inhibitor (TIU)	0.87	Raw tuber	Tamilnadu, India	Shanthakumari *et al*.,2008
	0.79	Soaking in distilled water		
	0.80	Soaking in sodium bicarbonate solution for 9 hours		
	0.78	Cooking 90 min		
	0.75	Autoclaving 45 min		
Saponin (mg/100g)	79.48	Uncooked (Dried at 65 ^{0}C in an oven)	Nigeria	Ogbuagu, 2008
	24.44	Cooked (After cooking dried at 65 ^{0}C)		
Alkaloid (%)	0.98	Uncooked (Dried at 65 ^{0}C in an oven)	Nigeria	Ogbuagu, 2008
	0.26	Cooked (After cooking dried at 65 ^{0}C)		
Oxalate (mg/100g)	2.46	Uncooked (Dried at 65 ^{0}C in an oven)	Nigeria	Ogbuagu, 2008
	0.69	Cooked (After cooking dried at 65 ^{0}C)		
TPC(g/100g)	3.37±0.15	Raw tuber	Tamilnadu, India	Arinathan *et al*.,2009
Tannin Content (g/100g)	2.55±0.07	Raw tuber	Tamilnadu, India	Arinathan *et al*.,2009
HCN (mg/100g)	0.17±0.02	Raw tuber	Tamilnadu, India	Arinathan *et al*.,2009
TPC (g/100g)	2.20±0.01	Raw tuber	Tamilnadu, India	Shajeela *et al*.,2011
Tannin (g/100g)	1.48±0.10	Raw tuber	Tamilnadu, India	Shajeela *et al*.,2011
HCN (mg/100g)	0.19±0.01	Raw tuber	Tamilnadu, India	Shajeela *et al*.,2011
Total oxalate (g/100g)	0.78±0.01	Raw tuber	Tamilnadu, India	Shajeela *et al*.,2011

Antinutritional components	**Amount**	**Sample type**	**Region**	**Reference**
Amylase inhibitor (AIU/mg soluble starch)	1.36	Raw tuber	Tamilnadu, India	Shajeela *et al*.,2011
Trypsin inhibitor (TIU/mg protein)	1.21±0.01	Raw tuber	Tamilnadu, India	Shajeela *et al*.,2011
Saponin	21.37	Oven dried Bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	32.28	Oven dried peel		
	24	Oven dried Whole tuber		
Tannin	4.21	Oven dried bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	4.79	Oven dried peel		
	4.21	Oven dried Whole tuber		
Pholabatanin	1.56	Oven dried Bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	1.87	Oven dried peel		
	ND	Oven dried Whole tuber		
Flavonoid	6.33	Oven dried bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	9.17	Oven dried peel		
	5.36	Oven dried Whole tuber		
Steroid	ND	Oven dried Bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	ND	Oven dried peel		
	ND	Oven dried Whole tuber		
Terpenoid	20.40	Oven dried Bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	8.48	Oven dried peel		
	8.48	Oven dried Whole tuber		
Alkaloid	ND	Oven dried Bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	ND	Oven dried peel		
	ND	Oven dried Whole tuber		
Anthraquinone	ND	Oven dried bulbils	Ondo-State, Nigeria	Adeosun *et al*.,2016
	ND	Oven dried peel		
	ND	Oven dried Whole tuber		

Antinutritional components	Amount	Sample type	Region	Reference
Cardiac glycosides	12.37	Oven dried Bulbils	Ondo-State, Nigeria	Adeosun *et al.*, 2016
	15.90	Oven dried peel		
	13.13	Oven dried Whole tuber		
Tannins (mg/100gm)	10.27±0.36	Air oven dried Light grey	Ghana	Polycarp *et al.*, 2012
	10.98±0.03	Deepgrey tuber		
Phytates (mg/100gm)	1.20±0.22	Air oven dried Light grey	Ghana	Polycarp *et al.*,2012
	2.24±0.23	Deepgrey tuber		
Oxalates (mg/100gm)	0.63±0.02	Air oven dried Light grey	Ghana	Polycarp *et al.*,2012
	0.58±0.02	Deepgrey tuber		
Flavonoids	1.80±0.14%	aqueous extract of peel of Db	Nigeria	Eleazu *et al.*,2013
	1.70±0.14%	Ethanolic extract of peel of		
Tannins	2.21±0.16%	aqueous extract of peel of Db	Nigeria	Eleazu *et al.*,2013
	1.47±0.03%	Ethanolic extract of peel of		
Alkaloids	0.38±0.04%	aqueous extract of peel of Db	Nigeria	Eleazu *et al.*,2013
	0.68±0.04%	Ethanolic extract of peel of		
Saponins	3.72±0.03%	aqueous extract of peel of Db	Nigeria	Eleazu *et al.*,2013
	6.07±0.10%	Ethanolic extract of peel of		
Total phenol	558 ± 3.46	Dried bulbils	Abrogoua	Achy, JY., *et al.*, 2016
Total oxalate	320 ± 2.65	Dried bulbils	Abrogoua	Achy, JY., *et al.*, 2016
Phytate	469.33±2.08	Dried bulbils	Abrogoua	Achy, J Y. *et al.*, 2016
Tannins	66 ± 1.73	Dried bulbils	Abrogoua	Achy, J Y *et al.*, 2016
α-amylase inhibitors	251 ± 2.65	Dried bulbils	Abrogoua	Achy, JY *et al.*, 2016
Trypsin inhibitors	197 ± 1.73	Dried bulbils	Abrogoua	Achy, JY *et al.*, 2016
Alkaloid (%)	2.06±0.34	Oven dried bulbs Sulfited flour	Enugu State, Nigeria	Igbokwe *et al.*, 2016
	3.63±0.99	Steam blanched Flour		
	2.04±0.31	Untreated flour		

Antinutritional components	Amount	Sample type	Region	Reference
Flavonoid (%)	11.23±0.14	Oven dried bulbs Sulfited flour	Enugu State, Nigeria	Igbokwe *et al.*,2016
	12.87±0.61	Steam blanched Flour		
	8.84±0.36	Untreated flour		
Tannin (mg/100g)	75.7±10.58	Oven dried bulbs Sulfited flour	Enugu State, Nigeria	Igbokwe *et al.*,2016
	53.3±0.79	Steam blanched Flour		
	112.9±3.78	Untreated flour		
Phytate (%)	0.82±0.48	Oven dried bulbs Sulfited flour	Enugu State, Nigeria	Igbokwe *et al.*,2016
	0.40±0.01	Steam blanched Flour		
	0.24±0.05	Untreated flour		
Carotenoid (%)	4.83±0.50	Oven dried bulbs Sulfited flour	Enugu State, Nigeria	Igbokwe *et al.*,2016
	0.38±0.16	Steam blanched Flour		
	0.08±0.02	Untreated flour		
Saponin (%)	0.53±0.01	Oven dried bulbs Sulfited flour	Enugu State, Nigeria	Igbokwe *et al.*,2016
	0.46±0.09	Steam blanched Flour		
	0.73±0.05	Untreated flour		
Hydrogen cyanide	49.70 ± 0.00	Fresh	Benue state, Makurdi	Anhwange *et al.*,2011
	32.40 ± 0.00	Dried		
Saponins	14.88±0.10	Sun dried for four days	Abia state, Nigeria	Okwu and Ndu,2006
Flavonoids	8.04±0.20	Sun dried for four days	Abia state, Nigeria	Okwu and Ndu,2006
Alkaloids	0.88±0.11	Sun dried for four days	Abia state, Nigeria	Okwu and Ndu,2006
Phenol	1.10	Green	Enugu state, Nigeria	Ogbonna *et al.*,2015
	1.22	Red		
	1.64	Yellow		
Saponin	14.03	Green	Enugu state, Nigeria	Ogbonna *et al.*,2015
	5.46	Red		
	8.49	yellow		
Oxalate	12.60	Green	Enugu state, Nigeria	Ogbonna *et al.*,2015
	9.00	Red		
	10.89	yellow		
Tannin	0.22	Green	Enugu state, Nigeria	Ogbonna *et al.*,2015
	0.19	Red		
	0.18	yellow		

1.2.6. Phytochemicals

Phytochemicals are secondary metabolites of the plant which are responsible for pharmacological potential as well as the toxicity of plant material. Many researchers identified and isolated a vast of phytochemicals of *D.bulbifera*. Some of those are depicted in table 1.5 and molecular structures of those are included in table 1.6.

Table 1.5. Various phytochemicals of the *Dioscorea bulbifera*.

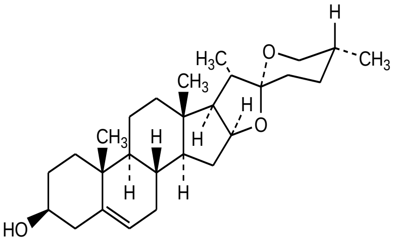
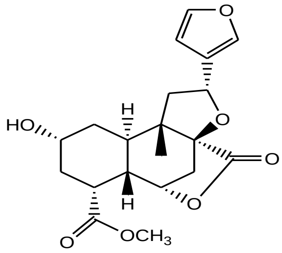
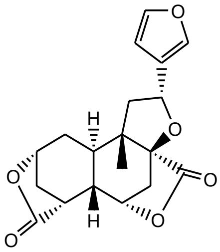
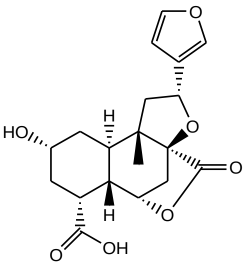
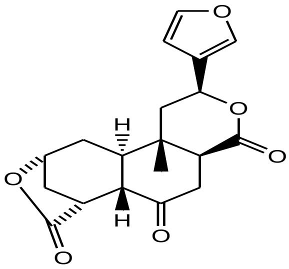
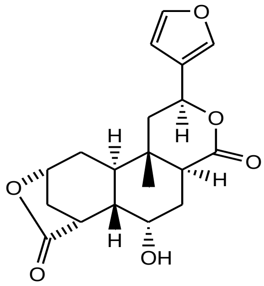
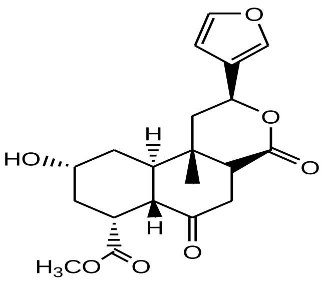
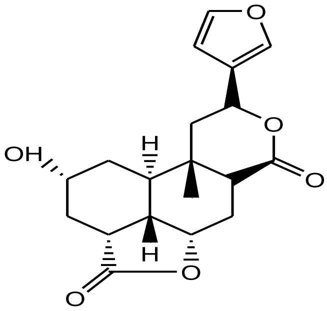
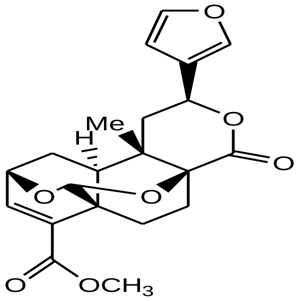
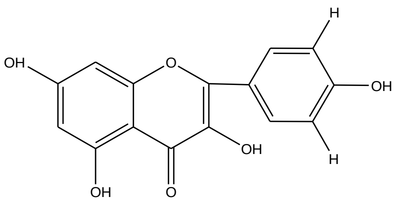
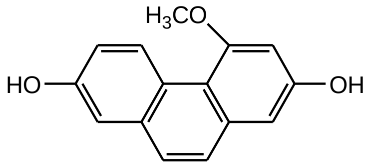
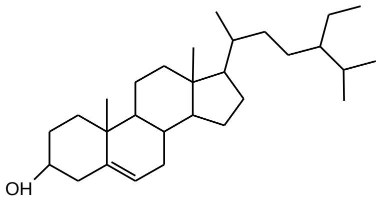
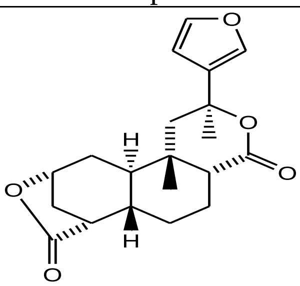
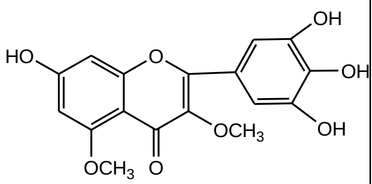
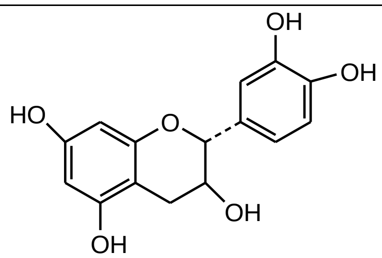
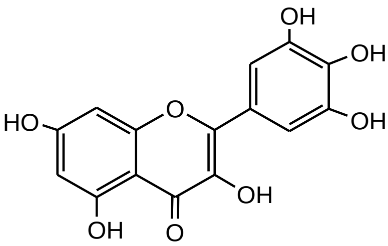
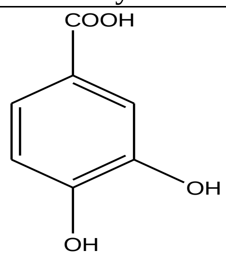
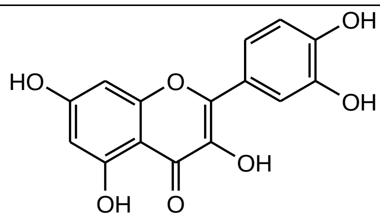
S.No	Chemicals	Sources	Region	References
1	Diosgenin		India	Ghosh *et al*.,2014
2	Diosbulbin A	Tuber, leaf and stem	Japan Hubei (China)	*Kawasaki et al.,(*1968), Tang *et al*., (2014) , Komari (1997)
3	Diosbulbin B	Tuber, rhizome, leaf and stem	Japan Shengyang (China) Qingyang (China)	Kawasaki *et al*.,(1968) wang *et al*.,(2009), Gao *et al*.,(2002) Gao *et al*.,(2007) and Liu *et al*.,(2010), Komari,(1997)
4	Diosbulbin C	Tuber, leaf and stem	Japan Hubei (China)	Kawasaki *et al*.,(1968), Tang *et al*., (2014), Komari,(1997)
5	Diosbulbin D	Tubers, leaf and stem	Hubei (China) Japan	Tang *et al*.,(2014), Wang *et al*.,(2009), Murry *et al*., (1984) Komari ,(1997)
6	Diosbulbin E	Leaf and stem rhizomes	Japan, Qingyang (China)	Komari,(1997) Liu *et al*.,(2010)
7	Diosbulbin F	Leaf and stem, tuber, rhizomes	Japan Hubei (China) Qingyang (China)	Komari,(1997) Tang *et al*.,(2014) Liu *et al*.,(2010)
8	Diosbulbin G	Leaf and stem, tuber, rhizomes	Japan, Hubei (China)	Komari,(1997), Tang *et al*.,*(*2014), Liu *et al*.,(2010)
9	Bafoudiosbulbin C	Bulbils	Cameroon	Kuete *et al*.,(2012) Teponno *et al*.,(2007), Teponno *et al*.,(2008)
10	Kaempferol	Rhizomes, bulbils	Shengyang (China) Qingyang (China) Cameroon	Gao *et al*., (2007), Liu *et al*., (2011),Teponno *et al*., (2008)
11	2,7-dihydroxy-4-methoxyphenanthrene	Bulbil, tuber	Cameroon	Kuete *et al*.,(2012), Teponno *et al*.,(2006)
12	β-Sitosterol	Rhizome, tuber	Shengyang(China)	Gao *et al*.,(2007),Teponno *et al*.,(2006)
13	8-epidiosbulbin E acetate	Tuber	India	Shriram *et al*., (2008) Murray *et al*., (1984)

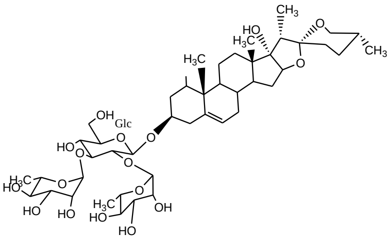
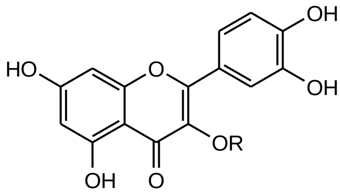
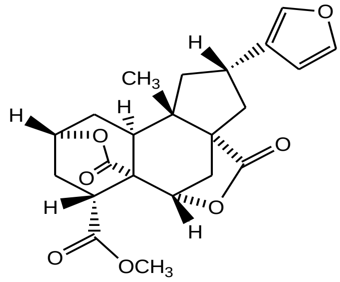
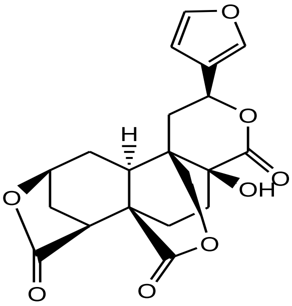
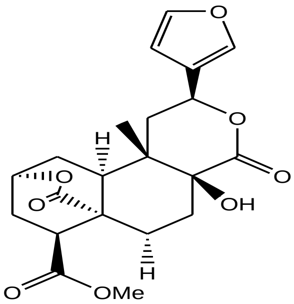
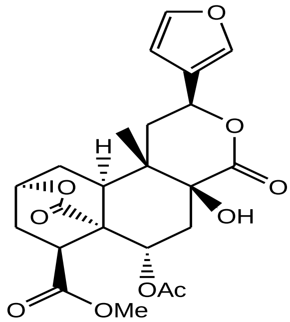
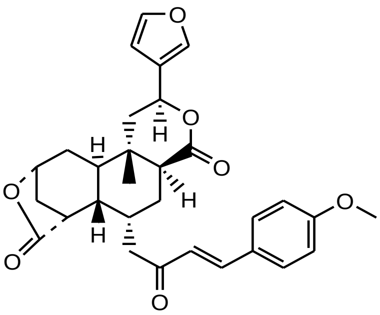
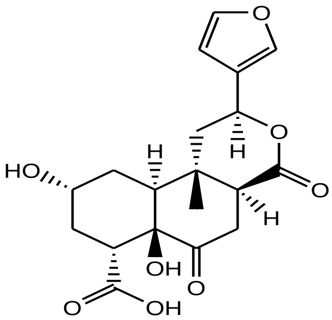
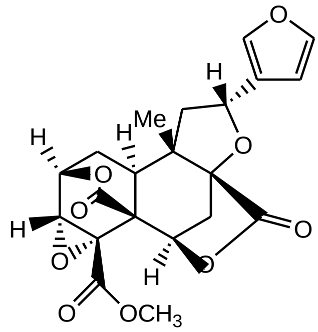
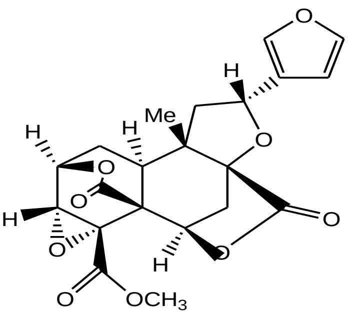
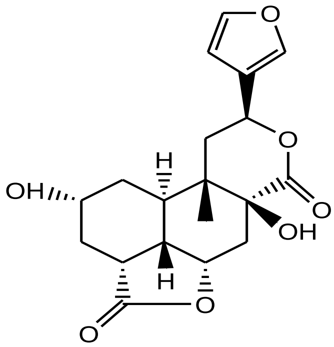
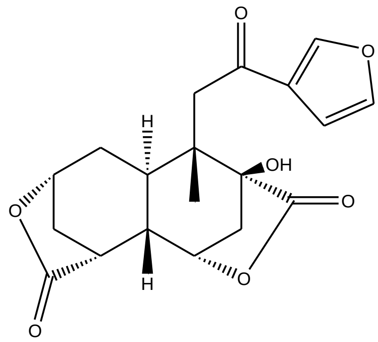
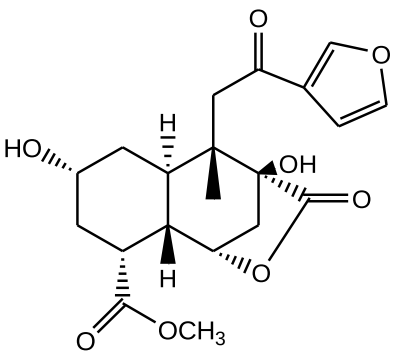
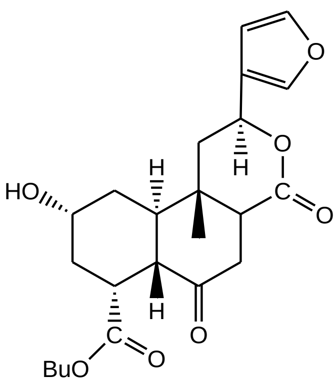
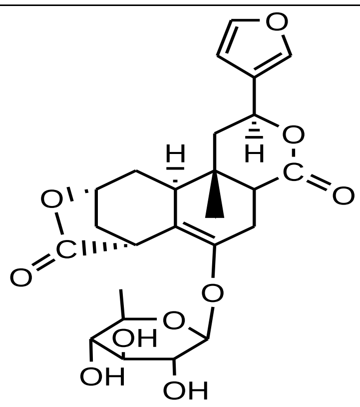
14	Caryatin	Rhizome	Shengyang(China)	Gao *et al*.,(2007), Gao *et al*.,(2002)
15	(+)catechin	Rhizome	Shengyang(China)	Gao *et al*.,(2002;2007), Liu *et al*.,(2011)
16	Myricetin	Rhizome, herb	Shengyang(China)	Gao *et al*.,(2002;2007), Tang *et al*.,(2006)
17	protocatechuic acid	Tuber, rhizome	Cameroon	Wang *et al*.,(2009),Gao *et al*.,(2007)
18	Quercetin	Tuber, rhizome	Cameroon Qingyang(China)	Teponno *et al*., (2006), Liu *et al*. (2011)
19	Spiroconazole A	Tuber , flower	Cameroon	Teponno *et al*., (2006) Tapondjou *et al*., (2013)
20	Quercetin-3-O-β-glucopyranoside	Tuber	Cameroon	Teponno *et al*., (2006).
21	Quercetin-3-O-β-galactopyranoside	Tuber	Cameroon	Teponno *et al*., (2006)
22	Bafoudiosbulbin A Bafoudiosbulbin B	Bulbil, tuber	Cameroon	Kuete *et al*., (2012) Teponno *et al*., (2006-2008)
23	Bafoudiosbulbin F Bafoudiosbulbin G	Bulbil	Cameroon	Kuete *et al*.,(2012) Teponno *et al*.,(2008)
24	Diosbulbin I, Diosbulbin J	Tuber	Anhui(China)	Wang *et al*.,(2009)
25	Bafoudiosbulbin D Bafoudiosbulbin E	Bulbil	Cameroon	Teponno *et al*., (2007)
26	Diosbulbin N Diosbulbin O Diosbulbin P	Tuber	Hubei(China)	Tang *et al*.,(2014)
27	Diosbulbin H, Diosbulbinoside D Diosbulbinoside F	Leaf & stem	Japan	Komari (1997)
28	Diosbulbin K, Diosbulbin L, Diosbulbin M, Diosbulbinoside G	Rhizomes	Qingyang(China)	Liu *et al*., (2010)
29	(+)Epicatechin, Isovanillic acid and Vanillic acid	Herb, rhizome	China, Cameroon	Tang *et al*.,(2006), Gao *et al*.,(2007)
30	3α-hydroxy-13β-furan-11-ketoapia-8-en-(20,6)-olide	Root	China	Zheng *et al*.,(2003)
31	13β-furan-11-keto-apian-3(4),8-dien-(20,6)-olide,	Root	China	Zheng *et al*.,(2003)
32	7α-methoxy-13β-furan-11-keto-apian-3(4),8-dien-(20,6)-olide 3	Root	China	Zheng *et al*.,(2003)
33	Hyperside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
34	Myricetin-3-0-β-D-glucopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)

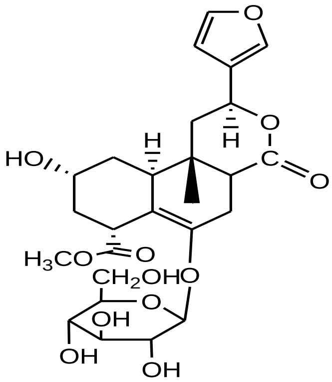
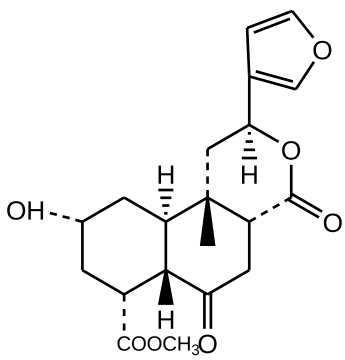
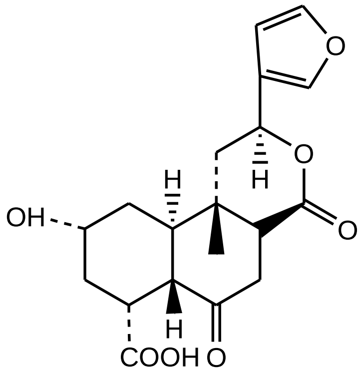
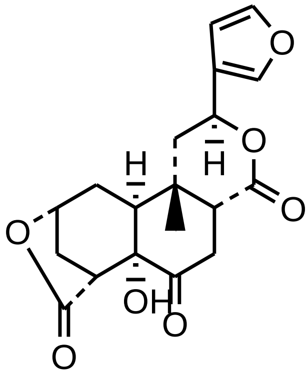
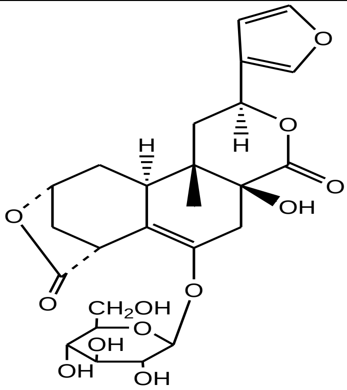
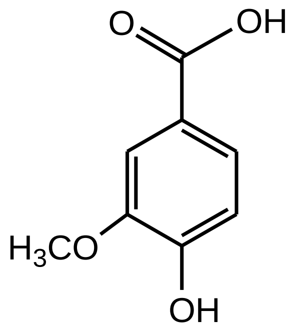
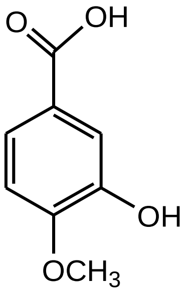
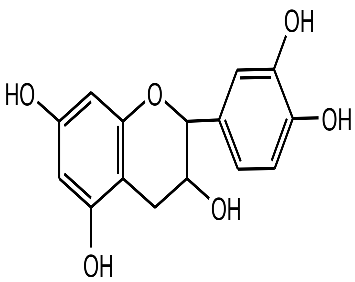
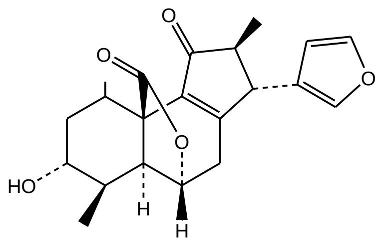
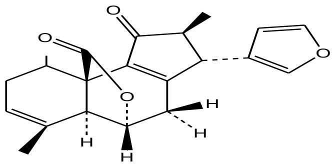
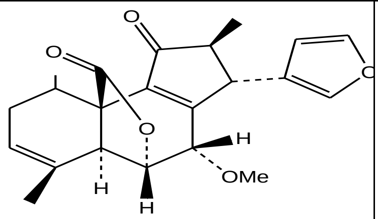
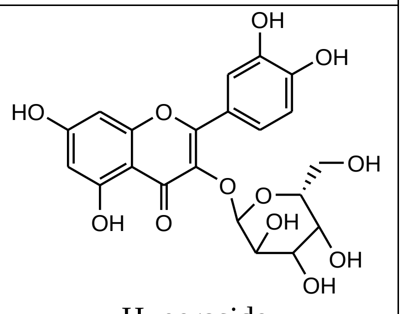
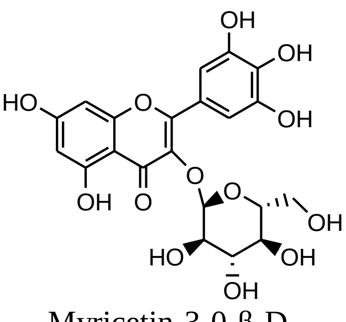
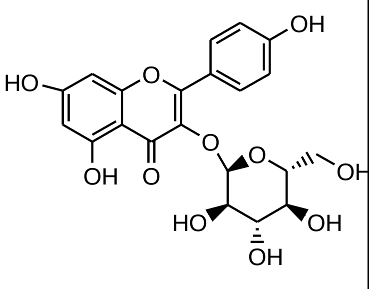
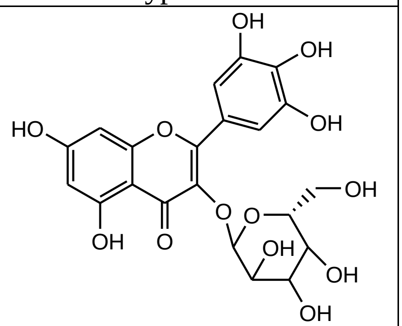
35	Myricetin-3-0-β-D-galactopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
36	3,5,3'-trimethoxy-quercetin	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
37	3,5-dimethoxykaempferol	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
38	Shikimic acid	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
39	Succinic acid	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
40	Palmatic acid	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
41	Daucosterol	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
42	kaempferol-3-0-β-D-galactopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
43	kaempferol-3-0-β-D-glucopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
44	Methyl-0-α-D-fructofuranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
45	Butyl-0-α-D-fructofuranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
46	Ethyl-0-β-D-fructopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
47	Butyl-0-β-D-fructopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
48	3-phenyl-2-propenyl-0-β-D-glucopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
49	2-(4-methoxyphenyl)-ethyl-0-β-D-glucopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
50	Phenyl-methyl-0-β-D-glucopyranoside	Rhizomes	Shengyang(China)	Gao *et al*.,(2007)
51	Dioscoreanoside A	Flowers	Cameroon	Tapondjou *et al*.,(2013)
52	DioscoreanosideB	Flowers	Cameroon	Tapondjou *et al*.,(2013)
53	Dioscoreanoside C	Flowers	Cameroon	Tapondjou *et al*.,(2013)
54	Dioscoreanoside D	Flowers	Cameroon	Tapondjou *et al*.,(2013)
55	Dioscoreanoside E,	Flowers	Cameroon	Tapondjou *et al*.,(2013)
56	Dioscoreanoside F,	Flowers	Cameroon	Tapondjou *et al*.,(2013)
57	Dioscoreanoside G	Flowers	Cameroon	Tapondjou *et al*.,(2013)
58	Dioscoreanoside H	Flowers	Cameroon	Tapondjou *et al*.,(2013)
59	Dioscoreanoside I	Flowers	Cameroon	Tapondjou *et al*.,(2013)
60	Dioscoreanoside J	Flowers	Cameroon	Tapondjou *et al*.,(2013)
61	Dioscoreanoside K	Flowers	Cameroon	Tapondjou *et al*.,(2013)
62	Stigmasterol	Tuber	China	Wang *et al*.,(2009)
63	Mono-arachidin	Tuber	China	Wang *et al*.,(2009)
64	1,7-bis-(4-hydroxyphenyl)-1E,4E,6E-heptatrien-3-one	Tuber	China	Wang *et al*.,(2009)

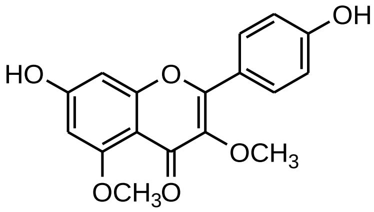
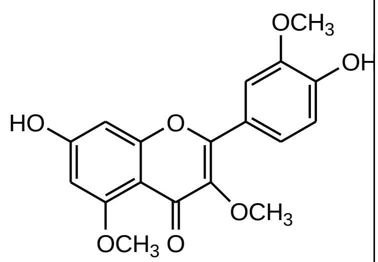
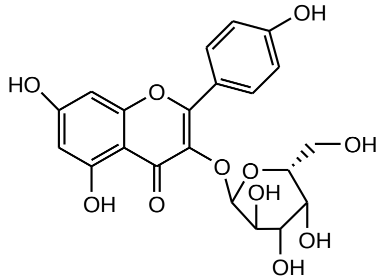
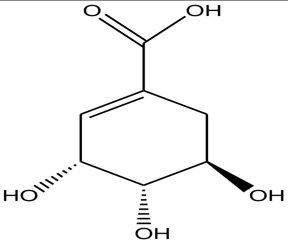
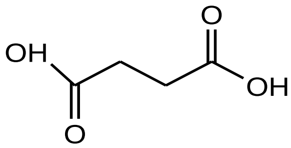
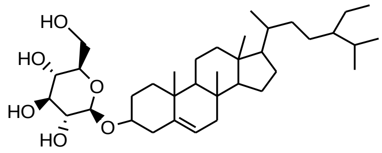
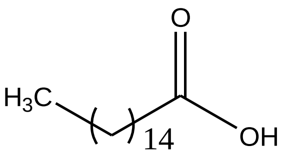
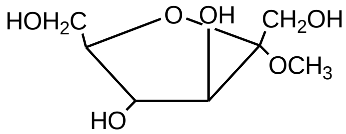
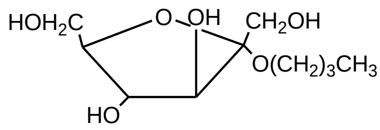
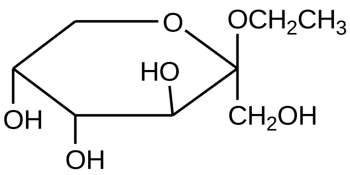
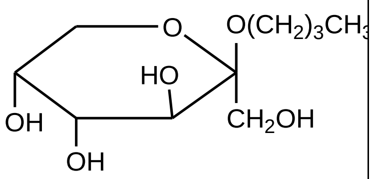
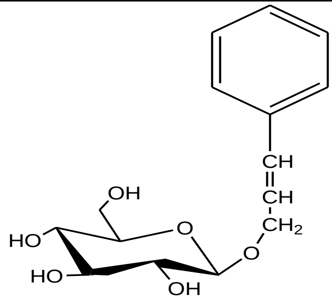
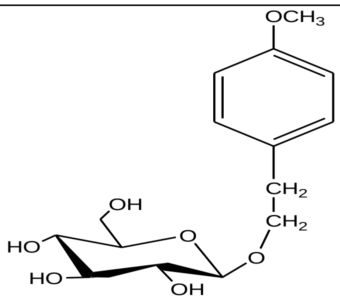
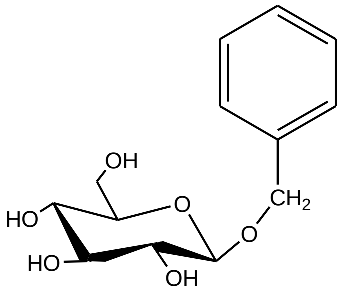
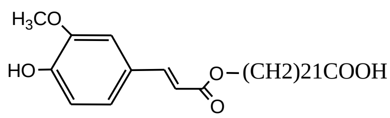
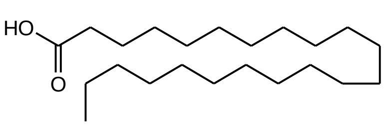
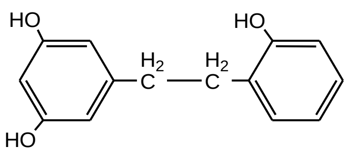
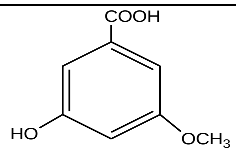
65	Behenic acid	Tuber	China	Wang *et al.*,(2009)
66	Demethyl batasin IV	Tuber	China	Wang *et al.*,(2009)
67	2,3'-dihydroxy-4',5'-dimethoxybibenzyl	Tuber	China	Wang *et al.*,(2009)
68	Docosyl ferulate	Tuber	China	Wang *et al.*,(2009)
69	7-bis-(4-hydroxyphenyl)-4E,6E-heptadien-3-one	Tuber	China	Wang *et al.*,(2009)
70	5,3,4-trihydroxy-3,7-dimethoxyflavone	Tuber	China	Wang *et al.*,(2009)
71	Tristin	Tuber	China	Wang *et al.*,(2009)
72	Adenosine	Tuber	China	Wang *et al.*,(2009)
73	C22 ω-hydroxy fatty acid	Rhizomes	Qingyang(China)	Liu *et al.*,(2011).
74	3-hydroxy 5-methoxybenzoic acid	Rhizomes	Qingyang(China)	Liu *et al.*,(2011).
75	Batatasin III	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
76	2,7-dihydroxy-3,4,6-trimethoxyphenanthrene	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
77	1,6-dihydroxy-2,5,7-trimethoxyphenanthrene	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
78	2,4,6,7-tetrahydroxy-9,10-dihydrophenanthrene	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
79	2,5,2',5'-tetrahydroxy-3'-methoxybibenzyl	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
80	Thunalbene	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
81	2,4-dimethoxyphenanthrene-3, 7-diol	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
82	Favanthrinin	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
83	Kaempferol	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
84	Isorhamnetin	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
85	Quercetin	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
86	3,5,3'-trimethoxyquercetin	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).
87	Isoquercitin	Rhizomes	Qingyang(China)	Liu *et al.*, (2011).

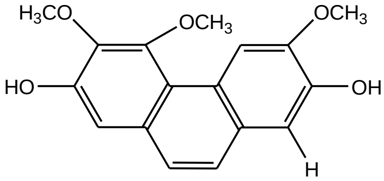
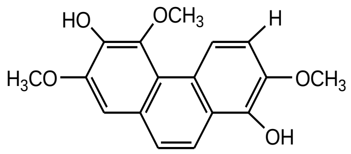
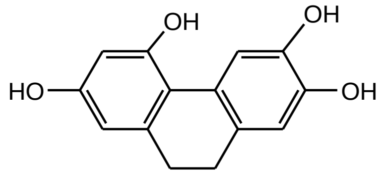
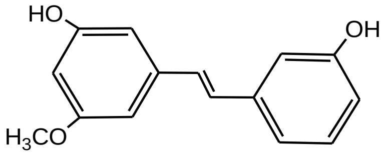
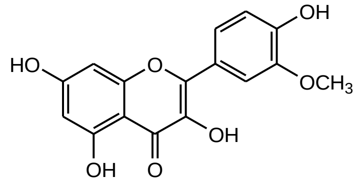
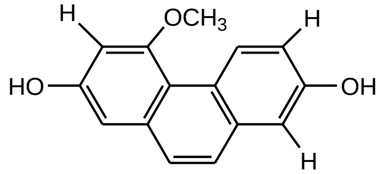
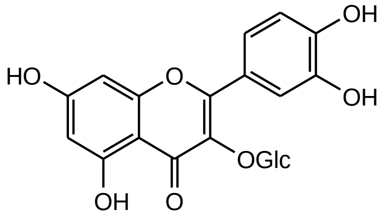
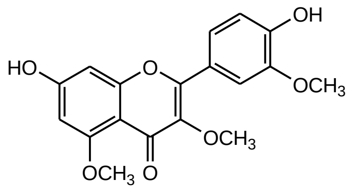
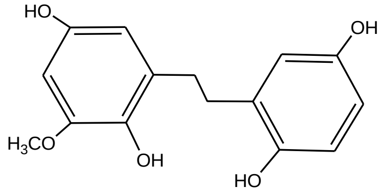
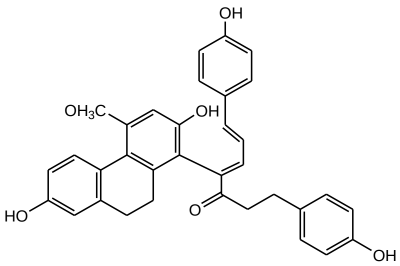
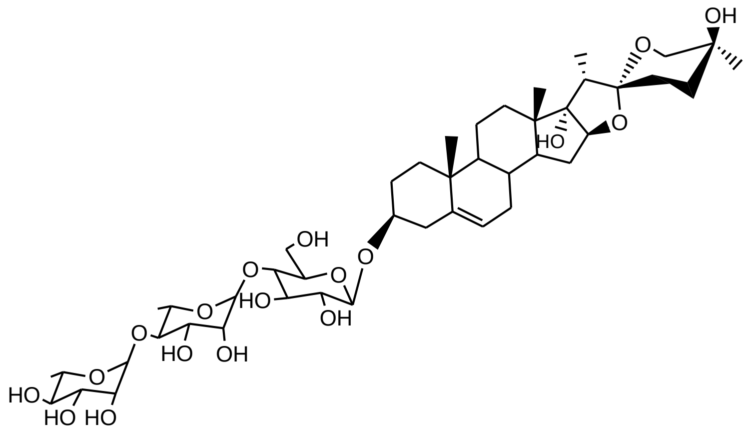
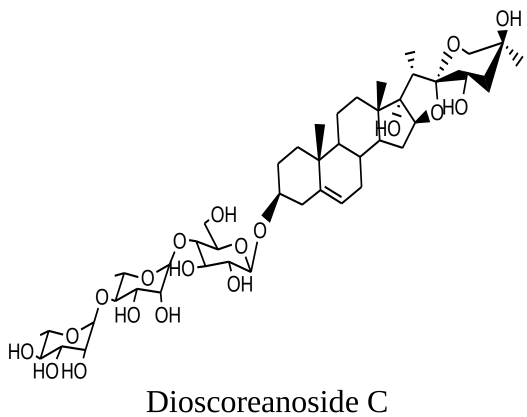
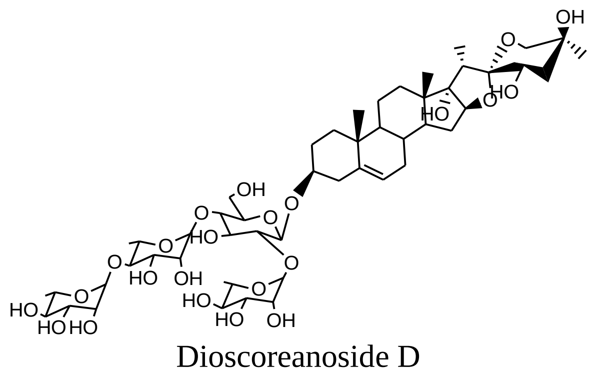
Table 1.6. Molecular structure of various phytochemicals of *Dioscorea bulbifera*

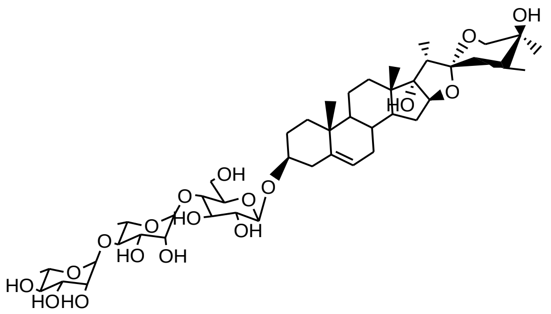
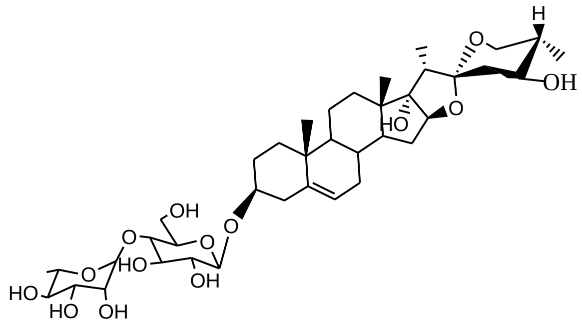
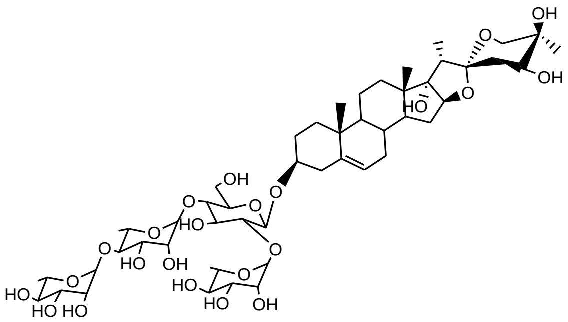
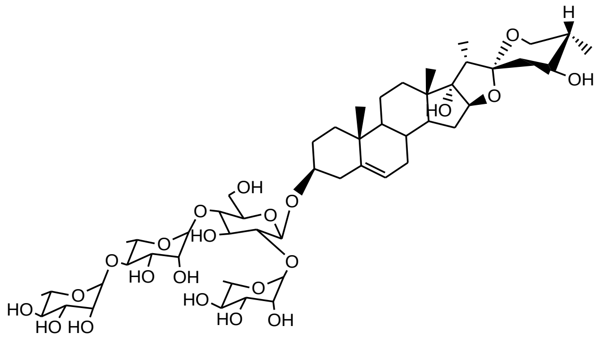
 Diosgenin	 Diosbulbin A	 Diosbulbin B
 Diosbulbin C	 Diosbulbin D	 Diosbulbin E
 Diosbulbin F	 Diosbulbin G	 Bafoudiosbulbin C
 Kaempferol	 2,7-dihydroxy-4-methoxyphenonthrene	 Beta-sitosterol
 8-epidiosbulbin E acetate	 Carytin	 (+) Catechin
 Myricetin	 Protocatechuic acid	 Quercitrin

 Spiroconazole A	 R=β-D-gluopyranosyl R=β-D-galactopyranosyl	 Bafoudiosbulbin A
 Bafoudiosbulbin B	 Bafoudiosbulbin F	 Bafoudiosbulbin G
 Diosbulbin I	 Diosbulbin J	 Bafoudiosbulbin D
 Bafoudiosbulbin E	 Diosbulbin N	 Diosbulbin O
 Diosbulbin P	 Diosbulbin H	 Diosbulbinoside D

 Diosbulbinoside F	 Diosbulbin K	 Diobulbin L
 Diosbulbin M	 Diosbulbinoside G	 Vanillic acid
 Isovanillic acid	 Epicatechin	 3α-hdorxy-13β-furan-11-keto-apian-8-en-(20,6)-olide
 13β-furan-11-keto-apian-3(4),8dien-(20,6)-olide	 7α-methoxy-13β-furan-11-keto-apian-3(4),8-dien-(20,6)-olide	 Hyperoside
 Myricetin-3-0-β-D-glucopyranoside	 Kaempferol-3-0-B-D-glucopyranoside	 Myricetin-3-0-B-D-galactopyranoside

 3,5-dimethoxy kaempferol	 3,5,3'-trimethoxy quercetin	 Kaempferol-3-0-B-D-galactopyranoside
 Shikimic acid	 Succinic acid	 Daucosterol
 Palmitic acid	 Methyl-0-α-fructo furanoside	 Butyl-0-α-fructo furanoside
 Ethyl-0-β-D-fructopyranoside	 Butyl-0-β-D-fructopyranoside	 3-phenyl-2-propenyl-0-β-D-glucopyranoside
 2-(4-methoxyphenyl)-ethyl-0-β-D-glucopyranoside	 Phenyl-methyl-0-β-D-glucopyranoside	 C22-ω-hydroxy fatty acid
 Behenic acid	 Demethyl batatasin IV	 3-hydroxy-5-methoxy benzoic acid

 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene	 1,6-dihydroxy-2,5-7-trimethoxy phenanthrene	 2,4,6,7-tetrahydroxy-9,10-dihydrophenanthrene
 Thunalbene	 Isorhamnetin	 Flavanthrinin
 Isoquercitrin	 3,5,3’-trimethoxy quercetin	 2,5,2’,5’-tetrahydroxy-3’-methoxybibenzyl
 Diobulbinone-A	 Dioscoreanoside A	
 Dioscoreanoside C	 Dioscoreanoside D	

 Dioscoreanoside E	 Dioscoreanside F
 Dioscoreanoside G	 Dioscoreanoside H

1.2.7. Pharmacological activity

Organic solvents extracts of *D.bulbifera* reported to inhibited the growth of the tumor and also prolonged the survival of tumor-bearing mice and human liver cancer, colon cancer and other tumor cells (Dutta, 2015; Ghosh *et al*., 2015; Hans, 1996; Li *et al.*, 1999; Zhao, 2009). Researchers found that *D.bulbifera* has potent antibacterial activity against many bacteria (Okigbo *et al.*, 2009; Kuete *et al*., 2012; Teponno *et al*., 2006; Shriram *et al*., 2008; Tang *et al.*, 2006; Seetharam *et al*., 2003; Ghosh *et al*., 2012 and Xu *et al*., 1988). *D.bulbifera* extracts have strong antioxidant potential (Song *et al*., 2010; liu *et al*., 2011; Ghosh *et al*., 2013).

1.2.8. Toxicity analysis

Bhandari and Kawabata (2005) identified furanoid norditerpenes, Diosbulbin B (0.151±0.02 g/kg) and Diosbulbin A (0.043±0.004 g/kg) as the bitter compound in Nepalian *Dioscorea bulbifera* tuber. It was reported that Diosbulbin A, Diosbulbin B, Diosbulbin C, Diosbulbin D and 8-epidiosbulbin E compounds of *Dioscorea bulbifera* have hepato-toxicity effect (Min, MA., *et al*., 2011, Ma, Y., *et al.*, 2014; Niu, C., *et al*., 2016; Wang, LL., *et al*., 2017; Zhao *et al*., 2018;). Min, MA., *et al*. (2011) observed toxicity effect of diterpene lactone, Diosbulbin D isolated from Chinese *Dioscorea bulbifera* tuber in L-02 human normal liver cell based on the increase of the liver enzymes, alanine aminotransferase and aspartate aminotransferase. Ma, Y., *et al*. (2014)

investigated the hepatotoxicity induced by Diosbulbin B, a diterpene lactone isolated from Chinese *D.bulbifera* L. in mice at the doses of 0, 16, 32 and 64 mg/kg once daily for 12 consecutive days. They observed enzymes ALT, AST and ALP were increased significantly as compared to control in mice treated with 32 mg/kg and 64 mg/kg where livers lost normal architecture with extensive acute hemorrhage, swelling hepatocytes and widespread necrosis. Niu C., *et al*. (2016) found Diosbulbin B as the main toxic compound but serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) decreased due to the administration of ferulic acid. Wang, LL., *et al*. (2017) administrated 2.10 and 8.40 g/kg of the Chinese *Dioscorea bulbifera* rhizome extract (80% ethanolic) until 12 days to observe hepatotoxicity in rats and identified Diosbulbin A (0.020%), Diosbulbin B (2.218%), Diosbulbin C (0.046%) as toxic compounds. ALT and AST levels were significantly increased in both 2.10 g/kg and 8.40 g/kg body weight treated groups compound to the control group. Zhao *et al*. (2018) also conducted a hepatotoxicity study where they administrated 80% ethanolic extract of Chinese *Dioscorea bulbifera* in two doses, 1.8 g/kg body weight (low dose) and 18 g/kg body weight (high dose) for 12 weeks. They reported main toxic components were Diosbulbin B (2.15%W/W) and 8-epidiosbulbin E (0.057% W/W). They found an increased level of aspartate aminotransferase (AST) in both the treated groups. Hepatic cell swelling and necrosis were observed in both low and high dose treated rats. However, bile duct proliferation was detected only in the high dose group compared with the control group. Total saponins extracted from Chinese rhizome *Dioscorea bulbifera* were given to rats (30 g/kg body weight) once daily for 10, 30 and 60 days. The serum levels of both ALT and AST markers of liver injury were elevated for 60 days. The proliferation of fibrous tissue was seen in 30 days. After 60 days it was show some toxic effects.

1.2.9. Detoxification process

Bitter compounds of *Dioscorea bulbifera* vary based on geographical region. In Central American, South African and Indian varieties, saponin and sapogenins are responsible for bitterness (Galani Varsha J., 2017). In Indochinese varieties, tannins and polyphenols are bitter compounds where as in China and Australia furanoidnor diterpenes (Diosbulbins) are major bitter compounds (Galani Varsha J, 2017). In Australian varieties Diosbulbins D (yield 0.07 mg/g) is a major bitter compound (Webster *et al*., 1984). Bhandari and Kawabata (2005) reported furanoidnor diterpens, Diosbulbin A (0.043 g/kg) and Diosbulbins B (0.151 g/kg) for the bitterness of Nepalian

Dioscorea bulbifera tuber. Boiling followed by leaching is the most effective method for the removal of bitterness and making tuber palatable (Webster *et al.*,1984; Bhandari & Kawabata, 2005). Boiling could reduce the bitterness in the range of 75-100% where as pressure cooking and baking reduced bitterness in the range of 50-75% only (Bhandari & Kawabata, 2005).

1.2.10. Food uses of *Dioscorea bulbifera*

After removal of bitterness sliced tuber consumed as such or taken with jaggery as a staple food. The charcoal roasted tuber is also consumed in some parts of Ghana (Sanful *et al*., 2003). Seal (2015) reported that tuber is eaten as a vegetable along with other vegetables. It can be prepared and processed into edible food by boiling, frying or roasting, or eaten as cooked vegetables (Nwosu and Justin, 2014). It can be made into a paste after boiling and eaten with soup or can as well be processed into various forms such as crisps and chips or flaskes (Nwosu and Justin, 2014).

1.2.11. Different products developed from *Dioscorea bulbifera*

The tuber of *Dioscorea bulbifera* is mainly consumed and also being used to produce various processed products by boiling, frying or roasting or eaten as mixed with other vegetables (Nwosu and Justina, 2014; Seal, 2015; Gurung, 1995). Amandikwa *et al*. (2015) prepared bread from *Dioscorea bulbifera* flour and found 25% substitution was suitable while more than 25% substitution was not acceptable. Composite flours containing blanched fermented sun-dried aerial yam and cassava flour in the different ratios were also used to make a paste. Based on sensory characteristics, the 60-80% aerial yam composite flour was found to be more suitable for the paste (Kayode *et al*., 2017). Aerial yam flour at different levels with wheat flour was used for biscuit production. Biscuits made from 10% to 50% aerial yam substituted wheat flour were found to be acceptable while more than 50% substitution was not appreciable by panelists (Sanful *et al*., 2016). Similarly, cookies were prepared from composite flour of aerial yam and wheat flour. But 50% of aerial yam substituted cookies were found to be the most convenient one by the panelist in terms of colour, taste, aroma and overall acceptance (Bansod *et al*., 2020).

1.3 Objectives of the proposed work

1. To ascertain the nutritional composition (viz. minerals, vitamins, total phenol, flavonoids, carbohydrates, protein, amino acids, lipid and proximate content) of *Dioscorea bulbifera* from Odisha for the formulation of nutritional supplements.
2. Phytochemical profiling of *Dioscorea bulbifera* from Odisha through GC-MS/HRMS.
3. To evaluate the antioxidant, antibacterial and anticancer activity *in vitro* of the extract of *Dioscorea bulbifera* from Odisha for pharmacological importance.
4. To evaluate the toxicity if anything of the processed tuber of *Dioscorea bulbifera* consume by the local tribes.
5. Development of different processed food products such as biscuits, cookies, etc. and their nutritional characterization.

1.4 Organization of the thesis works

The thesis includes five distinct chapters. Chapter 1, describes a general introduction on *Dioscorea bulbifera*, its distribution, traditional uses, nutritional and pharmacological importance, different food products developed from it and objectives of the study. Chapter 2 includes the nutritional characterization of both raw and boiled tubers of *Dioscorea bulbifera* in which the proximate contents, minerals and vitamins profiling have been investigated and discussed. Chapter 3 includes phytochemical profiling of tubers of *Dioscorea bulbifera* and identification of putative compounds by GC-MS analysis. Chapter 4 deals with the pharmacological importance of tuber of *Dioscorea bulbifera* in terms of evaluation of the antioxidant, antibacterial and anticancer activity. Chpater 5 describe the toxicity evaluation of tuber extract of *Dioscorea bulbifera* following OECD guideline based on histopathological study and blood biochemistry. Chapter 6 includes the development of different value added food products, their nutritional and sensory evaluation.

CHAPTER II

NUTRITIONAL COMPOSITION OF BOTH RAW AND BOILED DIOSCOREA BULBIFERA TUBER

2.1. Introduction

Plants producing starchy roots, tubers, rhizomes, corms and stems are important in nutrition and health. They play an important role in the diet of populations in developing countries. Nutritionally, roots and tubers have a great potential to provide economic sources of dietary energy, in the form of carbohydrates (Chandrasekara and Kumar, 2016).). Important agronomic advantages of root and tuber crops as staple foods in their favorable adaption to diverse soil and environmental conditions (Chandrasekara and Kumar, 2016).

Historically, tribal and rural people identified and collected wild tubers from the forests and developed a range of processing methods in accordance to their needs (Sujatha *et al.*, 2013). Dioscoreaceae family is the most used family among tribal people (Padhan and Panda, 2016). *Dioscorea bulbifera* that belongs to the family *Dioscoreaceae* produces both underground and aerial tubers. However, underground tubers are very rich in starch and mostly consumed as alternative foods. The tribal people used it as food as well as medicine (Kumar *et al*., 2013). The tubers were found with a high amount of carbohydrates, fibers, and low levels of fats and protein. A good proportion of essential amino acids also make it a good dietary source. It is generally consumed by boiled, steamed, baked or fried (Osman, 1990; Gurung, 1995). Conversely, the wider utilization of yam is limited; due to a lack of proper nutritional information on the yam. Studies of nutritional composition on yam revealed as a food of considerable significance to the tribal people in order to fulfill their nutritional requirement.

Detailed information on the nutritional profiling of tubers of *D. bulbifera* from the state of Odisha is very rare. In this regard, the present study was initiated to evaluate and understand the nutritive values of wild yam *D. bulbifera*, prevalently consumed by the local tribal people in the forest area of Sundargarh district, Odisha India for its utilization as food supplements.

2.2. Materials and methods

2.2.1. Collection of sample

The mature and dormant tubers of *Dioscorea bulbifera* were collected from the forest of different villages such as Hatikucha, Kherban, Barhadema of district Sundargarh, Odisha, India in the month of November to January (Figure 2.1).

Figure 2.1. Images for collection of *Dioscorea bulbifera* tuber from the forest of different villages.

2.2.2. Identification of sample

The plant is identified by taxonomist, Regional Plant Resource Centre, Bhubaneswar, Odisha, India and its voucher specimen (Figure 2.2) is kept in the herbarium of Regional Plant Resource Centre.

Figure 2.2. Herbarium sheet of *Dioscorea bulbifera* plant kept in the herbarium of Regional Plant Resource Centre, Bhubaneswar, Odisha.

2.2.3. Preparation of sample

Collected tubers were washed thoroughly under tap water to remove dirt and dust. Cleaned tubers were peeled and sliced about 1-2 mm in thickness and divided into two portions; one portion of the sliced tubers was boiled in water until the foam was formed. The boiled water was discarded and the tubers were steeped in fresh water for the whole night followed by dryness in a hot air oven at 80 ^{o}C until a constant weight was obtained. The other portion was taken fresh (without boiling) and dried in a hot air oven at 80 ^{o}C until a constant weight was obtained. The dried samples were pulverized and sieved through a 1.0 mm sieve to get the powder form of the sample. Both raw and boiled tuber powder samples were kept in an airtight glass bottle for further analysis. The entire process of processing of tuber samples is collated in Figure 2.3.

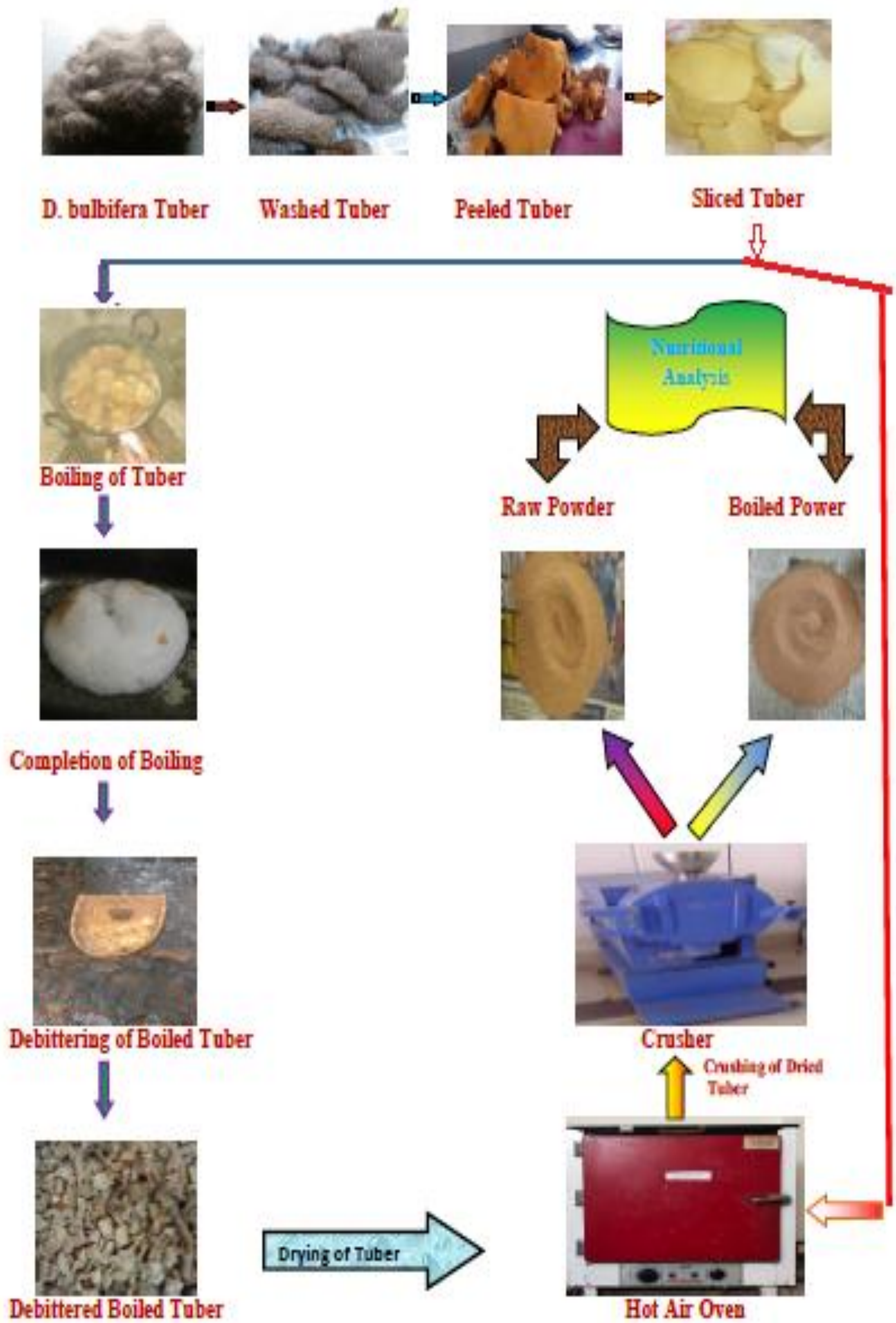


Figure 2.3. Flow chart of sample preparation

2.2.4. Methods of estimation

2.2.4.1. Moisture content

The moisture content of both the raw and boiled tuber samples was determined by the method of A.O.A.C (1970). Samples were accurately weighed and dried in an oven at 80^{o}C until a constant weight was obtained. The moisture content was estimated using the following formula.

Moisture content (gm/100gm) sample = (Initial weight-final weight)/(sample weight) ×100

2.2.4.2. Ash content

The ash content of both the raw and boiled tuber samples was determined by heating the samples in a muffle furnace at 550 ^{o}C for six hours or until a constant weight was reached (AOAC, 1990). The amount of ash formed was weighted in an electronic balance.

2.2.4.3. Total carbohydrate content

The total carbohydrate of both the raw and boiled tuber samples was estimated using Anthrone reagent method (Hodge and Hofreiter, 1962). Briefly, 100 mg of powder sample was hydrolyzed with 5 ml of 2.5 N HCl for three hours. After cooling, it was neutralized with solid sodium carbonate and centrifuged at 6000 rpm for 10 minutes. The supernatant was used to determine the amount of total carbohydrate in the sample.

2.2.4.4. Starch content

Starch content of both the raw and boiled tuber samples was estimated and calculated by the methods of Hodge and Hofreiter (1962) and Thayumanavan and Sadasivam (1984). About 100 mg of sample was washed with hot 80% ethanol till the washing didn’t give green colour with Anthrone reagent to remove free sugars. To the residue, 52% perchloric acid was added and incubated for 20 minutes at room temperature. The extraction process was repeated three times followed by centrifugation at 600 rpm for 10 minutes. The amount of glucose present was estimated using Anthrone reagent. The glucose content of the sample was then multiplied by 0.9 to obtain the starch content.

2.2.4.5. Reducing sugar content

Reducing sugar content of both the raw and boiled tuber samples was estimated by Dinitrosalicyclic acid method (Sadasivam and Manickam, 2008). Briefly, 100 mg of sample was used for hot solvent extraction using hydro-ethanol (80%) in a Soxhlet apparatus for 24 h. The extract was dried using a rotary evaporator at 60 $^{\circ}$C for 4 h and stored at -20 $^{\circ}$C for use in subsequent experiments. The extract was dissolved in 10 ml of distilled water and used as a stock solution. About 0.5 ml of extract was taken and the volume was adjusted to 3 ml. To it, 3 ml of DNS reagent was added, heated in a boiling water bath for 5 min and added 1 ml of 40% Rochelle salt solution. The absorbance was measured at 510 nm to determine the reducing sugar content.

2.2.4.6. Protein estimation

The protein content of both the raw and boiled tuber samples was estimated by the methods of Lowery *et al*. (1951). Briefly, 1 g of dry powder sample was homogenized with 10 ml of cold phosphate buffer (pH 7.5, 0.1 M). The homogenate was centrifuged at 5000 rpm for 15 min. The supernatant was collected and used to determine the protein content.

2.2.4.7. Total free amino acid estimation

The total free amino acid of both the raw and boiled tuber samples was determined by using ninhydrin reagent (Sadasivam and Manickam, 2008). Briefly, 100 mg of sample was extracted by 80% ethanol. To 0.1 ml of extract 1 ml of ninhydrin solution was added and volume was adjusted to 2 ml with distilled water. The mixture was boiled for 20 min in a boiling water bath. To it, 5 ml of diluent was added and incubated for 1 hour. The intensity of the purple colour was measured at 570 nm to determine the total free amino acid content in the sample.

2.2.4.8. Composition of amino acid

The amino acid composition of both the raw and boiled tuber samples was determined by using Shimadzu LC-30 AD HPLC, as previously reported (Pal *et al*., 2016). The system consisted of a fluorescence detector and a C-18 column of the dimension 150x4.6 mm and pore size of 5 µm. The peaks detected were analyzed using 5.54 SP 5LAB solutions software. The amino acid composition in mg/100g was determined by comparing the retention time of individual amino acid standards with the sample and quantified by comparing the area ratio.

2.2.4.9. Total fat content

The total fat content of both the raw and boiled tuber samples was estimated based on standard protocol (AOAC, 1970). Briefly, 5 g of sample was transferred to a thimble plugged with a wad of fat-free cotton and dropped into the bottom of the extraction tube. The bottom of the extraction tube was connected to the Soxhlet flask and the top to the condenser. Before connecting the flask, the weight of the empty flask was taken. To it, 200 ml of petroleum ether was poured and the extraction was continued up to 16 hours. At the end of the extraction period, the petroleum ether was evaporated and dried at 100 ^{o}C for 1 hour. The extract was cooled in desiccators and weighted to determine the total fat content.

2.2.4.10. Ascorbic acid content

The ascorbic acid content of both the raw and boiled tuber samples was estimated as previously reported (Harris and Ray, 1935; Sadasivam and Theymoli, 1987). Briefly, 10 ml of 4% oxalic acid was added to the 5 ml of ascorbic acid solution (0.1 mg/1ml of ascorbic acid dissolved in 4% oxalic acid solution) and titrated against the 2, 6-dichlorophenolindophenol dye in sodium bicarbonate until the pink colour appeared, which was persisted for few minutes. (V_1). The amount of the dye consumed (V_1 ml) is equivalent to the amount of ascorbic acid present. About 0.5 g of sample was extracted in 4% oxalic acids and the volume was adjusted to 100 ml. The mixture was centrifuged at 5000 rpm for 10 min. To 5 ml of supernatant 10 ml of 4% oxalic acid was added and titrated against the dye. The amount of dye consumed was noted (V_2 ml). The amount of ascorbic acid in the sample was calculated based on the following formula.

Amount of ascorbic acid (mg/100g of sample) = (0.5 mg/V_1 ml×V_2 ml/5 ml×100 ml/wt. of the sample) ×100.

2.2.4.11. HPLC analysis of vitamins

Both the raw and boiled tuber samples of *Dioscorea bulbifera* were analyzed for different vitamins (B1, B2, B3 and B6) using HPLC. The vitamins were extracted as per the method of Perales *et al.* (2005). The chromatographic system was equipped with a Shimadzu HPLC and photodiode array detector. Supelcosil LC 17 DB column (250 mm×4.6 mm, 5µm; Sigma, USA) was used for the separation of vitamins. The vitamins composition of the samples was investigated in triplicate.

2.2.4.12. Analysis of mineral composition

About 1g fine powder of raw and boiled tuber samples were taken in 30 ml glazed porcelain crucible and heated at 500 ^{o}C for 2 h in a muffle furnance. The crucible was taken out and to it added 3.0 ml of HNO_3. The sample was then heated on a hot plate at 100 °C until dry. The crucible was again kept into the muffle furnace and added 10 ml of HCL. The sample was then transferred to a 50 ml volumetric flask and diluted with deionized water. An atomic absorption spectrometer was used to analyze the minerals separately after acid digestion of the sample, as described in the official method of the association of official analytical chemists.

2.3. Results and discussion

2.3.1. Proximate composition

In the present study comparative analysis of nutritional composition between the raw and boiled tubers has been carried out and presented in Table 2.1 and Figure 2.4. The difference in proximate content between raw and boiled tubers was found to be statistically significant using a student t-test ($p \leq 0.05$). The moisture content of the raw tuber was found to be quite low (74.89±0.54%) compared to the boiled tuber (80.48±1.18%). The ash content of the raw tuber was found to be high (2.57±0.04%) compared to the boiled tuber (1.66±0.34%). We found a very little amount of fats in both raw tubers (0.19±0.01%) and boiled tuber (0.14±0.012%), which are not significantly different. Total carbohydrate and reducing sugar content of raw tuber was found to be quite high (31.62±0.46% and 0.018±0.012%) compared to the boiled tuber (23.94±0.50% and 0.012±0.008%). In contrast, it was found that boiled tuber contained albeit high amount of starch (11.67±0.65%) as compared to the raw tuber (8.6±0.54%). The protein content of the raw and boiled tuber was found to be 3.48±0.92% and 2.25±0.16% respectively. The total free amino acid content was found to be slightly high in the raw tuber (1.45±0.05%) compared to the boiled tuber (0.59±0.13%). A higher amount of carbohydrate, protein, fat and ash have been reported earlier by other researchers (Ogbuagu, 2008; Abara, 2011; Polycarp *et al*., 2012; Achy *et al*., 2016; Adeosun *et al*., 2016; Ifeanacho *et al*., 2017; Arinathan *et al*., 2009; Celestine and David, 2015; Shanthakumari *et al*., 2008; Shajeela *et al*., 2011; Ogbonna *et al*., 2015). Whereas a very similar amount of ash content has been observed as compared to the earlier report (Celestine and David, 2015). Starch content of present findings has been found lower than earlier reports (Shanthakumari *et al*., 2008; Arinathan *et al*., 209; Shajeela *et al*., 2011). Carbohydrate content of raw tuber was found to be higher than potato (22.6), colocasia (21.1) and near to the value of tapioca (38.1), while boiled tuber contained a quite similar amount of carbohydrate with potato and colocasia (Mudambi and Rajagopal, 2015). The protein content of both raw and boiled tuber was superior to potato (1.6) and tapioca (0.7). However, fat content of raw and boiled tuber was very much similar to potato (0.1), colocasia (0.1) (Mudambi and Rajagopal, 2015).

Table 2.1. Proximate content of both raw and boiled tubers of *Dioscorea bulbifera.*

Proximates	Raw tuber (%)	Boiled tuber (%)
Moisture	74.89±0.54	80.48±1.18
Ash	2.57±0.04	1.66±0.34
Total carbohydrate	31.62±0.46	23.94±0.50
Starch	8.6±0.54	11.67±0.65
Reducing sugar	0.018±0.008	0.012±0.008
Fat	0.19±0.01	0.14±0.012
Protein	3.48±0.92	2.25±0.16
Free amino acid	1.45±0.05	0.59±0.13

Comparison in the proximate content between both the tubers has been performed by student t-test and was found to be statistically significant using student t-test ($p \leq 0.05$).

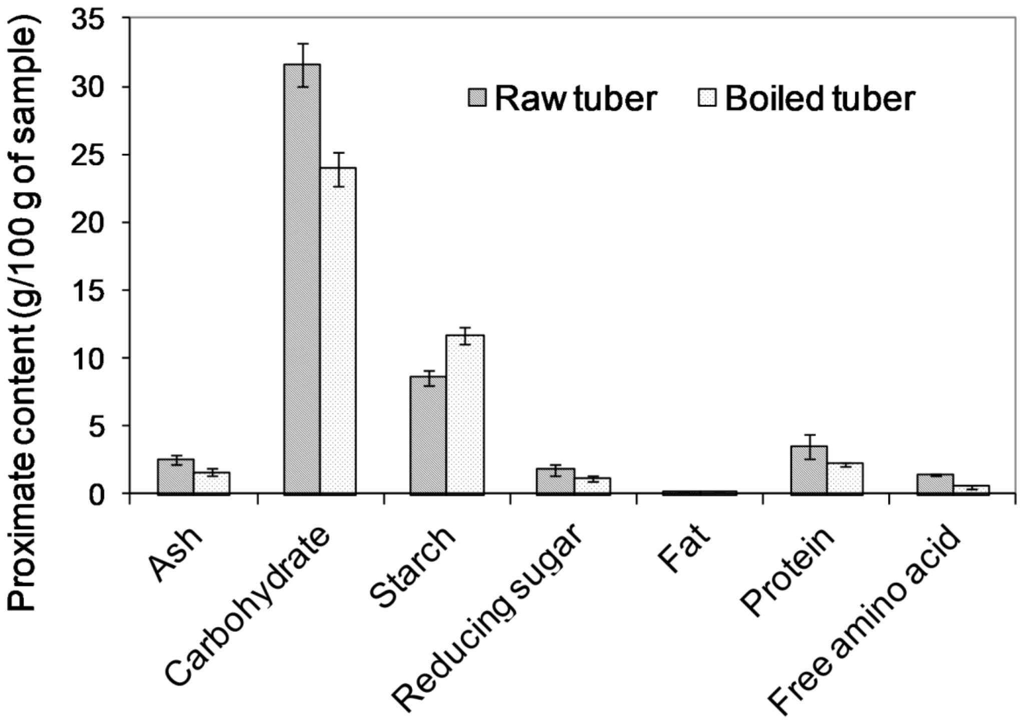


Figure 2.4. Comparison in proximate content between raw and boiled tubers of *Dioscorea bulbifera.*

2.3.2. Aminoacid profiling

HPLC analysis of both the raw and boiled tubers for different amino acids revealed that raw tuber contained a total of 15 amino acids out of which, six amino acids were essential amino acids (histidine, methionine, lysine, phenylalanine, threonine, and valine). While boiled tuber contained a total of 12 amino acids including 4 essential amino acids (lysine, threonine, valine and phenylalanine). The amino acid composition of both the raw and boiled tubers are collated in Table 2.2 and Figure 2.5(a&b). Phenylalanine was present in the highest amount in both raw and boiled tuber followed by valine. In contrast, histidine, methionine and cysteine amino acids were found in the least amount in the raw tuber and were not detected in the boiled samples. Bhandari *et al*., 2003 observed 17 amino acids including eight essential amino acids in *D.bulbifera*. They also reported that the amino acid composition of *Dioscorea bulbifera* was superior to other yam tubers. The difference in amino acid profile between raw and boiled tubers was found to be statistically significant using a student t-test ($p \leq 0.05$).

Table 2.2. Amino acid compositions of both raw and boiled tubers of *Dioscorea bulbifera.*

Parameters	Raw	Boiled
Glutamic Acid	14.25 ± 0.02	10.23 ± 0.01
Glutamine	8.95 ± 0.03	7.63 ± 0.05
Histidine	1.03 ± 0.005	-
Arginine	3.73 ± 0.15	3.35 ± 0.01
Alanine	5.1 ± 0.1	3.31 ± 0.025
Serine	6.54 ± 0.02	5.85 ± 0.02
Tyrosine	4.17 ± 0.01	3.6 ± 0.02
Cysteine	0.37 ± 0.005	-
Methionine	1.16 ± 0.005	-
Proline	4.01 ± 0.005	3.44 ± 0.01
Glycine	5.5 ± 0.1	6.3 ± 0.2
Lysine	3.52 ± 0.01	2.33 ± 0.01
Threonine	4.42 ± 0.01	2.06 ± 0.01
Valine	5.18 ± 0.01	4.42 ± 0.01
Phenylalanine	5.28 ± 0.01	4.81 ±0.01

Comparison in the amino acids profile between both the tubers has been performed by student t-test and was found to be statistically significant using student t-test ($p \leq 0.05$).

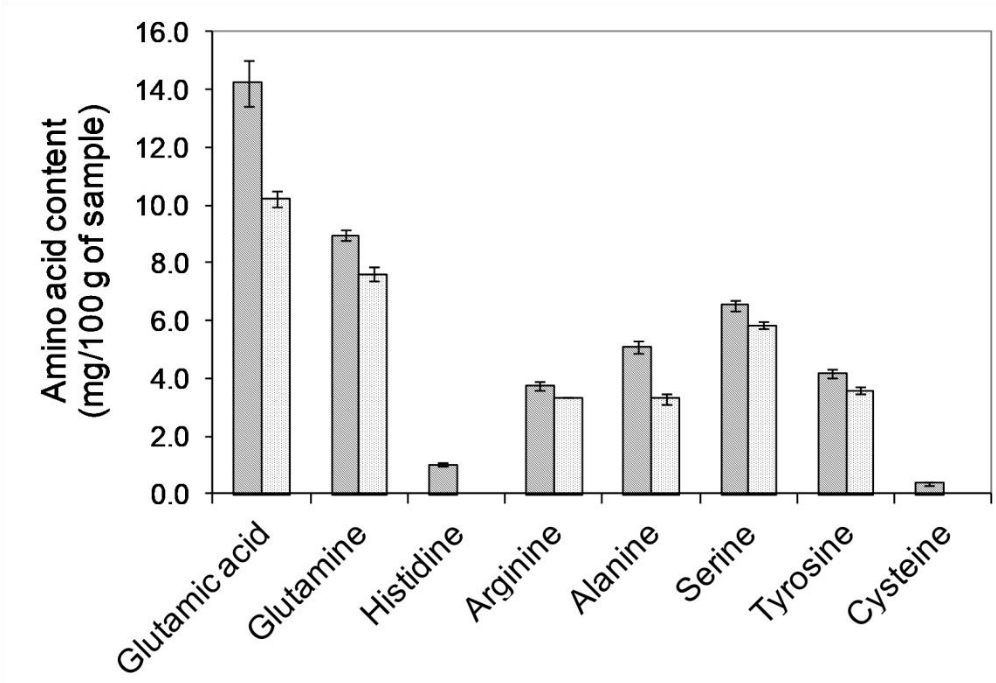


Figure 2.5a. Comparison in aminoacid compositions between raw and boiled tubers of *Dioscorea bulbifera.*

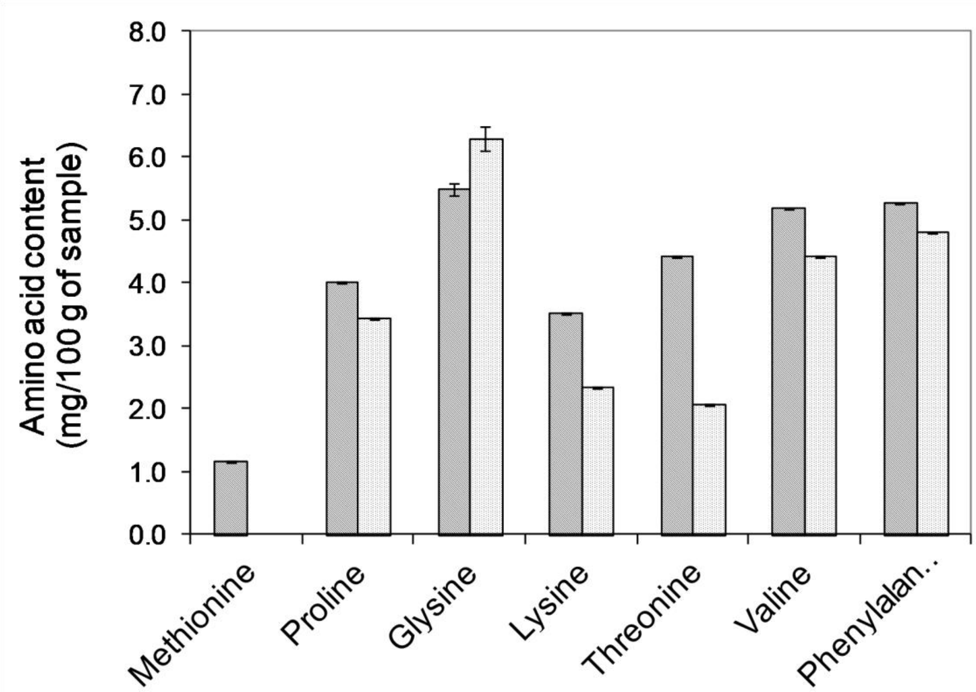


Figure 2.5b. Comparison in aminoacid compositions between raw and boiled tubers of *Dioscorea bulbifera.*

2.3.3. Mineral compositions

The tubers of *Dioscorea bulbifera* are very rich in mineral composition. The results of the mineral compositions of the raw and boiled tubers are included in Table 2.3

and Figure 2.6(a&b). The difference in mineral content between raw and boiled tubers was found to be statistically significant using a student t-test ($p \leq 0.05$). The sodium content of raw tubers and boiled tubers was 316±27.78 mg/100g and 119.36±16.25 mg/100g dry mass, respectively which is quite double that of the reported value of 150 mg/100g dry mass and 140 mg/100g dry mass for the uncooked and cooked tuber, respectively (Ogbuagu, 2008). In general lower amount of sodium content has been observed as compared to the earlier report (Celestine and David, 2015; Abara, 2011; Polycarb *et al*.,2012; Arnithan *et al*., 2009; Bhandari *et al.*, 2003). The potassium content of raw and boiled tuber was found to be 677.33±21.38 mg/100g dry mass and 232.33±12.50 mg/100g dry mass, respectively which were quite lesser than that of reported values of 1548 mg/100g dry mass (Shanthakumari *et al*., 2008), 1554 mg/100g dry mass (Shajeela *et al.*, 2011) and 1660 mg/100g dry mass (Arianthan *et al.*, 2009). In contrast, the potassium content of raw tuber of present findings was higher compared to the estimated values of 560 mg/100gm (Bhandari *et al.*, 2003) and 334.71 mg/100gm (Celestine and David, 2015). The phosphorus content of the raw and boiled tuber was found to be 153.20±17.17 and 60.43±1.72 mg/100g dry mass, respectively which are very much similar to the value of 154.42 mg/100g dry mass reported by Shajeela et al. (2011).

The iron content of raw and boiled tuber was found to be 6.16±0.89 and 3.24±1.06 mg/100g dry mass, respectively. Similarly, the calcium content of the raw tuber was estimated to be 290±4.13 mg/100g dry mass. In contrast, Sanful *et al*. (2013) has reported a very low value of calcium (24.02 mg/100g dry mass) in the solar dried raw tuber, whereas Shajeela *et al*. (2011) reported a very high value of calcium content (338.2 mg/100g dry mass) in the oven dried raw tuber. The boiled tuber contains less calcium (180±2.28 mg/100g dry mass) compared to the raw tuber. In compared to other tubers calcium and iron content of *D.bulbifera* were remarkably higher compared to Potato (10 & 0.7 mg/100gm), Colocasia (40 & 1.7 mg/100gm), Cassava (50 & 0.9 mg/100gm), respectively (Mudambi and Rajagopal, 2015).

The other essential elements such as magnesium, zinc, manganese and copper were found to be 203±6.42, 0.45±0.95, 4.2±2.16 and 0.79±0.62 mg/100g dry mass, respectively in the raw tuber. In contrast, the boiled tuber contained albeit lower amount of magnesium, zinc, manganese and copper elements (102±4.14, 0.18±0.83, 0.89±1.86 and 0.12±0.37 mg/100g dry mass, respectively). Previously it was reported that the magnesium content of the raw tuber ranges from 24.3 to 441.2 mg/100g dry mass, zinc content ranges from 0.53 to 6.35 mg/100g dry mass, manganese content ranges from 1.60 to 11.6 mg/100g dry mass and copper content ranges from 2 ppm to 2.74 mg/100g dry mass (Shanthakumari *et al*., 2008; Arinathan *et al*., 2009; Shajeela *et al*., 2011, Polycarp *et al.*, 2012 and Abara, 2011). Overall the results revealed that *Dioscorea bulbifera* tuber is a good source of minerals and could be used as food supplements.

Table 2.3. Mineral composition of both raw and boiled tubers of *Dioscorea bulbifera.*

S.No.	Minerals	Raw tuber (mg/100g dry mass)	Boiled tuber (mg/100g dry mass)
1	Sodium	316±27.8	199.66±16.25
2	Potassium	677.33±21.38	232.33±12.5
3	Phosphorus	153.20±17.17	60.43±1.72
4	Iron	6.16±0.89	3.24±1.06
5	Calcium	289 ± 1	180.32 ± 0.58
6	Magnesium	204.33 ± 1.52	102.55 ± 0.48
7	Zinc	0.43 ± 0.015	0.18 ± 0.005
8	Manganese	4.26 ± 0.030	0.88 ±0.005
9	Copper	0.75 ± 0.03	0.166 ± 0.005

Comparison in the minerals profile between both the tubers has been performed by student t-test and was found to be statistically significant using student t-test ($p \leq 0.05$).

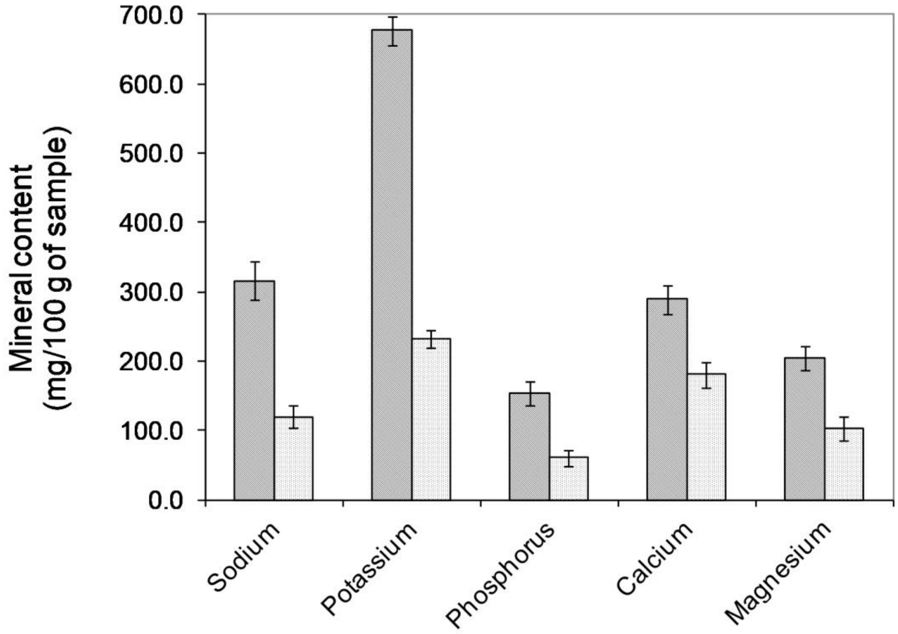


Figure 2.6(a). Comparison in minerals compositions between raw and boiled tubers of *Dioscorea bulbifera.*

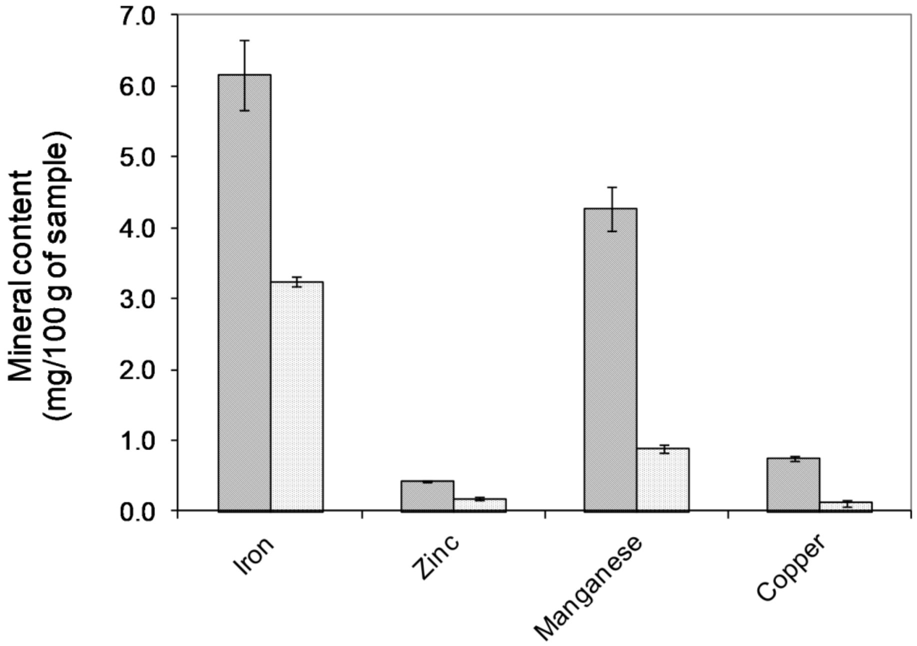


Figure 2.6(b). Comparison in minerals compositions between raw and boiled tubers of *Dioscorea bulbifera.*

2.3.4. Vitamin compositions

The tubers of *Dioscorea bulbifera* contain a variety of vitamins and could serve as food supplements. The details of vitamin analysis are shown in Table 2.4. The ascorbic acid content of the raw tuber was found to be 99.5±0.94 mg/100g dry mass, which is corroborated with the finding of Shajeela *et al*. (2011), who have reported the ascorbic acid content of the tuber as 91.04±0.86 mg/100g dry mass. In contrast, a lower amount of ascorbic acid content has been reported by Arinathan *et al.* (2009) and Sanful *et al*. (2013), while on the other hand a higher amount of ascorbic acid content has been observed by Okwu and Ndu (2006) and Ogbonna *et al*. (2015). The ascorbic acid content of the boiled tuber was found to be 70.7±1.19 mg/100g dry mass. The content of vitamin B1, vitamin B2, vitamin B3 and vitamin B6 for the raw tuber was found to be 0.007±0.0004, 0.027±0.007, 27.38±1.42 and 0.128±0.028 mg/100g dry mass, respectively which are comparable to the previously reported value of 0.009 mg/100g, 0.038 mg/100g and 23.69 – 33.74 mg/100g dry mass (Arinathan *et al*., 2009; Shajeela *et al*., 2011; Okwu and Ndu, 2006). The vitamin B1, vitamin B2, vitamin B3 and vitamin B6 content of the boiled tuber was found to be comparatively in lesser quantity than that of the raw tubers. The difference in vitamins content between raw and boiled tubers was found to be statistically significant using a student t-test ($p \leq 0.05$).

Table 2.4. Vitamins contents of both raw and boiled tubers of *Dioscorea bulbifera.*

Vitamins	Raw tuber (mg/100g)	Boiled tuber (mg/100g)	Reported value (mg/100g)
Vitamin C	99.46±0.05	70.64±0.29	91.04
Vitamin B1	0.008±0.001	0.005±0.005	0.009
Vitamin B2	0.027±0.001	0.014±0.005	0.038
Vitamin B3	27.86±0.03	14.62±0.02	23.69-33.74
Vitamin B6	0.128±0.001	0.08±0.001	NA

Comparison in the vitamins profile between both the tubers has been performed by student t-test and was found to be statistically significant using student t-test ($p \leq 0.05$).

2.4. Conclusion

In comparison to the boiled tuber, raw tubers are found richer in moisture, carbohydrate, protein, ash, free amino acid, minerals and vitamins which might be due to the leaching and degradation of most of the nutrients contents during boiling. Boiled tuber found to contain higher starch than raw tuber, may be due to bulking of starch due to boiling. In comparison to the previous reports, the variation observed in the nutritional content of the tuber in the current report might be due to the soil physico-chemical properties, soil fertility, geographical and climatic condition, genetic variation, etc. In conclusion, the tubers of *D. bulbifera* could be used as food due to its high nutritional content.

CHAPTER III

PHYTOCHEMICAL PROFILING OF DIOSCOREA BULBIFERA

3.1. Introduction

Dioscorea bulbifera is a potent medicinal plant used in both Indian and Chinese traditional medicine owing to its rich phytochemical diversity (Ghosh *et al*., 2015). It has been used historically as a traditional Chinese medicine to treat cancer and thyroid diseases for thousands of years (Tang *et al*., 1995). In Bangladesh, this plant is used by tribal people for the treatment of leprosy and tumors (Murray *et al*.,1984). Flavonoids norcleorodane diterpenoids and steroidal saponins have been found from the *D.bulbifera* species and most of them showed potent biological activities such as antitumor, antifungal and antiinflammatory effects (Gao *et al*.,2001; Teponno *et al*., 2007; Gao *et al*., 2002).

In spite of the recent domination of synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants to provide new and novel products for disease treatment and prevention is still enormous (Raskin *et al*., 2012). Compared with chemical synthesis, plant-derived natural products represent an attractive source of biologically active agents since they are natural and available at affordable prices (Ghosh *et al*., 2008). Also, plants derived agents may have different mechanisms than conventional drugs and could be of clinical importance in health care improvement (Eloff et al., 1998). Plant materials might have bioactive secondary metabolites that have the potential to treat different afflictions. Examples of these compounds include phenols, phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Mukherjee *et al*., 2011; Quiroga *et al*., 2011). The composition and concentration of active compounds of the plant are mostly affected by the factors such as the effects of climate, region of growth and season of harvest on the contents of active ingredients (Tang et al.,2006).

D.bulbifera tuber is being used as a medicinal crop in many parts of India without complete information on its chemical constituents. Therefore analysis of its phytochemicals is of great importance. *Dioscorea bulbifera* has been thoroughly studied by many Chinese workers and several phytochemicals presented in *Dioscorea bulbifera* have been isolated and identified. However, data on phytochemical profiling of Indian varieties is still limited. Therefore, the present study was designed to identify chemical constituents of *Dioscorea bulbifera* available in the local area.

3.2. Materials and Methods

3.2.1. Quantification of bioactive compounds

3.2.1.1. Tannins

Tannins are high molecular weight polyphenols responsible for the astringent taste of foods. The total tannins from the raw and boiled tuber powder samples were estimated as per the method of Okwu and Ndu (2006). Briefly, 500 mg of the sample with 50 ml of distilled water was shaken. Then it was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was mixed with 3 ml of 0.1 ml $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelengths within 10 min.

3.2.1.2. Diosgenin

Diosgenin content from the raw and boiled tuber powder samples was estimated by the gravimetric method (Behera *et al*., 2010). Briefly, a slurry of 10 g of dried tuber powder was made with 2.5N HCL for 2 hours. This hydrolyzed material was dried after washing with distilled water and then extracted with petroleum ether for 8 hours at 40-60 ^{o}C in a Soxhlet extractor. The solvent with diosgenin was concentrated, chilled in ice, filtered and dried in an oven at 100 ^{0}C for 2 hours and expressed in mg present in 100 g of sample.

3.2.1.3. Saponin

Saponin content from the raw and boiled tuber powder samples was estimated as per the method of Okwu and Ndu (2006). About 20 g of powder sample with 100 ml of 20% aqueous ethanol was taken in a conical flask. Then it was heated over a hot water bath for 4 hours with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue was reextracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml in a water bath at about 90 ^{o}C. The concentrate was transferred into a 250 ml separating funnel and to it, 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was discarded and the purification process was repeated. To it, 60 ml of n-butanol was added. The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the Saponins content was calculated as a percentage.

3.2.1.4. GC-MS analysis

The GC-MS analysis was carried out following the methods of Uthirapathy *et al*., 2021. The methanolic extract of *D.bulbifera* tuber was injected for Gc-ms analysis. The extract of *D.bulbifera* (10mg) was dissolved in methanol. The sample was analysed by Gc-Ms on 500 perkin Elmer using the following experimental conditions. Column type-elite-5 (5% diphenyl 95% dimethyl polysiloxane), Column dimension (30m ×0.32mm), carrier gas-helium 1ml min-^{1,} column temperature from 50^{0}c up to 285^{0}c, injector and detector temperature 290^{0}c, injection mode split, volume injected; 0.5µl of a solution prepared from 2mg/100ml in methanol. The total run time was 30min. The mass spectrum was taken using a mass detector-turbo mass gold Perkin Elmer. Transfer line temperature 230^{0}c, source temperature 230^{0}c, scan range is from 40-450 amµ, ionization technique- Electron ionization technique. The component identification was confirmed by comparing the mass spectra of compounds with available NIST and Wiley mass spectra libraries. The quantitative composition was obtained by peak area normalization.

3.3. Results and Discussion

3.3.1. Quantification of Bioactive compounds

Bioactive compounds are secondary metabolites of the plant that have pharmacological or toxicological effects in humans and animals. The result of the analysis showed that raw tuber contained 160.2±0.84 mg/100gm while boiled tuber contained 12.5±0.11mg/100gm, of diosgenin (Table 3.1). Diosgenin is a steroidal saponin and is a major active ingredient in *Dioscorea species*. After oral administration structure of diosgenin became similar to cholesterol. It is metabolized in the liver and eliminated via bile (Kumar *et al*., 2013). Ghosh *et al*. (2014) reported diosgenin exhibited potent inhibition against both porcine pancreatic alpha-amylase and alpha-glucosidase as well as against crude murine amylase and glucosidase and acts as lead candidate in managing Type II Diabetes Mellitus. Jayachandran *et al*. (2016) isolated diosgenin from *D.bulbifera* and proved diosgenin is a potent cardioprotective agent. Behera *et al*., 2010 reported 1383 mg/100gm of diosgenin for *Dioscorea bulbifera* tuber.

Tannin is one of the phenolic compounds which gives astringent and bitter taste. Tannins are acts as antidiarrheal, haemostatic and antihemorrhoidal, antiinflammatory, antiviral and antibacterial, antiseptic and antioxidant. Tannin content was found to be 180.11±0.32 mg/100gm and 12.09±0.12 mg/100gm for raw and boiled tubers, respectively (Table 3.1). Shajeela, *et al*. (2011) reported a remarkably higher amount of tannin (1.48 g/100g) compared to the present findings. In contrast, Polycarp *et al*. (2012)

reported a significantly lower amount of tannin (10.98 mg/100g) compared to the present findings.

Saponins are surface-active glycosides with detergent, wetting, emulsifying and foaming properties (wang *et al*., 2005; Sarnthein *et al*., 2004; Mitra *et al*., 1997). They are abundant in many foods consumed by animals and men (Cheeke 1971). Saponins are effective against drug resistant cancer cells (Cheung *et al*., 2005). The saponin content of raw and boiled tuber was found to be 150.34±0.67 mg/100gm and 21.26 mg/100gm, respectively (Table 3.1). Ogbonna *et al*. (2015) found 8.49- 14.03 mg/100gm of saponin content of three cultivars of *Dioscorea bulbifera*.

Table 3.1. Bioactive compounds of both raw and boiled *D.bulbifera*

Parameters	Raw	Boiled
Tannin(mg/100gm)	180.11±0.32	12.09±0.12
Diosgenin(mg/100gm)	160.2±0.84	12.5±0.11
Saponin(mg/100gm)	150.34±0.67	21.26±0.89

Values are expressed as mean ±SD of triplicate determination

3.3.2. Phytochemical profiling of methanolic extract of raw *D.bulbifera* tuber by GC-MS.

The phytochemical screening of methanolic extract of *Dioscorea bulbifera* revealed the presence of various secondary metabolites. The GC-MS analysis of the *D.bulbifera* tuber revealed the presence of 24 compounds namely Glycerin, 2, 2'-Bioxirane, 2-Furanmethanol, Butanic acid, 2-ethy-3-oxo-methyl ester-, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Pentanoic acid, 4-oxo-, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, Catechol, 5-Hydroxymethyl furfural-, 2-Furoic acid, TBDMS derivatives-, n-Hexadecanoic, 9-12-Octadecadienoic acid(Z,Z)-, Ethyl oleate, Octadecanoic acid, (Z)1-Allyl-2-methylcyclohexanol, Oxiraneoctanoic acid, 3-octyl-cis, Campesterol, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Bis(2-ethylhexyl)-phthalate, Docosanoic acid, 1H, 3H-Furo[3,4-c]Furan,1,4-bis(3,4-dimethoxyphenyl)tetra hydro-, [IR-(1.alpha, 3a.al alpha., 4. Beta., 6a. alpha.)], E, Z-1,3, 12-Nonadecatriene, Gamma sitostirol, 3,4-seco-5.alpha.-cholestan-3-oic, 4-hydroxy-4-methyl-,epsilon.-lactone,(4R) . The structure of compounds was based on the analysis of fragmentation pattern of mass spectra and direct comparison of their spectral data with the chemical profiles, using the NIST library, and comparisons of published mass spectra (Figure 3.1). The chemical profiles of the identified compounds with their retention time, percentage peak area, molecular formula, molecular weight, structure are depicted in Table 3.2.). Uthirapathy *et al*., 2021 also identified glycerin, 2-furanmethanol, hexadecanoic acid and 9, 12- Octadecadienoic acid from 70% ethanolic extract of *D.bulbifera* tuber of region Tamilnadu India. However, Linatoc *et al.* (2021) revealed the presence of n-hexadecanoic acid, Octadecanoic acid in methanolic extract of Malaysian *D.bulbifera* leaf. While Ghosh *et al*., (2013) found n-hexadecanoic acid in petroleum ether extract of bulb of *D.bulbifera* from Maharastra, India. Phytochemical profiling differs excessively on the basis of geographical variation. *Dioscorea bulbifera* is a rich source of phytochemicals, which are may be responsible for various pharmaceutical potentials of the tuber.

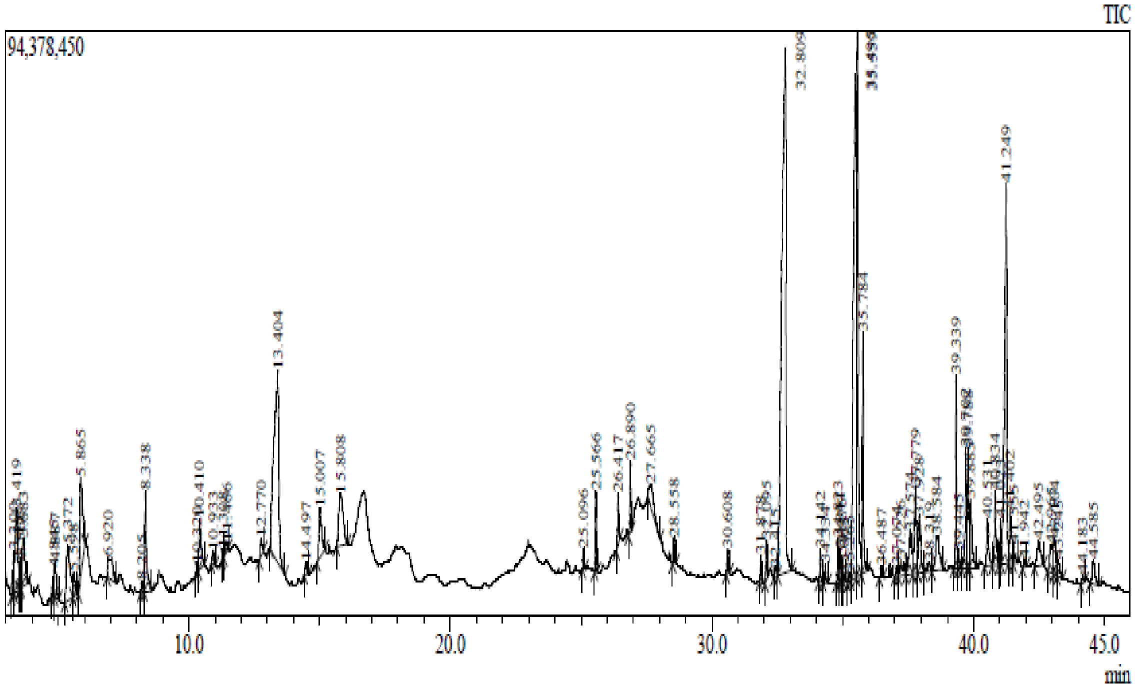
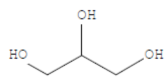
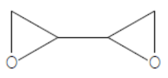
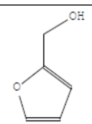
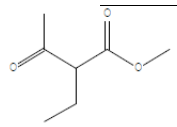
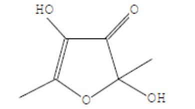
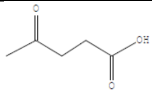
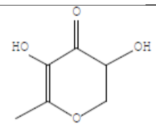
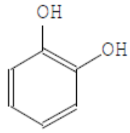
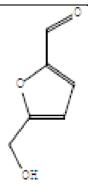
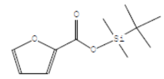
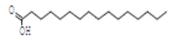
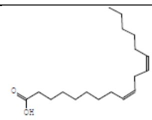
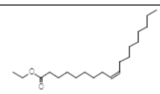
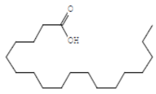
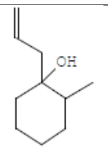
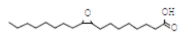
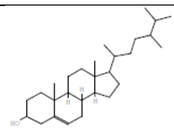
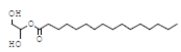
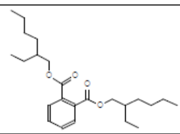
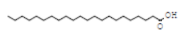
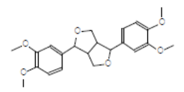
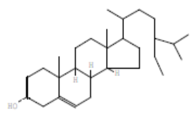
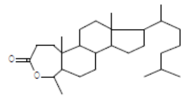


Figure 3.1. GC-MS chromatogram of *Dioscorea bulbifera* tuber

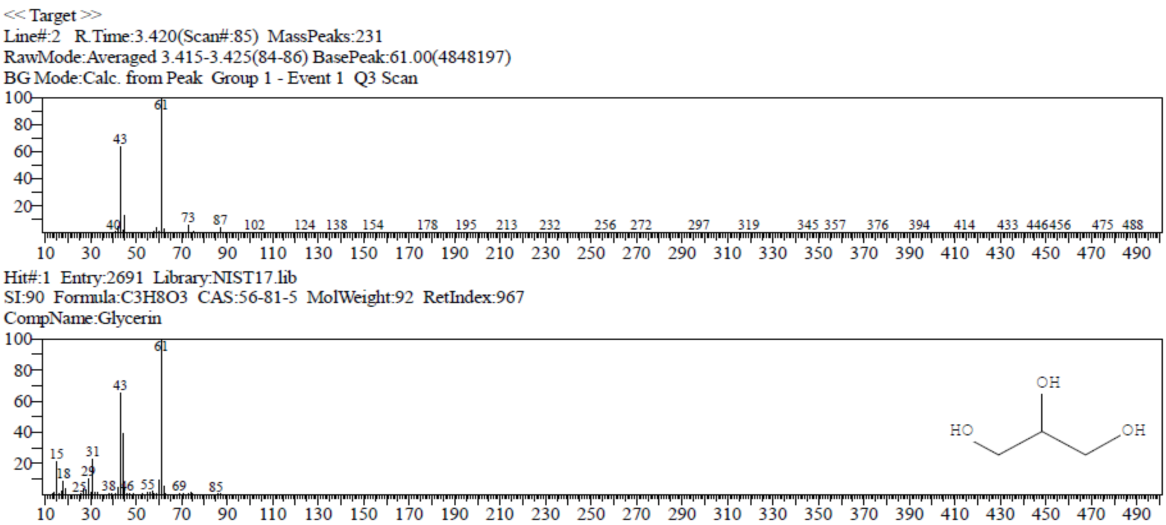
Table 3.2. Phytochemical identified in *Dioscorea bulbifera* tuber.

Peak	R.Time	Area	Area%	MW	MF	Name	Chemical structure
2	3.42	92004535	1.91	92	$C_3H_8O_3$	Glycerin	
5	3.685	65728282	1.37	86	$C_4H_6O_2$	2,2'-Bioxirane	
8	5.37	93793577	1.95	98	$C_5H_6O_2$	2-Furanmetha nol	
10	5.865	118969443	2.48	144	$C_7H_{12}O_3$	Butanoic acid, 2-ethyl-3-oxo-, methyl ester	

13	8.34	73624179	1.53	144	$C_6H_8O_4$	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	
15	10.41	47838807	1	116	$C_5H_8O_3$	Pentanoic acid, 4-oxo	
20	13.405	402751972	8.38	144	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihy dro- 3,5-dihydr oxy-6-methyl-	
22	15.005	58832705	1.22	110	$C_6H_6O_2$	Catechol	
23	15.81	99883193	2.08	126	$C_6H_6O_3$	5-Hydroxy methylfurfural	
28	27.665	48696877	1.01	226	$C_{11}H_{18}O_3Si$	2-Furoic acid, TBDMS derivative	
34	32.81	887452606	18.47	256	$C_{16}H_{32}O_2$	n-Hexadeca noic acid	
42	35.495	583484649	12.14	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-	
43	35.56	375280083	7.81	310	$C_{20}H_{38}O_2$	Ethyl Oleate	
44	35.785	108739037	2.26	284	$C_{18}H_{36}O_2$	Octadecanoic acid	
48	37.575	56195142	1.17	154	$C_{10}H_{18}O$	(Z)1-Allyl-2-methylcyclohexan ol	
49	37.78	77167718	1.61	298	$C_{18}H_{34}O_3$	Oxiraneoctanoic acid, 3-octyl-, cis-	
52	38.585	62707667	1.31	400	$C_{28}H_{48}O$	Campesterol	
53	39.34	67209909	1.4	330	$C_{19}H_{38}O_4$	Hexadecanoic acid,2-hydro xy-1-(hydroxyl methyl)ethyl ester	
55	39.7	81566186	1.7	390	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	
56	39.79	66600737	1.39	340	$C_{22}H_{44}O_2$	Docosanoic acid	

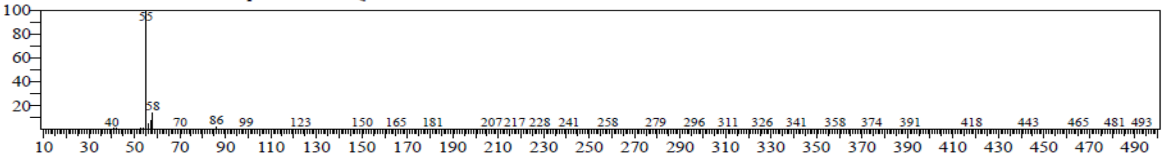
57	39.885	68154486	1.42	386	$C_{22}H_{26}O_6$	1H,3H-Furo[3,4-c]furan, 1,4-bis(3,4-dimethoxyphenyl) tetrahydro-, [1R-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-	
59	40.835	49021686	1.02	262	$C_{19}H_{34}$	E,Z-1,3,12-Nonadecatriene	
61	41.25	381687646	7.94	414	$C_{29}H_{50}O$	Gamma.-Sitosterol	
62	41.4	53507871	1.11	416	$C_{28}H_{48}O_2$	3,4-Seco-5.alpha.-cholestan-3-oic acid, 4-hydroxy-4-methyl-, .epsilon.-lactone, (4R)-	

Peak 2

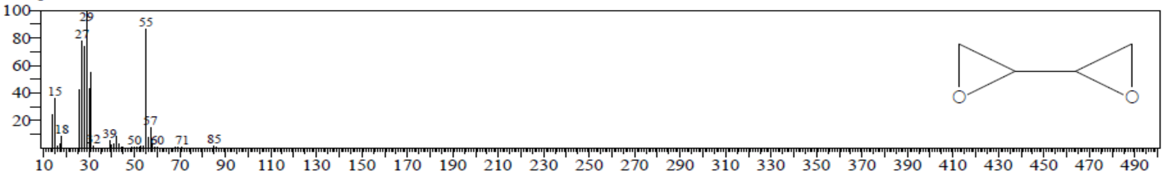


Peak 5

<< Target >>
Line#:5 R.Time:3.685(Scan#:138) MassPeaks:214
RawMode:Averaged 3.680-3.690(137-139) BasePeak:55.00(4646567)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

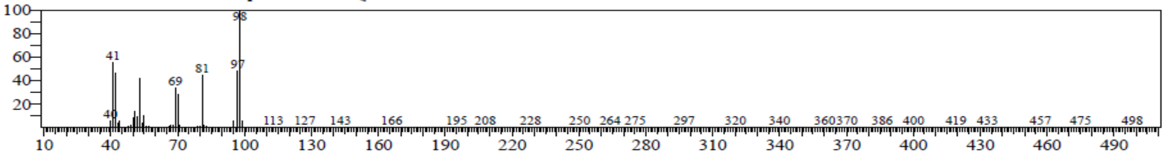


Hit#:1 Entry:1821 Library:NIST17.lib
SI:90 Formula:C4H6O2 CAS:1464-53-5 MolWeight:86 RetIndex:600
CompName:2,2'-Bioxirane

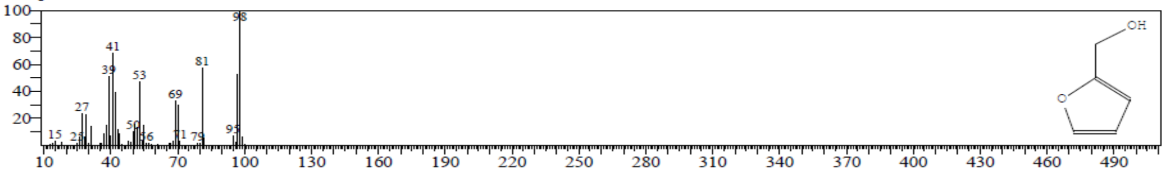


Peak 8

<< Target >>
Line#:8 R.Time:5.370(Scan#:475) MassPeaks:231
RawMode:Averaged 5.365-5.375(474-476) BasePeak:98.00(1656722)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

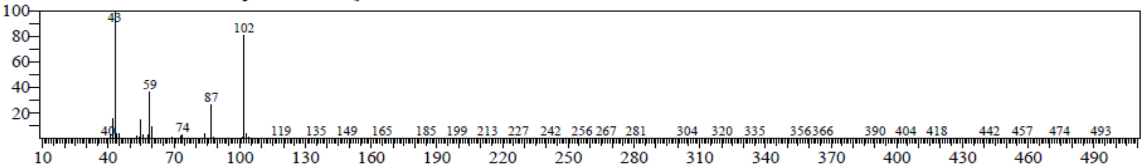


Hit#:1 Entry:3436 Library:NIST17.lib
SI:96 Formula:C5H6O2 CAS:98-00-0 MolWeight:98 RetIndex:885
CompName:2-Furanmethanol

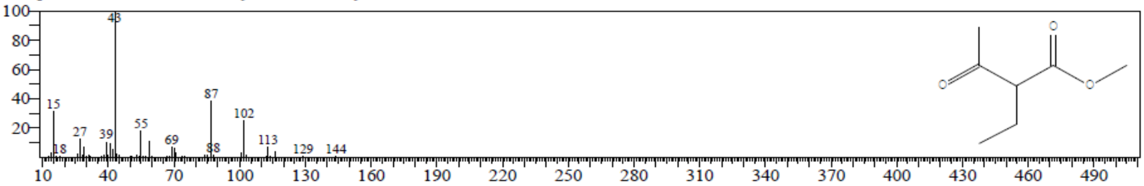


Peak 10

<< Target >>
Line#:10 R.Time:5.865(Scan#:574) MassPeaks:314
RawMode:Averaged 5.860-5.870(573-575) BasePeak:43.00(4951223)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

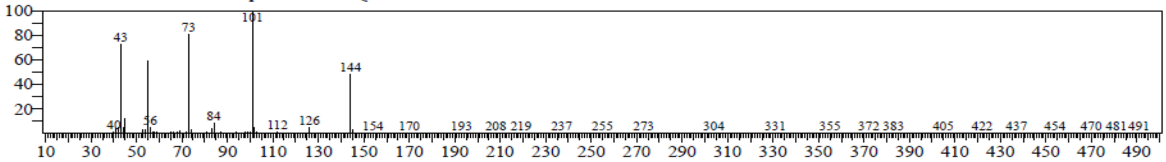


Hit#:1 Entry:23071 Library:NIST17.lib
SI:82 Formula:C7H12O3 CAS:51756-08-2 MolWeight:144 RetIndex:956
CompName:Butanoic acid, 2-ethyl-3-oxo-, methyl ester

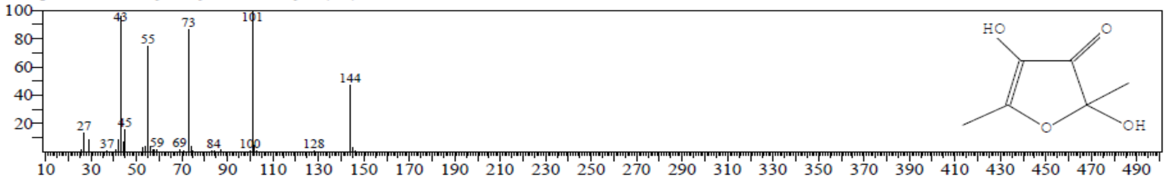


Peak 13

<< Target >>
Line#:13 R.Time:8.340(Scan#:1069) MassPeaks:245
RawMode:Averaged 8.335-8.345(1068-1070) BasePeak:101.00(3473390)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

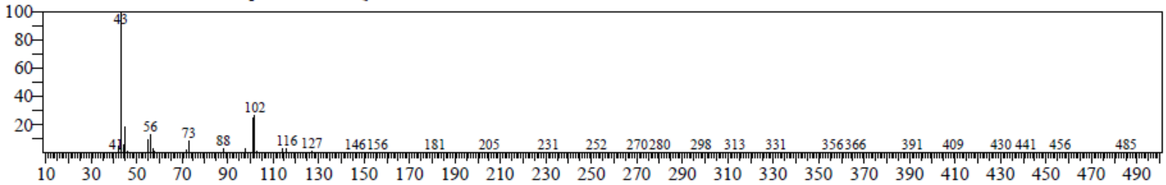


Hit#:1 Entry:22907 Library:NIST17.lib
SI:94 Formula:C6H8O4 CAS:10230-62-3 MolWeight:144 RetIndex:1173
CompName:2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one

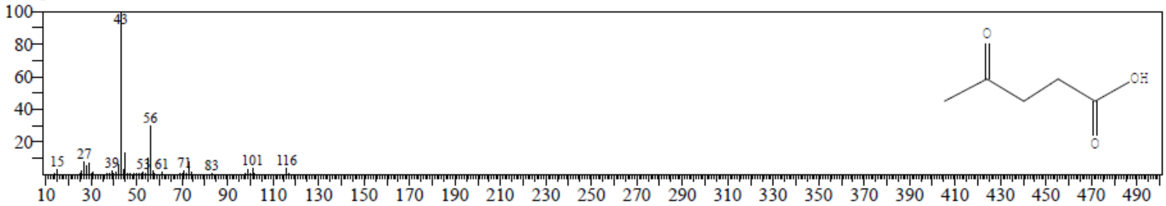


Peak 15

<< Target >>
Line#:15 R.Time:10.410(Scan#:1483) MassPeaks:210
RawMode:Averaged 10.405-10.415(1482-1484) BasePeak:43.00(3075413)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

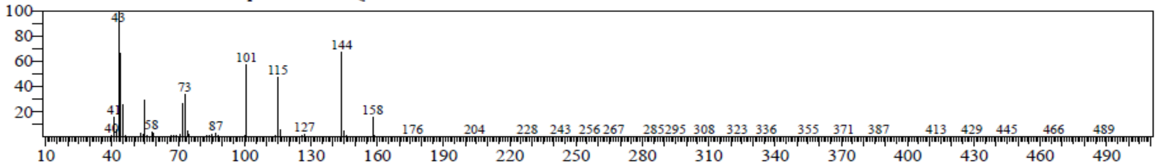


Hit#:1 Entry:8885 Library:NIST17.lib
SI:86 Formula:C5H8O3 CAS:123-76-2 MolWeight:116 RetIndex:1011
CompName:Pentanoic acid, 4-oxo-

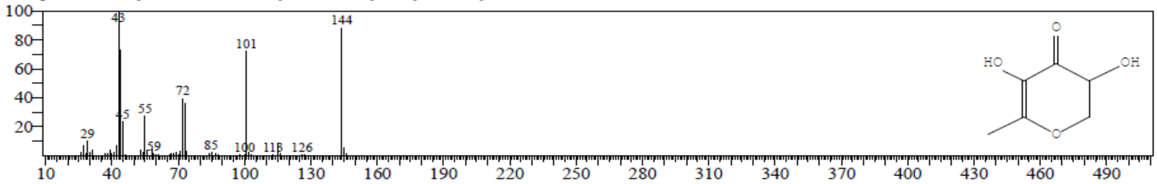


Peak 20

<< Target >>
Line#:20 R.Time:13.405(Scan#:2082) MassPeaks:289
RawMode:Averaged 13.400-13.410(2081-2083) BasePeak:43.00(5692088)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

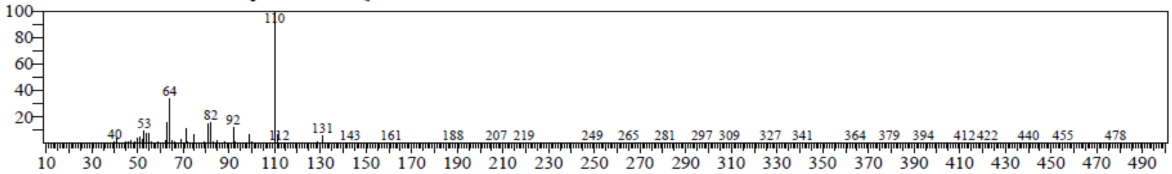


Hit#:1 Entry:22919 Library:NIST17.lib
SI:91 Formula:C6H8O4 CAS:28564-83-2 MolWeight:144 RetIndex:1269
CompName:4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

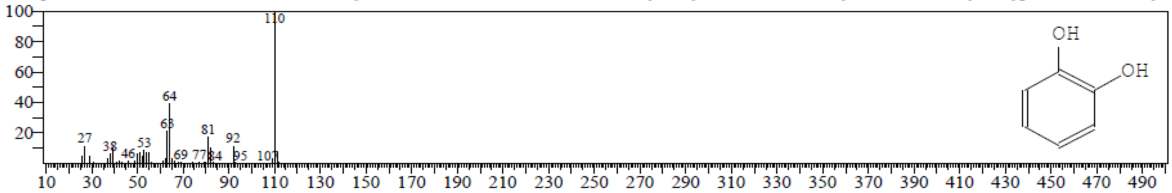


Peak 22

<< Target >>
Line#:22 R.Time:15.005(Scan#:2402) MassPeaks:263
RawMode:Averaged 15.000-15.010(2401-2403) BasePeak:110.00(2742157)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

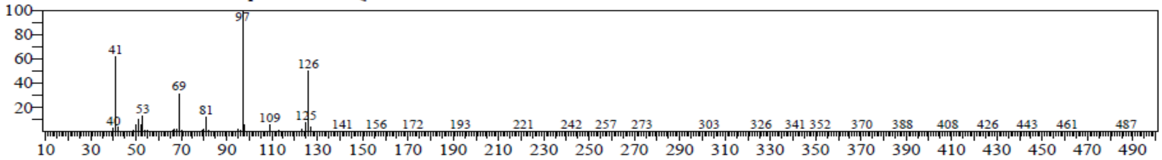


Hit#:1 Entry:2725 Library:NIST14s.lib
SI:90 Formula:C6H6O2 CAS:120-80-9 MolWeight:110 RetIndex:1122
CompName:Catechol $$ 1,2-Benzenediol $$ Pyrocatechol $$ o-Benzenediol $$ o-Dihydroxybenzene $$ o-Dioxybenzene $$ o-Hydroxyphenol $$ o-Phenyle

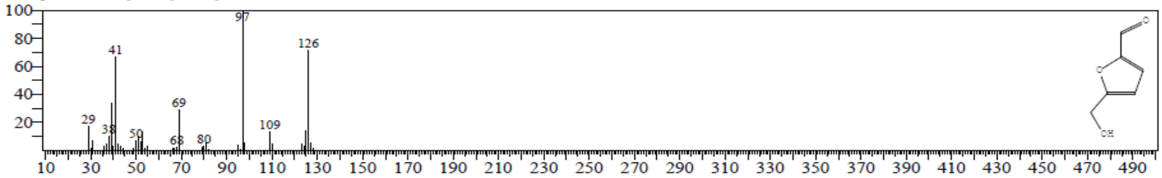


Peak 23

<< Target >>
Line#:23 R.Time:15.810(Scan#:2563) MassPeaks:199
RawMode:Averaged 15.805-15.815(2562-2564) BasePeak:97.00(2502365)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

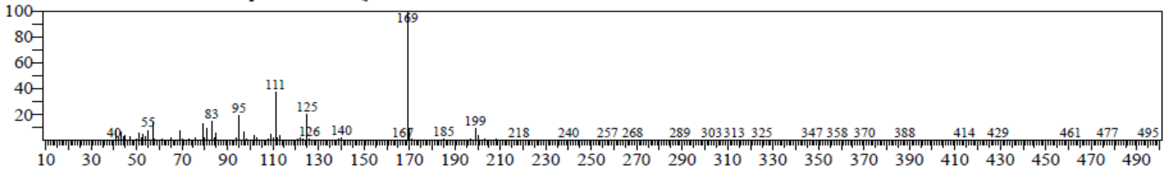


Hit#:1 Entry:12382 Library:NIST17.lib
SI:94 Formula:C6H6O3 CAS:67-47-0 MolWeight:126 RetIndex:1163
CompName:5-Hydroxymethylfurfural

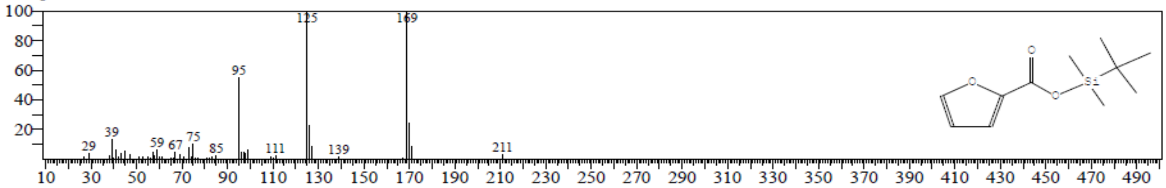


Peak 28

<< Target >>
Line#:28 R.Time:27.665(Scan#:4934) MassPeaks:246
RawMode:Averaged 27.660-27.670(4933-4935) BasePeak:169.05(1072285)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

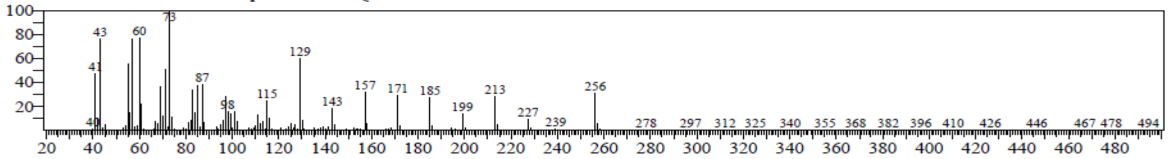


Hit#:1 Entry:97657 Library:NIST17.lib
SI:69 Formula:C11H18O3Si CAS:0-00-0 MolWeight:226 RetIndex:1232
CompName:2-Furoic acid, TBDMS derivative

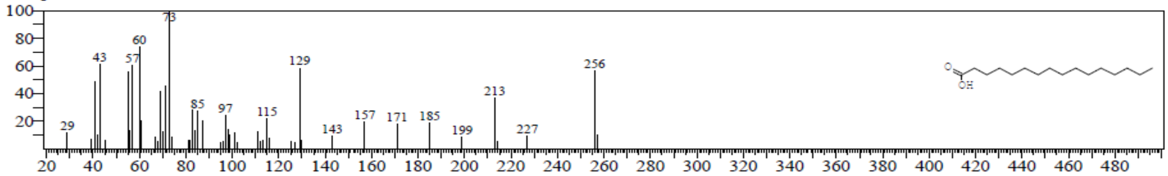


Peak 34

<< Target >>
Line#:34 R.Time:32.810(Scan#:5963) MassPeaks:306
RawMode:Averaged 32.805-32.815(5962-5964) BasePeak:73.00(6460532)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

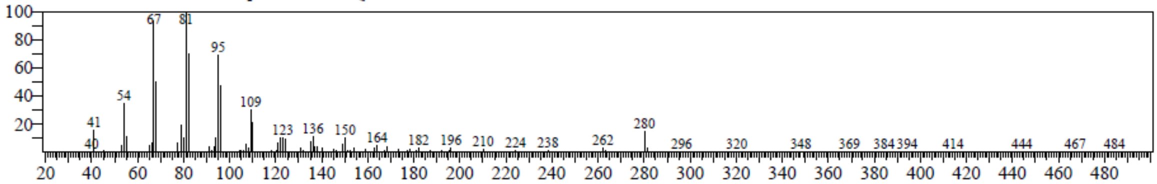


Hit#:1 Entry:129355 Library:NIST17.lib
SI:94 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid

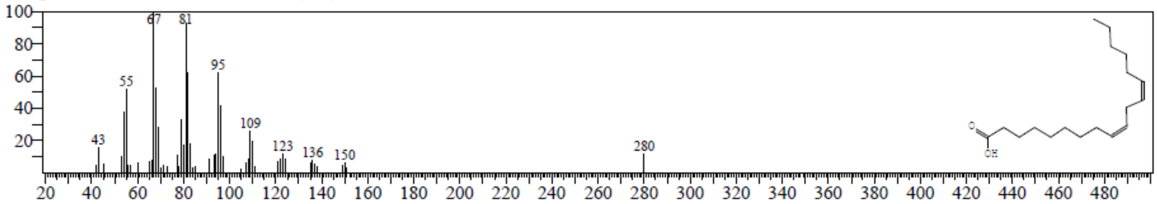


Peak 42

<< Target >>
Line#:42 R.Time:35.495(Scan#:6500) MassPeaks:232
RawMode:Averaged 35.490-35.500(6499-6501) BasePeak:81.05(3661616)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

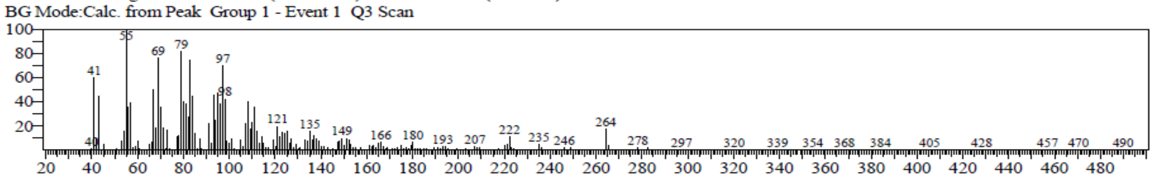


Hit#:1 Entry:154772 Library:NIST17.lib
SI:84 Formula:C18H32O2 CAS:60-33-3 MolWeight:280 RetIndex:2183
CompName:9,12-Octadecadienoic acid (Z,Z)-

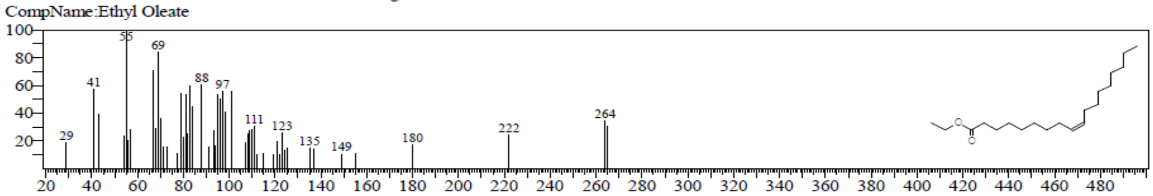


Peak 43

<< Target >>
Line#:43 R.Time:35.560(Scan#:6513) MassPeaks:307
RawMode:Averaged 35.555-35.565(6512-6514) BasePeak:55.05(1775209)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

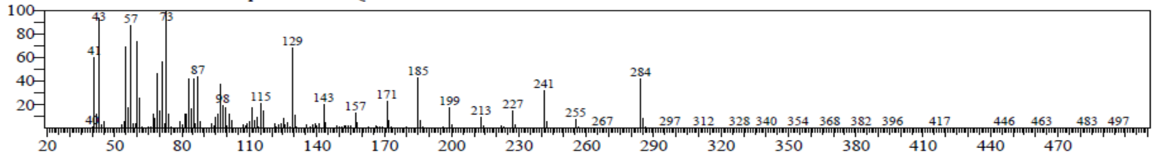


Hit#:1 Entry:187761 Library:NIST17.lib
SI:87 Formula:C20H38O2 CAS:111-62-6 MolWeight:310 RetIndex:2185
CompName:Ethyl Oleate

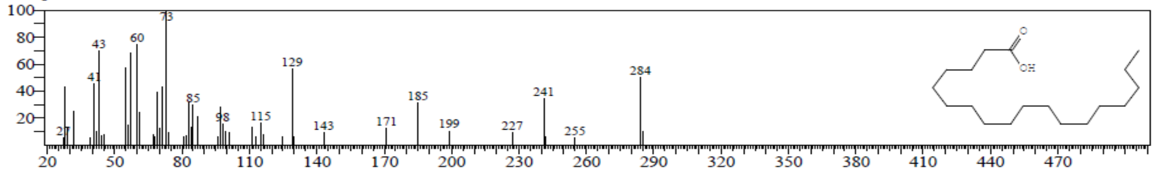


Peak 44

<< Target >>
Line#:44 R.Time:35.785(Scan#:6558) MassPeaks:351
RawMode:Averaged 35.780-35.790(6557-6559) BasePeak:73.00(2358105)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

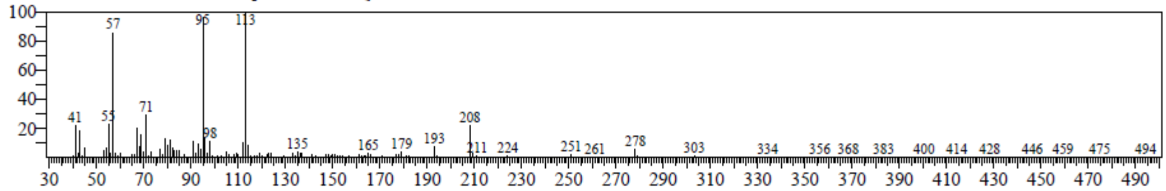


Hit#:1 Entry:159524 Library:NIST17.lib
SI:91 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:2167
CompName:Octadecanoic acid

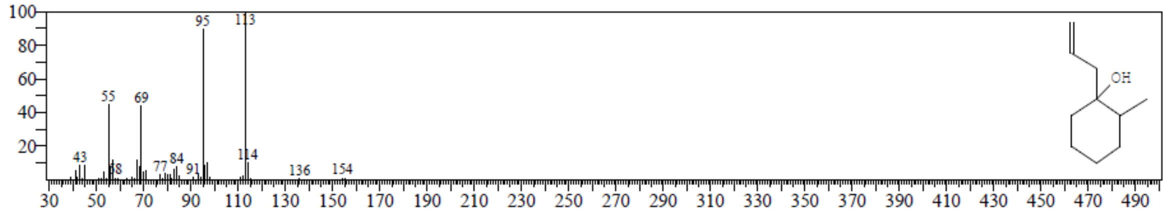


Peak 48

<< Target >>
Line#:48 R.Time:37.575(Scan#:6916) MassPeaks:319
RawMode:Averaged 37.570-37.580(6915-6917) BasePeak:113.10(652698)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

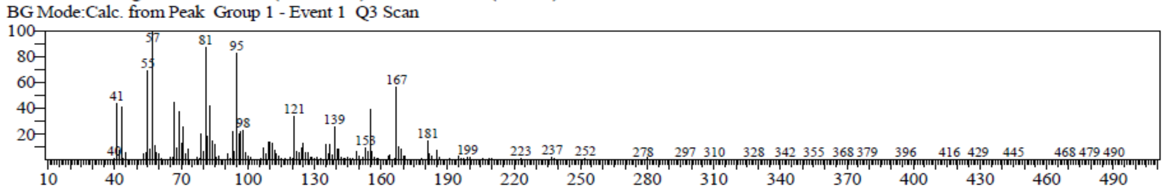


Hit#:1 Entry:18169 Library:NIST14.lib
SI:79 Formula:C10H18O CAS:0-00-0 MolWeight:154 RetIndex:1181
CompName:(Z)1-Allyl-2-methylcyclohexanol

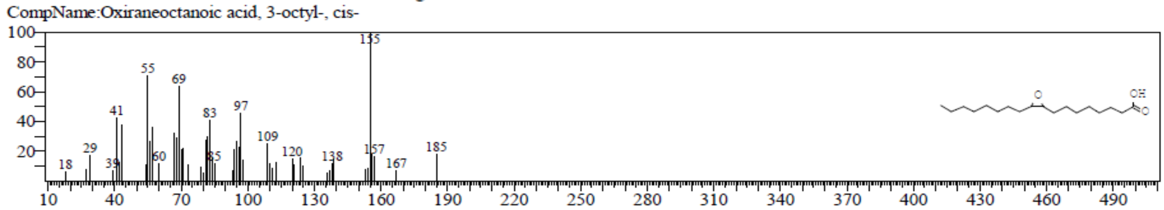


Peak 49

<< Target >>
Line#:49 R.Time:37.780(Scan#:6957) MassPeaks:349
RawMode:Averaged 37.775-37.785(6956-6958) BasePeak:57.05(823917)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

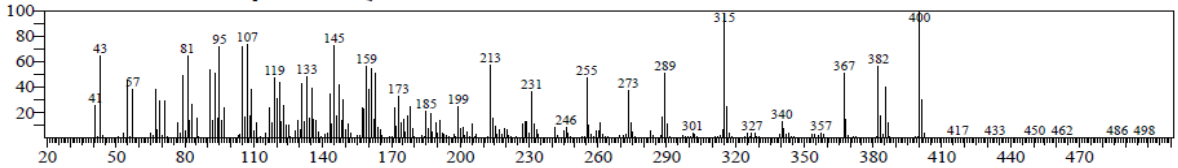


Hit#:1 Entry:174715 Library:NIST17.lib
SI:79 Formula:C18H34O3 CAS:24560-98-3 MolWeight:298 RetIndex:2219
CompName:Oxiraneoctanoic acid, 3-octyl-, cis-

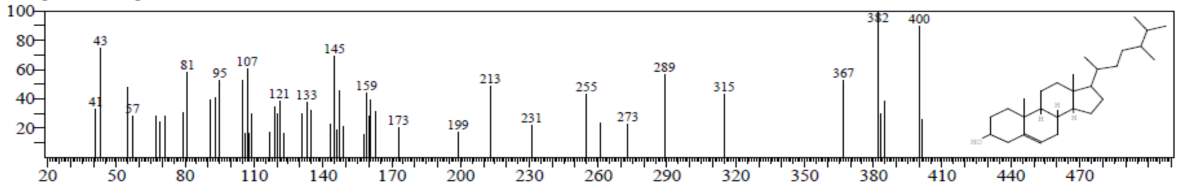


Peak 52

<< Target >>
Line#:52 R.Time:38.585(Scan#:7118) MassPeaks:346
RawMode:Averaged 38.580-38.590(7117-7119) BasePeak:400.35(170449)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

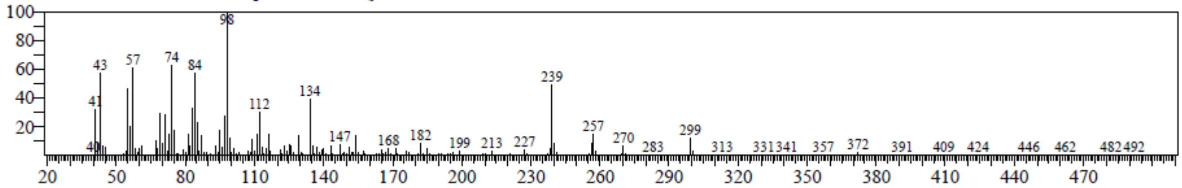


Hit#:1 Entry:265739 Library:NIST17.lib
SI:88 Formula:C28H48O CAS:474-62-4 MolWeight:400 RetIndex:2632
CompName:Campesterol

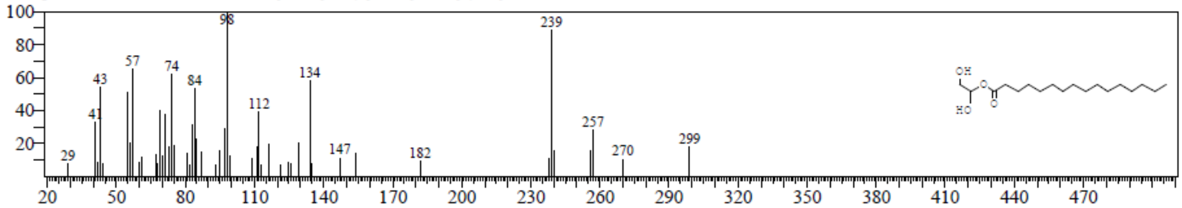


Peak 53

<< Target >>
Line#:53 R.Time:39.340(Scan#:7269) MassPeaks:359
RawMode:Averaged 39.335-39.345(7268-7270) BasePeak:98.05(2416536)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

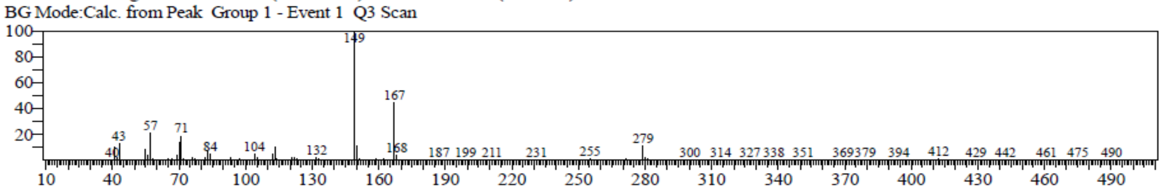


Hit#:1 Entry:209013 Library:NIST17.lib
SI:93 Formula:C19H38O4 CAS:23470-00-0 MolWeight:330 RetIndex:2498
CompName:Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

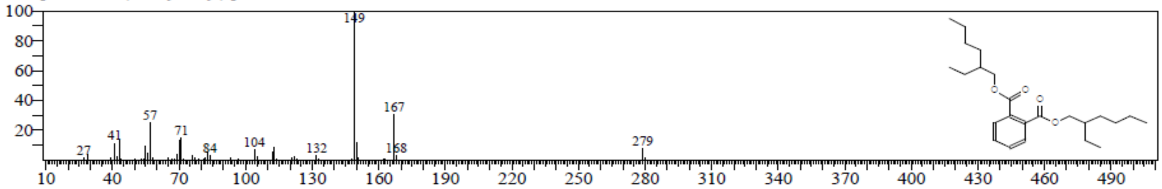


Peak 55

<< Target >>
Line#:55 R.Time:39.700(Scan#:7341) MassPeaks:329
RawMode:Averaged 39.695-39.705(7340-7342) BasePeak:149.00(3322598)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

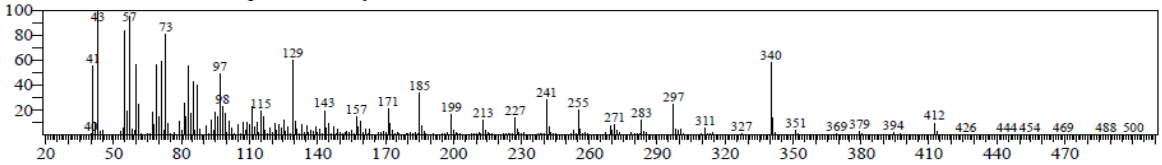


Hit#:1 Entry:259671 Library:NIST17.lib
SI:95 Formula:C24H38O4 CAS:117-81-7 MolWeight:390 RetIndex:2704
CompName:Bis(2-ethylhexyl) phthalate

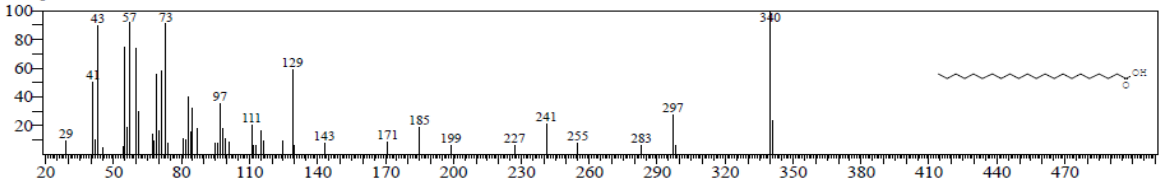


Peak 56

<< Target >>
Line#:56 R.Time:39.790(Scan#:7359) MassPeaks:365
RawMode:Averaged 39.785-39.795(7358-7360) BasePeak:43.05(503922)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

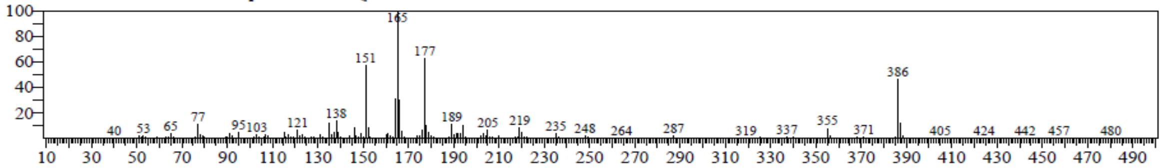


Hit#:1 Entry:219526 Library:NIST17.lib
SI:90 Formula:C22H44O2 CAS:112-85-6 MolWeight:340 RetIndex:2564
CompName:Docosanoic acid

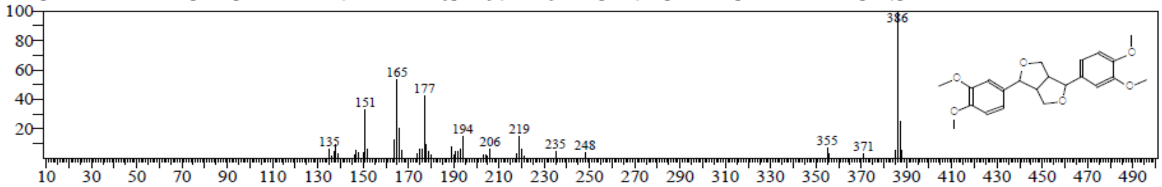


Peak 57

<< Target >>
Line#:57 R.Time:39.885(Scan#:7378) MassPeaks:251
RawMode:Averaged 39.880-39.890(7377-7379) BasePeak:165.05(931242)
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan



Hit#:1 Entry:256846 Library:NIST17.lib
SI:78 Formula:C22H26O6 CAS:4375-03-5 MolWeight:386 RetIndex:2865
CompName:1H,3H-Furo[3,4-c]furan, 1,4-bis(3,4-dimethoxyphenyl)tetrahydro-, [1R-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-



Peak 59

<< Target >>
Line#:59 R.Time:40.835(Scan#:7568) MassPeaks:369
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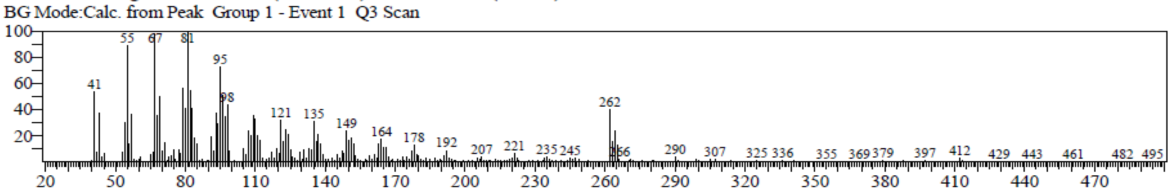
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan



Hit#:1 Entry:135759 Library:NIST17.lib
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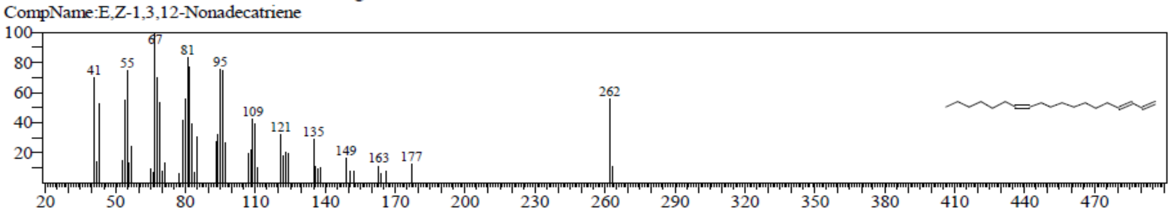
CompName:E,Z-1,3,12-Nonadecatriene



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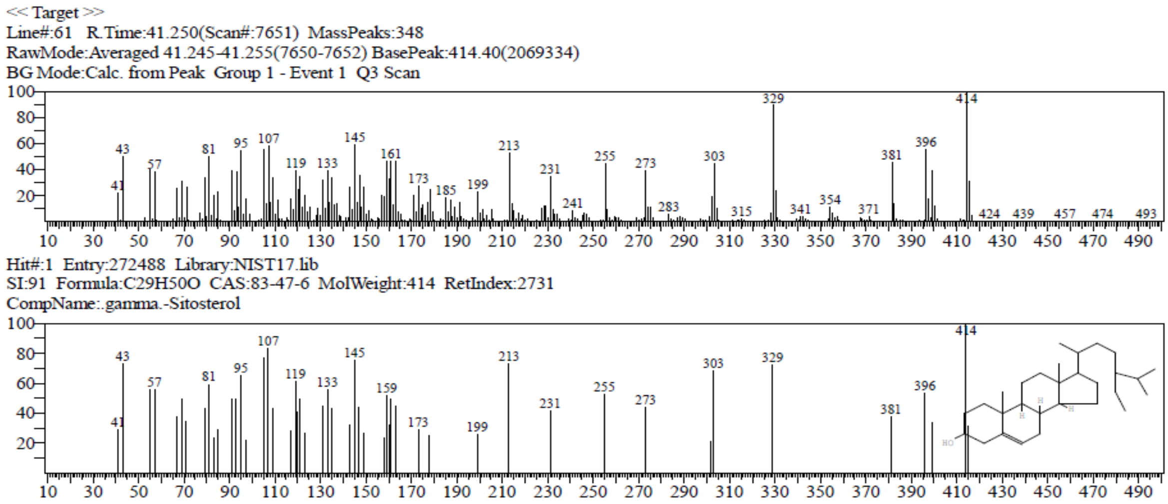

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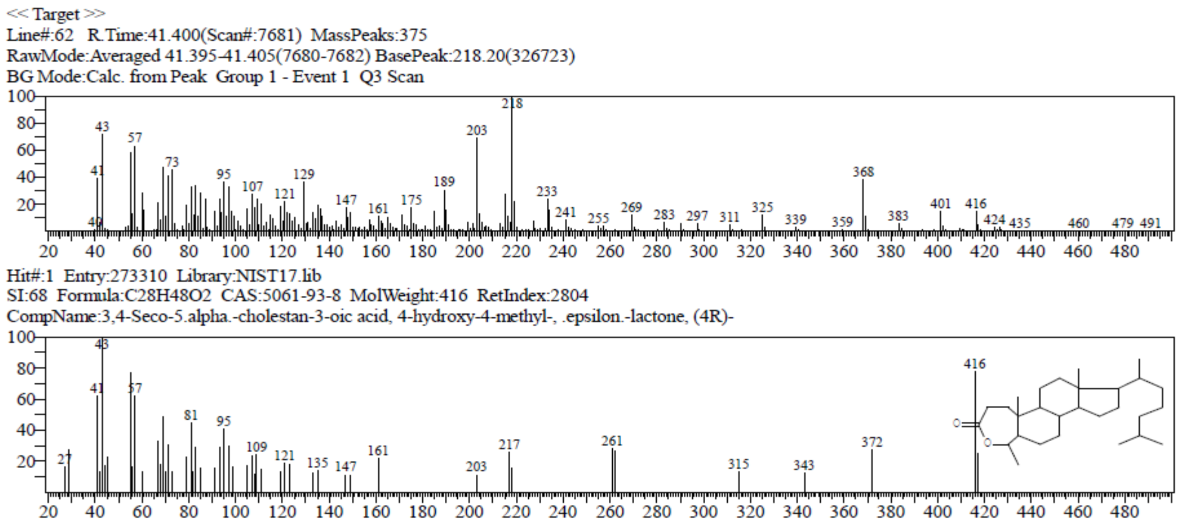


Conclusion

The phytochemical components like tannin, saponin and diosgenin were found to be lower in the case of boiled tubers, which makes it more suitable for human consumption because of de-bittering conditions. The GC-MS spectrum confirmed the presence of 24 peaks of different compounds which have been identified by searching with the NIST library.

CHAPTER IV

ANTIOXIDANT, ANTICANCER AND ANTIBACTERIAL ACTIVITY

4.1. Introduction

Dioscorea bulbifera is a potent medicinal plant used in both Indian and Chinese traditional medicine owing to its rich phytochemical diversity (Ghosh et al., 2015). *Dioscorea bulbifera L*. has been proven as a pharmacopotential natural product as it is effective against several diseases including diabetics, malaria, inflammation and cancer (Ghosh *et al*., 2012; Okon and Ofeni, 2013; Rego *et al*., 2014; Teponno *et al*., 2006; Ghosh *et al*., 2012; Mbiantcha *et al*., 2010; Chen x *et al*., 2013; Gao *et al*., 2002; Gao *et al*., 2007; Cui H., 2012; Trivedi *et al*., 2021; Ngan *et al*., 2021). Further, it is reported to exhibit antimicrobial, antioxidant plasmid curing, analgesic, anti-inflammatory, antihyperglycemic, antihyperlipidemic, antidiabetic, antinociceptive, and antitumor activities (Ghosh *et al*., 2015). *Dioscorea bulbifera* is also reported to have good radical scavenging and singlet oxygen quenching ability hence could be useful for treatment against various diseases that occurred due to oxidative stress (Ghosh *et al*., 2013). *D. bulbifera* exhibits higher antioxidant capacities with lower IC_{50} values as compared to the other species (Padhan *et al*., 2020). Crude extracts and compounds isolated from *D. bulbifera*, showed better antimicrobial activities against various pathogenic microorganism and was reported to treat conjunctivitis, diarrhea and dysentery (Kuete *et al*., 2012). *D.bulbifera* tuber extract and fractions remarkably inhibited the survivability of human colorectal carcinoma, human colorectal adenocarcinoma, human lung carcinoma (Hidayat *et al*., 2018). Methanolic and chloroform extract of *D.bulbifera* tuber exhibited potent activity against T47D breast cancer cell (Nur and Nugroho, 2018).

Detailed information on the pharmacological activity of tubers of *D. bulbifera* from the state of Odisha is very rare. In this regard, the present study was therefore aimed to evaluate and understand the antioxidant, antimicrobial and anticancer properties of wild yam *D. bulbifera*, prevalently used by the local tribal people in the forest area of Sundargarh district, Odisha India for its utilization as a medicinal plant.

4.2. Materials and Methods

4.2.1. Extract preparation

The powder samples of tuber were extracted using six different organic solvents (i.e. petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water) successively following the standard method described by Abubakar and Haque (2020). Extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressures at 40 ºC using a rotary evaporator and dissolved in methanol to make a stock solution of 50 mg/ml. Extracts were used for the analysis of antioxidant, antimicrobial and anticancer activity. The entire process of sample extraction is included in Figure 4.1.

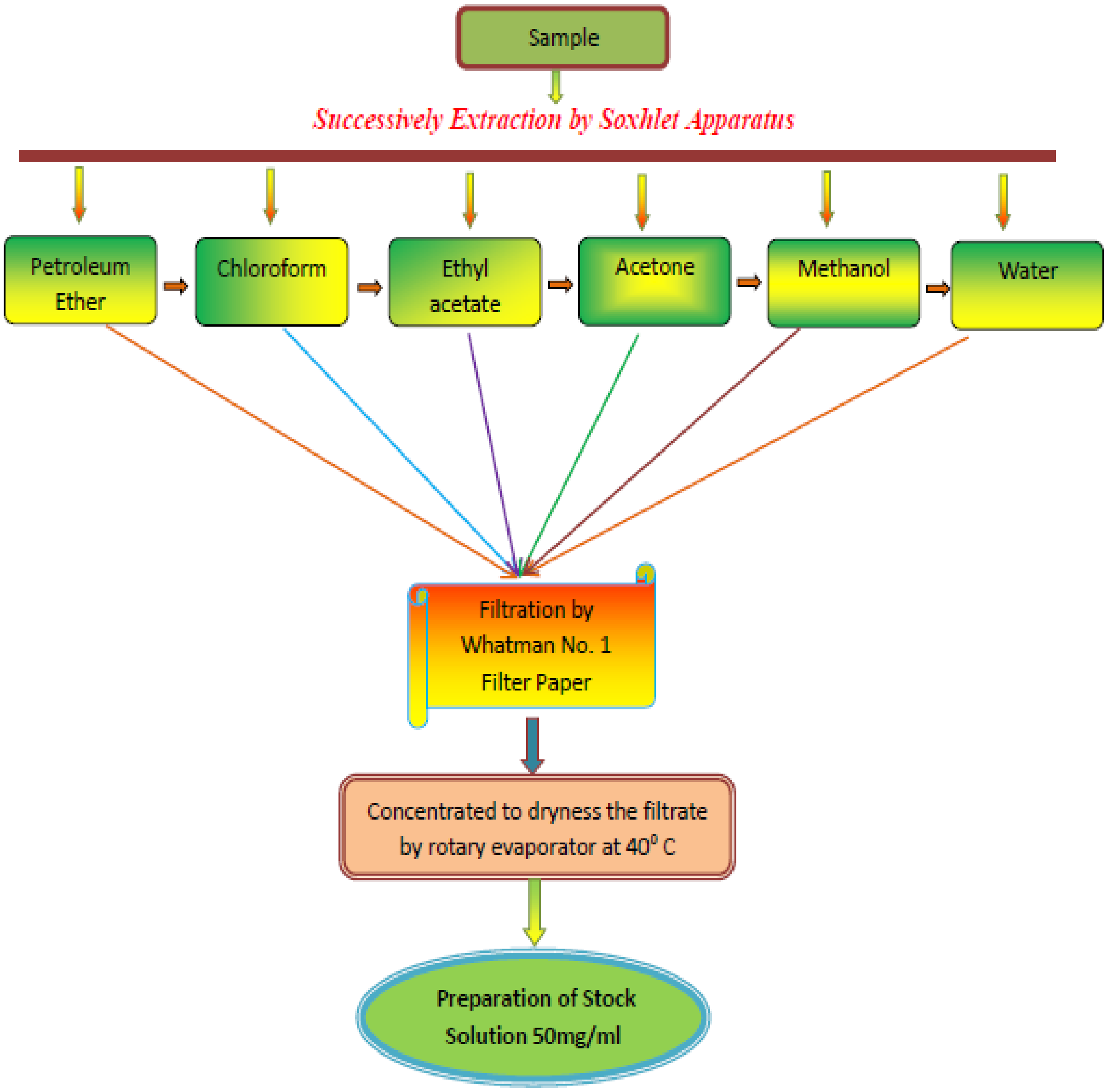


Figure 4.1. Flow chart describing the different steps of sample preparation

4.2.2. Percentage of yield

The percentage yield of the extracts was determined as the percentage of the weight of extracts to the original weight of the dried sample used, using the formula;

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100$$

4.2.3. Antioxidant activity

4.2.3.1. Total phenolic content

The total phenolic content of both the raw and boiled tuber powder samples was determined by the modified Folin-Ciocalteau method (Wolfe, 2003) with little modification. Briefly, 0.5 ml of diluted extract (1 mg/ml) was taken and mixed with 2.5 ml of 10 times diluted Folin-ciocalteau reagent. The mixture was incubated at room temperature for 5 min and then added 2 ml of prepared 7.5 % Na_2CO_3 (w/v) solution. The reaction mixture was then incubated at 40 $^{\circ}$C for 30 min and then cooled to room temperature. The absorbance was measured at 765 nm by taking distilled water as blank with the help of a UV-visible spectrophotometer. Different concentrations (10, 20, 40, 60, 80, 100 µg/ml) of Gallic acid solution were taken to make a standard calibration curve. The total phenolic content of the sample was expressed as mg of Gallic acid equivalents/100g of dry mass.

4.2.3.2. Total flavonoids content

The total flavonoids content of both the raw and boiled tuber powder samples was carried out using the method of Ordon *et al*. (2006). Briefly, to the 1 ml of aliquots, 4 ml of distilled water and 0.3 ml of 5% sodium nitrite solution were added. After 5 minutes, 0.3 ml of 10% aluminum chloride was added and after 6 minutes, 2 ml of 1M sodium hydroxide was added. The final volume was adjusted to 10 ml. The intensity of orange yellowish colour appeared was measured at 510 nm. The total flavonoids content was expressed as mg of quercetin equivalents/100g of dry mass

4.2.3.3. DPPH radical scavenging assay

The effect of the extracts on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was estimated using the method of Liyana-Pathiranan and Shahidi (2005). The absorbance of the mixture was measured spectrophotometrically at 517 nm using BHT (butylated hydroxytoluene) as reference. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs Control – Abs Sample)]/(Abs Control)] x 100 where; Abs Control is the absorbance of DPPH radical+methanol; Abs Sample is the absorbance of DPPH radical+sample extract/standard.

4.2.4. Antibacterial activity

4.2.4.1. Collection of bacterial sample

Strains of 2 GPs, *Staphylococcus aureus*, *Enterococcus faecalis*; 5 GNs, *Acinetobacter baumannii, Citrobacter freundii, Klebsiella pneumonniae, Proteus mirabilis* and *Pseudomonas aeruginosa* were isolated from clinical samples of hospitalized patients (IMS & SUM Hospital). Growth of isolated bacteria on suitable media, and biochemical identifications of isolated strains were done along with corresponding reference strains from Microbial Type Culture Collections (MTCC), Chandigarh, viz., with MTCC numbers *S. aureus* 7443 *E. faecalis* 439, *A. baumannii* 1425, *C. freundii* 1658, *P. mirabilis* 739, *K.pneumoniae* 1771 and *P. aeruginosa* 1688 were taken for reference (Figure 4.2) For the identification of bacterial strains, along with individual colony characters, biochemical tests and VITEK 2 system were considered.

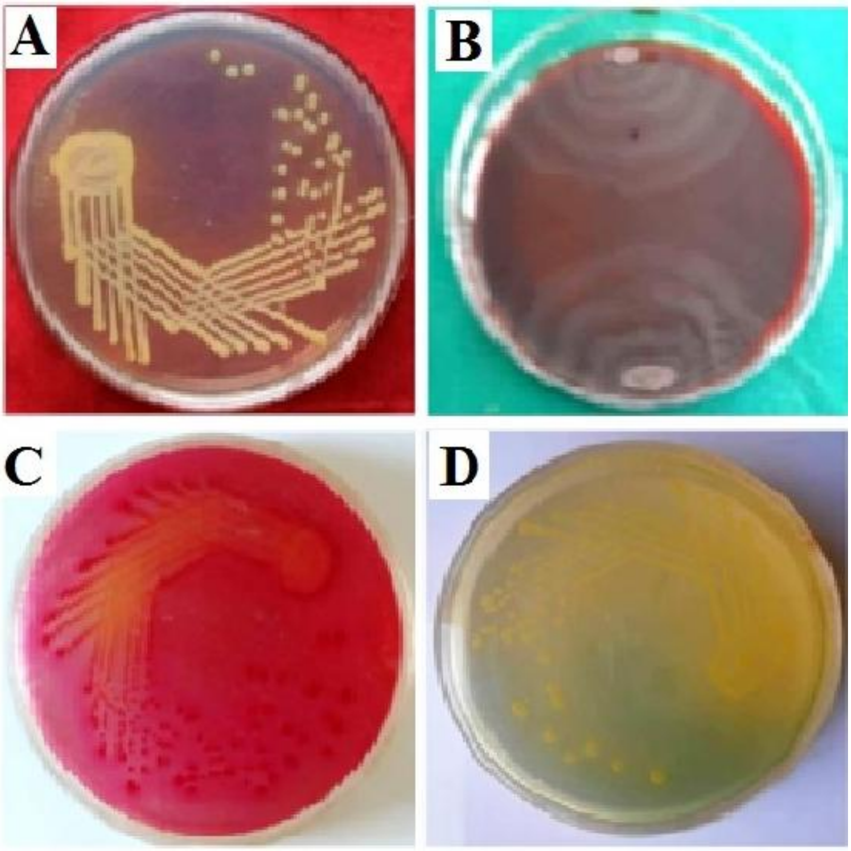


Figure 4.2. A. *S. aureus* on NA agar; B, *P. mirabilis* on Blood agar C. *C. freundii* on Mac Conkey, D. *A. baumanni* on CLED Aagar

4.2.4.2. Antibiotic susceptibility test by Kirby-Bauer's method

All bacterial strains including MTCC standard strains were subjected to antibiotic sensitivity tests by the Kirby-Bauer's/ disc-diffusion method (Figure 4.3), using a 4 mm thick Mueller–Hinton (MH) agar (HiMedia, Mumbai) medium, in duplicates. An aliquot of 0.1 mL of 0.5 McFarland equivalents, approximately from an exponentially growing culture was spread on agar for the development of lawn of a bacterium at 37 °C in a BOD incubator (Remi CIM-12S). Further, on the lawn-agar of each plate, 8 high potency antibiotic discs (HiMedia) of 16 prescribed antibiotics were placed, separately at equal distances from one another. Plates were incubated for 18 h at 37 °C and were examined for the size of zones of inhibition around each disc, following the standard antibiotic susceptibility test chart of Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2011). Experiments were done three times and data of the third set of experiments were presented (Forbes *et al*., 2007). The plates were then incubated at 37 °C for 24-48 h. Diameters of the zone of inhibition around the disc were measured using a zone measurement scale (caliper) and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI guidelines.

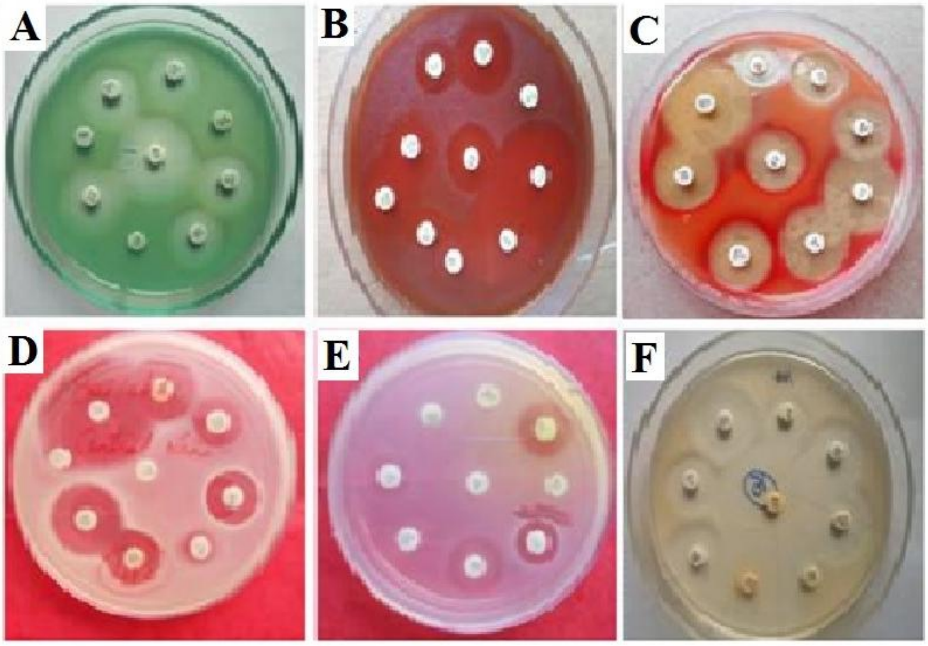


Figure 4.3. Antibiotic sensitivity of clinically isolated bacteria; A, *P. aeruginosa* B, *E. fecalis*; C..*P. mirabilis* D, *K. pneumoniae*; E. *A. baumanni and F. C. freundii*.

For Gram negative bacteria; ampicillin (AMP) (10 μg/disc), amoxicillin-clavulanic acid (AMC) (20/10 μg/disc), gentamicin (GEN) (30 μg/disc), amikacin (AK) (30 μg/disc), norfloxacin (NX) (10 μg/disc), levofloxacin (LV) (10 μg/disc), ciprofloxacin (CIP) (5 μg/disc), nitrofurantoin (NIT) (30 μg/disc), ceftriaxone (CTR) (30 μg/disc), ceftazidime (CAZ) (30 μg/disc), piperacillin/azobactam (PIT) (100/10 μg/disc), cefoperazone (CS) (75 μg/disc), cefoperazone sulbactam (CFS) (75/10 μg/disc), cefepime (CPM) (30 μg/disc) were used. Similarly, for Gram positive bacteria; ampicillin (AMP) (10 μg/disc), amoxicillin-clavulanic acid (AMC) (30 μg/disc), gentamicin (GEN) (10 μg/disc), amikacin (AK) (30 μg/disc), ciprofloxacin (CIP) (5 μg/disc), cefotaxime (CTX) (30 μg/disc), linezolid (LZ) (30 μg/disc), azithromycin (AZ) (30 μg/disc), oxacillin (Ox) (1 μg/disc), vancomycin (VA) (30 μg/disc), piperacillin (PI) (10 μg/disc), penicillin-G (P) (10 μg/disc) were used.

4.2.4.3. Agar-well diffusion method for antibacterial assays of seven crude extracts

Antibacterial activities of six crude leave solvent extracts were done by the agar well diffusion method. One strain from each bacterial species having resistance to maximum numbers of antibiotics was used, for monitoring the antibacterial activity of crude extracts. Bacterial lawns were prepared with agar of 6 mm thick that was fully punched and 6-8 wells were prepared when a lawn was 30 min old, and each well was based on 50 µl molten MH agar, in duplicates. Further, wells were filled with 100 µl aliquots of 30 mg/ml crude extracts diluted from the original stock of individual organic solvents with the aqueous extract, by 10% DMSO solution. Plates were incubated at 37 °C for 18-24 h. Antibacterial activities were evaluated by measuring the diameter values of zones of inhibition. The experiment of each solvent extract was conducted thrice and data of the third repeated experiment were presented. An aliquot of 100 µl of Linezolid /Imipenem 30 µg/ml with an average diameter of zone of inhibition of 21 mm and 1% DMSO solution were used as reference controls; 1% DMSO solution had no antibacterial activity (Perez *et al*., 1990).

4.2.4.4. Determinations of MIC and MBC values

Minimum inhibitory concentration (MIC) and *minimum bactericidal concentration* (MBC) of potential extracts which have the best zone of inhibition against selected bacterial strains were determined, by suitable dilutions from original stock solutions of each sample, for concentrations, 0, 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 mg plant-extract/ml in aliquots of 10% DMSO solution. Separate experiments were conducted for each sample. An aliquot of 80 µl of each dilution of a solvent-extract was released to a well on a 96-welled (12×8) micro-titer plate, along with an aliquot of 100 µl MH broth (HiMedia), an aliquot of 20 µl bacterial inoculum (10^9 CFU/ml) and a 5 µl aliquot of 0.5% 2,3,5-triphenyl tetrazolium chloride (TTC). After pouring all the above materials into a well, the micro-titer plate was incubated at 37 °C for 18 h. The development of pink colour due to TTC in a well indicated bacterial growth and the absence of the colour was taken as the growth inhibition. The first well of the microtiter plate was the control, without any plant extract (Eloff *et al*., 1998). The MIC value was noted at the well, where the pink colour was not manifested. Further, bacteria from each well of the microtiter plate were sub-cultured on nutrient agar; the level of dilution, where no bacterial growth on the nutrient agar was observed, was noted as the MBC value.

4.2.5. Anticancer activity

4.2.5.1. Cell culture and reagents

All the chemical reagents and media used for cell culture were procured from Sigma. Hela and MDAMB-231 cancer cell lines were obtained from the cell repository of the National Center for Cell Science Pune, Maharastra.

4.2.5.2. Anticancer evaluation by using MCF-7 and MDAMB-231 cell lines

The anticancer evaluation assay was performed by using two human breast cancer cell lines, MCF7 and MDAMB-231. In brief, cells were allowed to grow in culture medium (MEM, DMEM) supplemented with 10% FBS, 1% penicillin/streptomycin, with the maintenance of temperature at 37 °C and 5% CO_2. In a 96-well plate cancer cells were seeded at a density of $4x10^3$ cells per well and were treated with increasing concentrations from (6.25 to 100 µg/ml) of methanolic extract of *Dioscoria bulbifera* for 72 h. The cells were then stained with 0.56% of sulphorhodamine B in 1% acetic acid. The unbound stain was removed by washing with 1% acetic acid. About 10 mM Tris base with 10.5 pH was added to the 96 well plate containing fixed cells with protein-bound stain and the absorbance was taken at 495 nm wavelength using a Bio Red 96 well plate reader. The IC_{50} value for the extract was calculated from the plate reader data by using an online IC_{50} value Calculator (AAT Bioquest, Inc., Sunnyvale, CA, USA.

4.2.5.3. DAPI staining for detection of apoptosis

Apoptotic cells were visualized by DAPI staining method with fluorescence microscopy. MDAMB-231 cells were grown on 6-well plates and were treated with the methanolic extracts of *Dioscorea bulbifera* at a conc. of 50 µg/ml for 72 h. After incubation, 6 well plates were fixed in 3% formaldehyde and washed with PBS, stained with DAPI having a conc. of 1 mg/ml, and washed after 5 minutes by using PBS to remove the unbound stain. Images were captured using a fluorescent microscope (Nikon Eclipse Ts2R-FL). Apoptotic cells were identified based on the morphology of cells. e.g.

nuclear condensation, formation of membrane blebs and apoptotic bodies etc. compared to untreated cells.

4.2.5.4. Acridine Orange and ethidium bromide staining for detection of apoptosis

Apoptotic cells were visualized by Acridine Orange (AO) and ethidium bromide (EtBr) staining method with fluorescence microscopy. MDAMB-231 cells were grown on 6-well plates and were treated with the methanolic extracts of *Dioscorea bulbifera* at a conc. of 50 µg/ml for 72 h. After incubation, 6 well plate was fixed in 3% formaldehyde and washed with PBS, stained with AO with a conc. of 3 mg/ml and washed after 5 minutes by using PBS to remove the unbound stain. Images were captured using a fluorescent microscope (Nikon Eclipse Ts2R-FL). Apoptotic cells were identified based on the stain taken by the cells. The cells stained with green represent both live and pre-apoptotic cells stained with AO, cells stained with EtBr and AO having mixed red-green, displayed membrane blabbing forming apoptotic bodies as well as nuclear condensation.

4.3. Result and discussion

4.3.1. Percentage yield of solvents extracts

The extraction yield of methanol was found to be significantly highest among all extracts followed by water, ethylacetate, acetone, chloroform and petroleum ether (Table 4.1). Results showed that with the increase in polarity percentage yield of extract increases which indicated that tuber of *D. bulbifera* contained more polar compound.

Table 4.1. The percentage yield of extract using different solvents.

Name of the solvents	Polarity index of solvents	% of yield
Petroleum ether	0.1	4.14±0.05
Chloroform	4.1	5.44±0.3
Ethylacetate	4.4	7.7±0.02
Acetone	5.1	8.33±0.05
Methanol	5.1	12.36±0.15
Water	10.2	10.3±0.1

Values are expressed as mean ±SD of triplicate.

4.3.2. Antioxidant properties

4.3.2.1. Total phenolics and flavonoids content of *Dioscorea bulbifera* tuber

Halliwell and Gutteridge (2007) state that, “an antioxidant is a substance that, when present at a low concentration compared with that of an oxidizable substrate in the medium, inhibits oxidation of the substrate”. Under this classification, phenolic compounds, which are derived from the secondary metabolism of plants, protect multiple organs from oxidation and, therefore, phenolic compounds are regarded as natural antioxidants. Total phenolics content (699.47 µgGAE/100mg) and total flavonoids content (705.43 µgQE/100mg**)** were found remarkably high in methanolic extracts compared to other solvents. This corroborated the findings of Ghosh *et al*. (2013). Lowest TPC (50.18 µgGAE/100mg) content was found in petroleum ether while the lowest TFC (20.53 µgQE/100mg) content was found in water extracts.TFC content of acetone extract (37.58µgQE/100mg), ethylacetate extract (117.53 µgQE/100mg) and petroleum ether extract (38.46 µgQE/100mg) were significantly lower than TPC content of acetone extract (111.98±0.09 µgGAE /100mg), ethylacetate extract (295 µg GAE /100mg) and petroleum ether extract (50.18 µgGAE/100mg). However, TPC (151.42 µg GAE/100mg) and TFC (140.38 µgQE/100mg) content of chloroform extract were moderated. Many researchers have been estimated the phenolic content of *D.bulbifera* (Barman *et al.*, 2017; Bhandari and Kawabata, 2004; Ghosh *et al*., 2013). Observations of TPC and TFC content of *Dioscorea bulbifera* tuber extracts are presented in Table 4.2. Ghosh *et al*. (2013) estimated a very similar amount of phenolic content compared to present findings in petroleum ether. However, TPC and TFC of *D.bulbifera* in different solvents were deviated largely compared to present findings (Ghosh *et al*., 2013). Previously, Bhandari and Kawabata (2004) have reported 166 mg GAE/100gm fresh weight of total phenols for the acetone extract of *Dioscorea bulbifera* tuber. Okwu and Ndu (2006) and Adeosun *et al.* (2016) reported 8.04 mg and 5.36 mg quercetin equivalent/100g of flavonoid contents of *Dioscorea bulbifera* tuber on the dry weight basis.

Table 4.2. TPC and TFC of *Dioscorea bulbifera* tuber extracts

Solvents	**TPC (µgGAE/100mg)**	**TFC (µgQE/100mg)**
Petroleum ether	50.18±0.35	38.46±0.30
Chloroform	151.42±0.41	140.38±0.17
Acetone	111.98±0.09	37.58±0.06
Ethylacetate	295.23±0.25	117.53±0.25
Methanol	699.47±0.34	705.43±0.30
Water	82.80±0.01	20.53±0.37

4.3.2.2. DPPH scavenging activity

DPPH scavenging potential of methanolic and acetone extract was found to be very high while ethyl acetate, chloroform and water exhibited moderate scavenging potential. Ghosh *et al*., 2013 also reported the highest DPPH radical scavenging activity for the methanolic extract of *D.bulbifera*. In contrast, petroleum ether extract possessed the lowest DPPH scavenging activity. DPPH scavenging activity of different solvents was presented in Table 4.3. Out of six solvents extracts, methanolic extracts possessed excellent DPPH inhibition potential. Hence DPPH inhibition activity of methanolic extracts of both raw and boiled tuber was compared with standard ascorbic acid and BHT. The relationship between DPPH inhibition potential of methanolic extract of *D.bulbifera* with its phenolic and flavonoid content was also verified. The IC_{50} value (the concentration of the sample that reduced 50% of the absorbances of DPPH) of methanolic extract of tuber, ascorbic acid and BHT are included in Figure 4.4 and Table 4.4. Higher the IC_{50} value signifies less antioxidant activity and *vice-versa*. It was found that the methanolic extract of the raw tuber has significantly higher antioxidant activity (IC_{50} value is 46.11 µg/ml) compared to the ascorbic acid (IC_{50} value is 92.86 µg/ml), BHT (IC_{50} value is 54.35 µg/ml) in contrast after boiling antioxidant potential of tuber significantly reduced (455.37 µg/ml). Among boiled and raw tuber, the latter one showed excellent radical scavenging activity which is significantly higher than that of ascorbic acid and BHT. The present findings also support the observation of Murugan and Mohan (2012) who observed similar radical scavenging activity (79.3%) of *Dioscorea* sp. extract with IC_{50} value of 38.33 µg/mL in DPPH assay. However, Odeghe *et al*. (2021) found 261.09 µg/ml and 10 µg/ml of IC_{50} value for *D.bulbifera* and ascorbic acid. The lower scavenging activity observed in boiled tuber may be due to leaching out of phenol and flavonoid during boiling.TPC and TFC of tuber positively correlated with DPPH scavenging activity at the 0.01 level (R^2=0.9762) **(**Figure 4.5**)** and (R^2=0.9717) (Figure 4.6) respectively.

Table 4.3. Percentage of inhibition of DPPH with different solvent extracts.

Name of solvents	% of inhibition
Petroleum ether	32.97±0.24
Chloroform	63.20±0.08
Ethyl acetate	73.13±0.04
Acetone	84.61±0.02
Methanol	93.81±0.02
Water	57.65±0.03

Table 4.4. IC_{50} Value of tuber extract, ascorbic acid and BHT

Samples	**IC_{50}**(µg/ml)
Methanolic extract raw	46.11
Methanolic extract boiled	455.37
Ascorbic acid	92.86
BHT	54.35

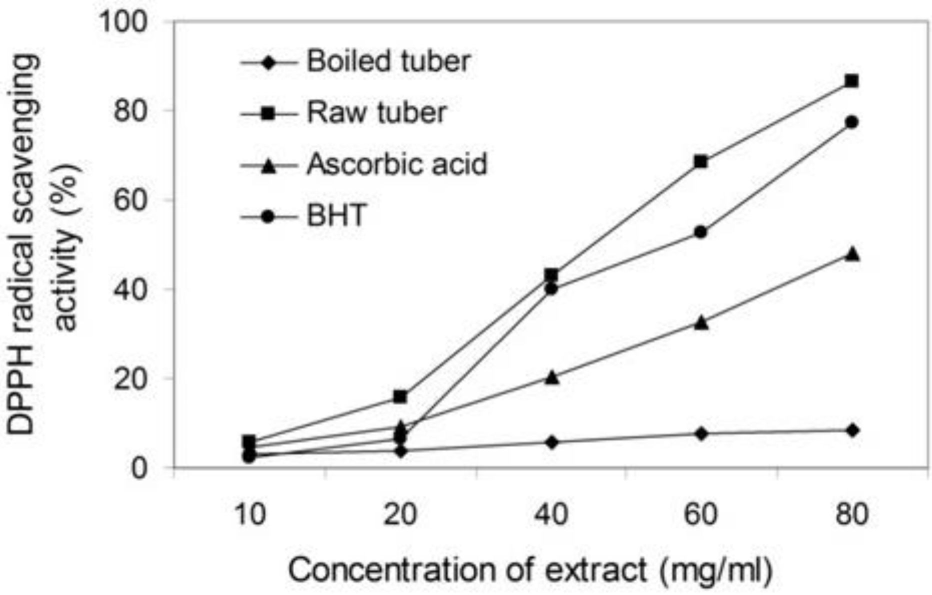


Figure 4.4. Comparison of DPPH scavenging activity(%) of tuber with ascorbic acid and BHT

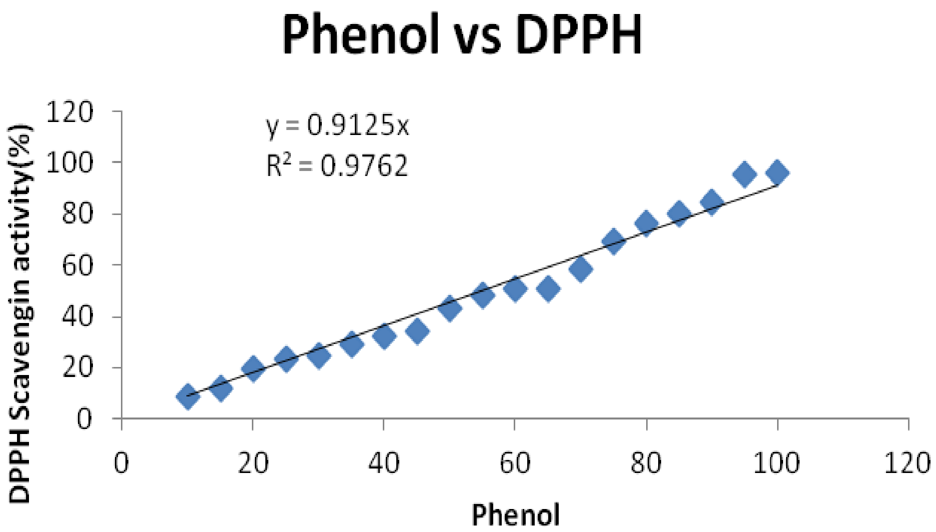


Figure 4.5. Correlation between Phenol and DPPH Scavenging activity.

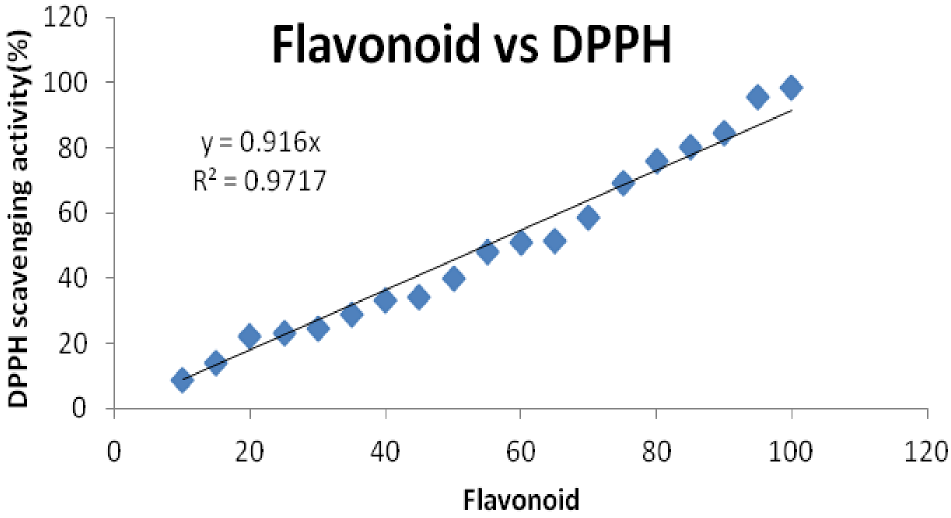


Figure 4.6. Correlation between flavonoid and DPPH scavenging activity.

4.3.3. Antibacterial activity

4.3.3.1. Antibacterial assay by agar-well diffusion method

Antibacterial activities of 6 different solvent-extracts were monitored by the agar-well diffusion method on lawns of 7 bacterial isolates. It was found that extracts with petroleum ether and chloroform had the least antibacterial activity. Extracts with methanol had the highest antibacterial activity on all bacterial strains, whereas extracts with acetone, ethyl acetate and water had moderate antibacterial activities. Methanolic extract of tubers registered the maximum size of the zone of inhibition of 30 mm against *S. aureus* and *C. freundii.* In contrast, methanolic extract revealed the least value of 19 mm against *P. mirabilis*. Furthermore, ethyl acetate and water extract of tubers had maximum zones of inhibition of 29 mm against *S. aureus* and *E. faecalis*. Inhibition zone of aqueous and chloroform extracts were found to be 17 mm and 10 mm against *K. pneumoniae* (Dahiya, 2017). We found that water and chloroform extracts possessed 26 mm and 10 mm of inhibition zone against *K. pneumonniae.* Dahiya (2017) reported that aqueous and chloroform extracts of *D.bulbifera* tuber were more capable to inhibit the pathogenic bacteria compared to other extracts. Sizes of zones of inhibition of all solvent extracts against 7 bacteria were recorded (Table 4.5). Furthermore, the best 3 active tuber extracts were used to determine their MIC and MBC values.

Table 4.5. Antibacterial assay by agar-well diffusion method of hot solvent tuber-extracts of *D. bulbifera* against MDR strains of bacteria as diameter size of zone of inhibition (mm).

Bacteria	Petroleum ether	Chloro form	Acetone	Ethyle acetate	Metha nol	Water	Linezolid/ imipenem (30/10μg/ml)
S. aureus	10	21	23	29	30	29	29
E. fecalis	17	15	19	29	29	24	33
A .baumannii	08	12	22	19	26	23	31
C. freundii	11	18	24	23	30	27	26
K. pneumonniae	10	19	23	26	22	26	29
P. mirabilis	18	18	25	19	19	23	26
P. aeruginosa	09	23	22	25	27	26	29

4.3.3.2. MIC and MBC values of extracts

MIC and MBC values of three tuber extracts, ethyl acetate, methanol and water that revealed maximum antibacterial activities were determined (Table 4.6). The MIC value of 1.56 mg/ml of ethyl acetate extract was registered against *S. aureus*, *E. faecalis*, *C. freundii* and *P. mirabilis*, while the value of 3.125 mg/ml against *A. baumannii, K. pneumoniae* and *P. aeruginosa* were recorded. Similarly, MIC value of 1.56 mg/ml for methanol extract was registered against *S. aureus, E. faecalis, C. freundii* and *P. mirabilis,* while the value of 3.125 mg/ml against *A. baumannii, K. pneumoniae* and *P. aeruginosa* were recorded. Likewise, the MIC value of 1.56 mg/ml of water extract was registered against *S. aureus*, *A.baumannii*, *K. pneumoniae* and *P. mirabilis,* while the value of 3.125 mg/ml against *E.faecalis, C.freundii, K.pneumonniae* and *P. aeruginosa* were recorded. Further, MBC values of three leading extracts (ethyl acetate, methanol and water) were determined. The MBC value of 6.25 mg/ml of the ethyl acetate extract was found against *S. aureus*, *E. faecalis*, *C. freundii*, *K.pneumoniae* and *P. mirabilis* while the value of 12.5 mg/ml against *A. baumannii* and *P. aeruginosa* was recorded. Similarly, the MBC value of 6.25 mg/ml of the methanol was registered against *S.aureus*, *E. faecalis*, *C. freundii*, *K. pneumoniae* and *P. mirabilis*, while the values of 12.5 mg/ml and 25 mg/ml against *A.baumannii* and *P. aeruginosa* were registered. Further, the MBC value of 6.25 mg/ml of the water extract was recorded against *S. aureus*, *A. baumannii, K. pneumoniae* and *P. aeruginosa,* while the value of 12.5 mg/ml against *E. faecalis* and *C. freundii* were registered. The value of 3.125 mg/ml was registered against *P. mirabilis*.

The findings of the present study were very much supported by the findings of Kuete *et al*. (2012) who reported antimicrobial activities of the methanol extracts of *D.*

bulbifera against mycobacteria and Gram-negative bacteria. Similarly, Seetharam *et al*. (2003) observed antimicrobial activity of *D. bulbifera* extracts against pathogenic strains *S. aureus*, *R. nigricans, K. pneumonia* and *Aspergillus fumigates.*

Table 4.6. MIC and MBC values of the best 3 solvent extracts of *D. bulbifera* against isolated MDR strains (mg/ml).

Strains	***D.bulbifera* leaf extract (mg/ml)**					
	Ethyl acetate		**Methanol**		**Water**	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	1.56	6.25	1.56	6.25	1.56	6.25
E. faecalis	1.56	6.25	1.56	6.25	3.125	12.5
A.baumannii	3.125	12.5	3.125	12.5	1.56	6.25
C. freundii	1.56	6.25	1.56	6.25	3.125	12.5
K. pneumoniae	3.125	6.25	3.125	6.25	1.56	6.25
P. mirabilis	1.56	6.25	1.56	6.25	1.56	3.125
P. aeruginosa	3.125	12.5	6.25	25.0	3.125	6.25

Note: MIC: minimum inhibitory concentration; MBC: *minimum bactericidal concentration.*

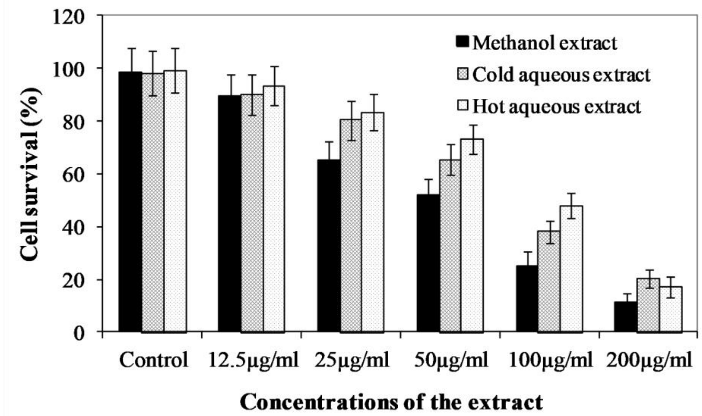
4.3.4. Anticancer activity

Natural products are gaining popularity in cancer therapy because they are regarded to be more physiologically friendly, meaning they are more co-evolved with their target areas and less harmful to normal cells (Mishra, 2011). Furthermore, anticancer drugs derived from natural ingredients have been shown to cause cell death in several ways. As a result, to fulfill the demand for anticancer drugs and stay sustainable, exploitation of these agents must be carried out. There are currently improvements in the synthesis of high-value plant metabolites using modern approaches and sustainable alternative ways for therapeutic purposes in cancer. Due to the rising cancer death rate each year, particularly in underdeveloped nations, current research is concentrating on alternative cancer therapies. Breast cancer is one of the most lethal cancers on the planet. *Dioscorea bulbifera L*. has been proven as a pharmacopotential natural product as it is effective against several diseases including diabetics, malaria, inflammation and cancer (Ghosh et al., 2012; Okon and Ofeni, 2013; Rego et al., 2014; Teponno et al., 2006; Ghosh et al., 2012; Mbiantcha et al., 2010; Chen x et al., 2013; Gao et al., 2002; Gao et al., 2007; Cui H., 2012). As a result, it was crucial to investigate *D. bulbifera*'s as anti-cancer agents. The goal of this study was to see if methanol and aqueous extracts of *Dioscorea bulbifera* L. were toxic to breast cancer (MCF-7, MDAMB-231) cells, and to identify the anticancer chemicals as an early step in the development of anti-cancer drugs.

4.3.4.1. Evaluation of Anticancer potential

Tuber extracts with different solvents were tested to demonstrate inhibition of proliferation of cancer cells (MCF-7 and MDAMB-231) at dosages ranging from 12.25 µg/ml to 200 µg/ml. The tuber extracts were inhibited cancer cell proliferation in a concentration-dependent manner (Figure 4.7). The IC_{50} (the concentration of extracts inhibiting 50% of cell death) value of the different extracts was determined and collated in Table 4.7. The methanolic extract has the lowest IC_{50} value of 55 µg/ml 75 µg/ml, respectively using MCF-7 and MDAMB-231 cancer cell lines. Previously Nur and Nugroho (2018) reported IC_{50} value of 2235.32µg/ml for the methanolic extract and 372.14 µg/ml for chloroform extract of tuber against T47D breast cell. Similarly, Hidayat *et al.* (2018) showed anticancer activity of *D.bulbifera* tuber extract and fractions and found demonstrated that ethyl acetate fraction remarkably inhibited the survivability of human colorectal carcinoma, human colorectal adeno carcinoma, human lung carcinoma.

(a) MCF-7



(b) MDAMB-231

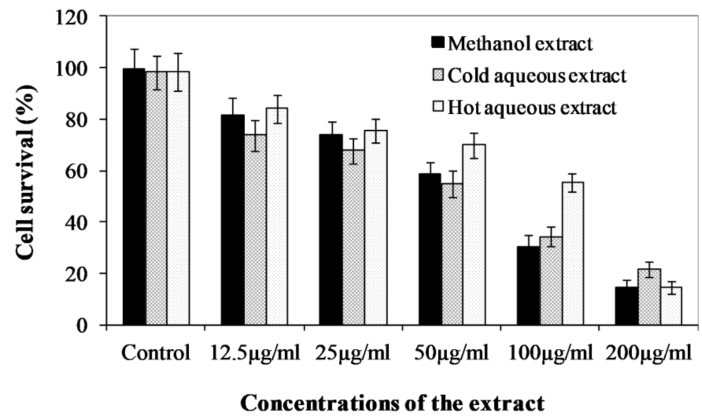


Figure 4.7. Inhibition to proliferation of cancer cells (A) MCF-7 and (B) MDAMB-231 with increasing concentration of different extracts of *D.bulbifera.*

Table 4.7. IC_{50} value of extracts treated against MCF-7 and MDAMB-231 breast cancer cell lines.

IC_{50} value (µg/ml) of different solvent extract of *D.bulbifera*			
	Methanolic	**Cold Aqueous**	**Boiled aqueous**
MCF-7	55±3.4	72±2.3	105±2.7
MDA-MB-231	75±2.8	86±2.4	126±1.8

4.3.4.2. Induction of apoptosis in cancer cells

Apoptosis was used to explore the mechanism of cell death in response to the tuber extract. Apoptosis is characterized morphologically by changes in the membrane and nuclear structure of the treated cells. MCF-7 and MDAMB-231 cancer cells treated with IC_{50} concentration of methanolic extract (55 µg/ml) revealed induction of apoptosis by staining with DAPI, Acridyne orange and Ethidium bromide. The treated cells appeared condensed chromatin, membrane blebs, and many shattered nuclei, all of which suggested induction of apoptosis to cancer cells (Figure 4.8-4.10.

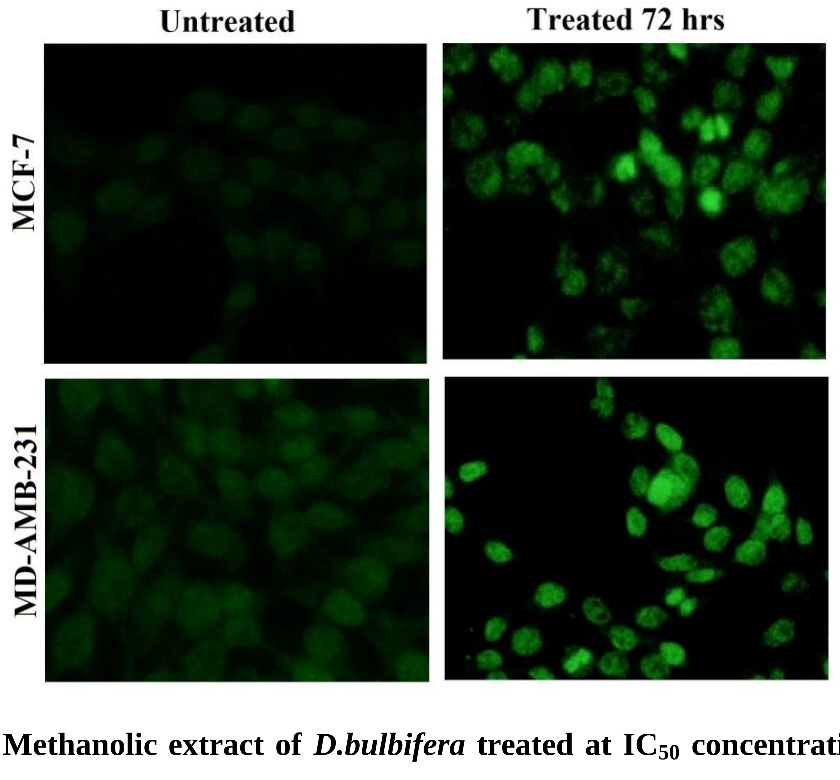


Figure 4.8. Methanolic extract of *D.bulbifera* treated at IC_{50} concentration against two breast cancer cell lines, stained with Acridine orange showing apoptotic cells.

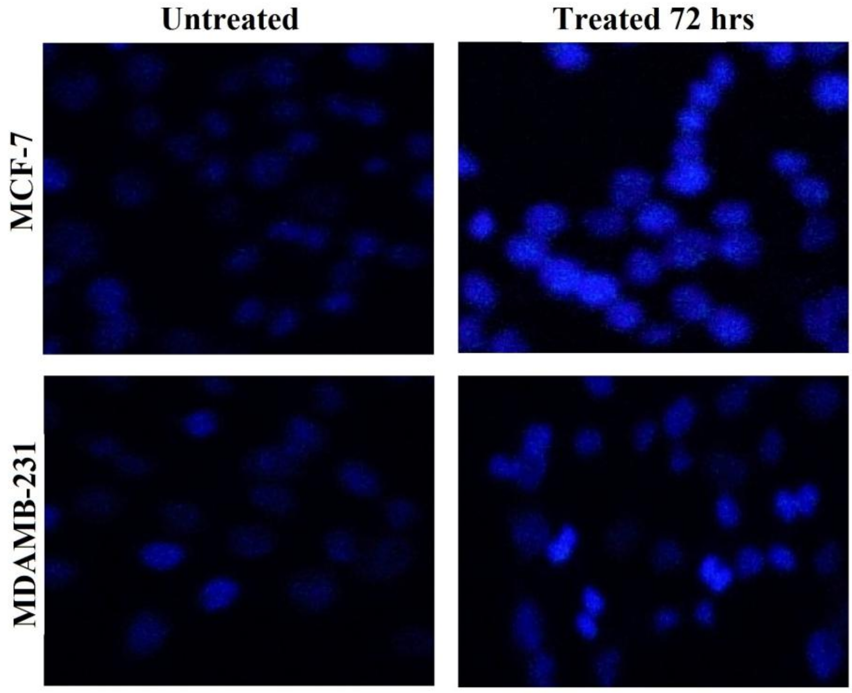


Figure 4.9. Methanolic extract of *D.bulbifera* treated at IC_{50} concentration against two breast cancer cell lines, stained with DAPI showing apoptotic cells.

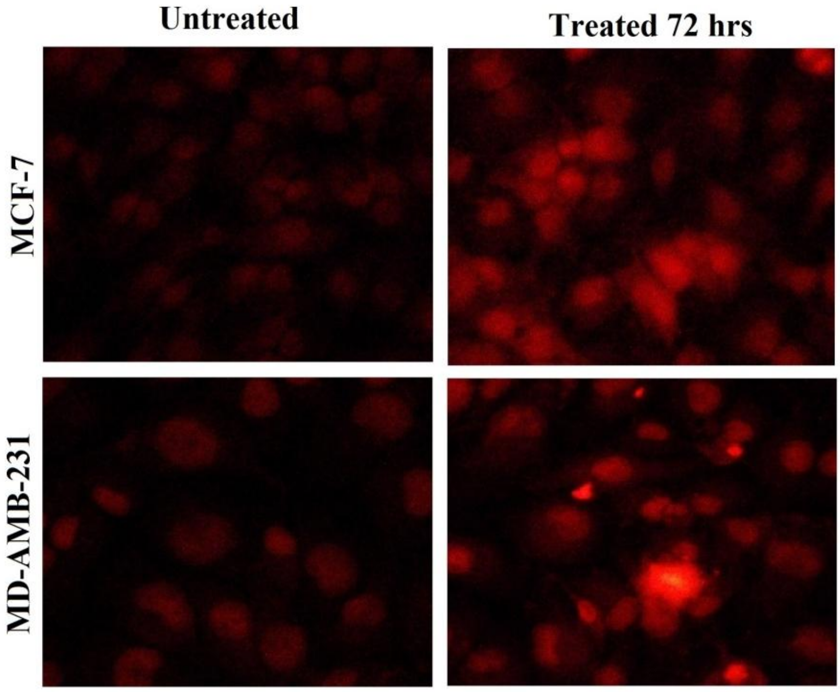


Figure 4.10. Methanolic extract of *D.bulbifera* treated at IC_{50} concentration against two breast cancer cell lines, stained with ethidium bromide showing apoptotic cells.

4.4. Conclusion

Overall, for treating radical-related pathological damage, bacterial infection and cancer related problems, the tuber of *Dioscorea bulbifera* can be used as a therapeutic agent. The current report also suggests that in *Dioscorea bulbifera* polyphenol are important components which not only responsible for its antioxidant activities but some of its pharmacological effects also.

CHAPTER V

TOXICITY ANALYSIS

OF DIOSCOREA BULBIFERA TUBER

5.1. Introduction

Generally, *Dioscorea* species are known as yam throughout the world. However, in Odisha Ban alu is the alternate name of *Dioscorea* species (Kumar *et al*., 2017). *Dioscorea* species constitute an important place in the diet of rural dwellers as a major staple medicinal food in the different states of India (Kumar *et al*., 2017). Out of 10 bitter wild tubers of *Dioscorea* species found in Odisha, *Dioscorea bulbifera* is the most commonly used species among rural people (Kumar *et al*., 2017). Bitter compounds of *Dioscorea bulbifera* vary on the basis of geographical distribution. In Central American, South African and Indian varieties, saponin and sapogenins are responsible for bitterness (Varsha and Divyeshkumar, 2017). In Indo-Chinese varieties, tannins and polyphenols are bitter compounds whereas in China and Australia furanoidnorditerpenes (Diosbulbins) are major bitter compounds (Varsha and Divyeshkumar, 2017). In Austrilian varieties Diosbulbins D (yield 0.07 mg/g) is a major bitter compound (Webster *et al*., 1984). While Bhandari and Kawabata, 2005 reported furanoidnorditerpens, Diosbulbin A (0.043 g/kg) and Diosbulbins B (0.151g/kg) are responsible for bitterness of Nepalian *Dioscorea bulbifera* tuber. The tribals of the central mountain area and the Western hilly region of the Odisha used *Dioscorea bulbifera* as food as well as medicine. The tribal people have much knowledge about the detoxification of the wild tubers before consumption (Padhan and Panda, 2016). It is bitter in taste, therefore locally known as Pitaalu in Odia and bitterness is removed by boiling followed by leaching of the sliced tubers in running water overnight (Webster *et al*., 1984).

Tubers of *D.bulbifera* possess significant activities like purgative, deflatulent, aphrodisiac, anthelminatic, rejuvenating and tonic. It is used in haematological disorders, scrofula, syphilis, haemorrhoids, flatulence, diarrhea, dysentery, worm infections, general debility, diabetic disorders, polyuric and skin disorders.

D.bulbifera is an important medicinal plant used in the prevention of many diseases and consumed as food after the removal of bitterness. But many researchers reported bitter compounds of *D.bulbifera* to have a toxic effect (Min, MA., *et al*., 2011, Ma, Y., *et al.*, 2014; Niu, C., *et al*., 2016; Wang, LL., *et al*., 2017; Zhao *et al*., 2018). Therefore an attempt has been made to investigate the toxicity of raw tuber and to check the effectiveness of the debittering process.

5.2. Materials and Methods

5.2.1. Toxicity evaluation

We have performed the acute and sub-acute toxicity of the aqueous extract of *Dioscorea bulbifera* tuber. The study was conducted on albino rats of Wister strain, according to OECD guidelines 423 and 407 respectively. The animal experiment was conducted at the School of Pharmacy, Siksha 'O' Aunsandhan University, and the protocol (Figure 5.1) used was approved by the Animal Ethics Committee (Protocol IAEC/SPS/SOA/18/2019).

5.2.2. Preparation of aqueous extract

About 100 g of raw and boiled *D. bulbifera* tuber was macerated each in 1 liter of distilled water with micro-vortex stirring at 350 rpm for 24 hours and the filtrate was collected. Again residue left after filtration macerated in 0.5 liter of distilled water for another 24 hours. The macerate was then filtered by poplin cloth and combined both the filtrate. Then the filtrate was dried in a rotary evaporator at 40 ^{o}C until the powder was obtained.

5.2.3. Animal protocol

The male and female rats of (180-230 g), aged 6-8 weeks were purchased from Neelachal Tirati, Kolkata, Saha Enterprises (1828/po/Bt/S/15/CPCSEA). Animals were kept in a temperature controlled environment (23 ± 20 ^{o}C) with a 12 hours light-dark cycle. Food and water were freely available (Burger *et al*., 2005). The control group received water only. Aqueous extract of *D.bulbifera* tuber was given to the animal by the rubber tubing. There was one control group and three treated group. Each group is containing six animals.

5.2.4. Dose level

There were two types of extracts given to animals, one aqueous extract of raw *D.bulbifera* tuber (RDBE) and another one aqueous extract of boiled *D.bulbifera* tuber (BDBE). RDBE was given to doses level of 200 mg/kg, 400 mg/kg and 800 mg/kg body weight. Whereas BDBE was given to 2000 mg/kg, 4000 mg/kg and 8000 mg/ kg body weight.

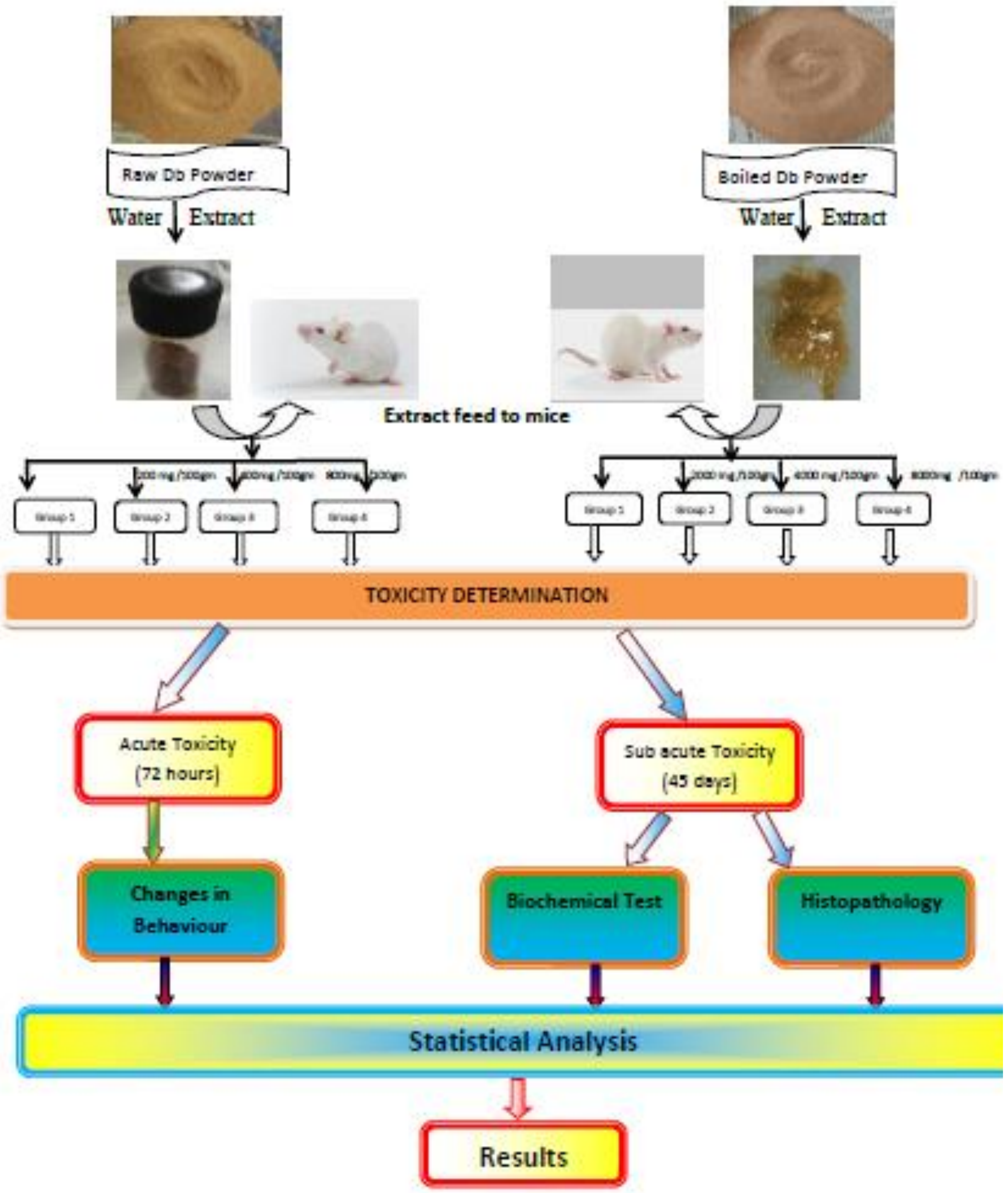


Figure 5.1. Flow chart is demonstrating the experimental plan for the toxicity evaluation of the extracts of *Dioscorea bulbifera*.

5.2.5. Acute toxicity

The acute toxicity study was carried out using male albino Wister rats (180-230g) as per OECD guidelines. Twenty four animals were divided into four groups, each group containing 6 rats, one control group and three treated groups. The control group received water only and each treated group received a single oral dose of extract. After administering the extract the animals were observed for any changes in the general behaviour, physiological activities and survival in 72 hours.

5.2.6. Sub acute toxicity

The sub-acute toxicity study was carried out using male albino Wister rats (180-230 g) as per OECD guidelines. Twenty six animals were divided into four groups, each group containing 6 rats, one control group and three treated groups. The control group received water only, whereas the treated group received extracts by rubber tubing for 45 days. After 45 days, the animals were anesthesized with formalin and the animals were sacrificed to collect their blood and organs (liver, kidney) for biochemical and histological analysis.

5.2.6.1. Blood biochemical analysis

On days 46, the control and treated groups of animals were given an overdose (0.2 ml) of 3.5% formaldehyde. Then blood was taken from the heart for analysis. The biochemical parameters analyzed from serum were glucose (G), total cholesterol (TC), triglycerides (TG), aspartate amino transferase (AST), alanine amino transferase (ALT), urea (Ur), creatinine (Cr) and total protein.

5.2.6.2. Histopathology and clinical biochemistry

On days 46, the control and treated groups of animals were given an overdose (0.2 ml) of 3.5% formaldehyde. The vital organs such as liver and kidney were removed and processed for histopathological analysis. Tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The tissues were observed under the microscope for toxicity evaluation.

5.3. Result and discussion

5.3.1. Acute toxicity study

Oral administration of the raw *D. bulbifera* aqueous extract (200 to 800 mg/kg body weight) and boiled *D. bulbifera* aqueous extract (2000 to 8000 mg/kg body weight) neither caused any death nor produced significant changes in the spontaneous type, alertness, awareness, sound response, touch response, pain response, righting reflex, pinna reflex, grip strength in the experimental rats, during 72 hours of the experimental period. All groups of animals showed neither any toxic effect nor any lethal effect. Administration of doses up to 800 mg/kg for raw *D. bulbifera* aqueous extract and 8000 mg/kg for boiled *D. bulbifera* aqueous extract did not reveal any signs of toxicity or mortality in rats during the entire observation period. Therefore, LD_{50} of raw *D. bulbifera* aqueous extract and boiled *D. bulbifera* aqueous extract may be considered to be greater than 800 mg/kg and 8000 mg/kg body weight, respectively.

Table 5.1. Effect of aqueous raw and boiled tuber extract of *Dioscorea bulbifera* on acute toxicity.

Behaviour Type	**Treatments**			
	Control	**200 & 2000 mg/kg body weight**	**400 & 4000 mg/kg body weight**	**800 & 8000 mg/kg body weight**
Spontaneous type	Normal	Normal	Normal	Normal
Alertness	Normal	Normal	Normal	Normal
Awareness	Normal	Normal	Normal	Normal
Sound response	Normal	Normal	Normal	Normal
Touch response	Normal	Normal	Normal	Normal
Pain response	Normal	Normal	Normal	Normal
Righting reflex	Normal	Normal	Normal	Normal
Pinna reflex	Normal	Normal	Normal	Normal
Grip strength	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal
Mortality	Absent	Absent	Absent	Absent

5.3.2. Sub acute toxicity

Blood biochemical parameters and histopathology of kidney and liver of the control and experimental animals were observed to investigate any side effects to the animal.

5.3.2.1. Biochemical parameters

The blood biochemical parameters between treated and untreated groups of animals were analysed to examine whether the raw tuber and boiled tuber of *D. bulbifera* aqueous extract have any side effects on animals. The data were collated in Table 5.2 and

5.3 as well as Figure 5.2 and 5.3. Briefly, the parameters examined include glucose, urea, creatinine, protein, cholesterol, triglycerides, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). After 45 days of daily doses of raw tuber and boiled tuber aqueous extract of *D. bulbifera* failed to reveal any significant difference (using one way anova test at p≤0.05) in various blood biochemical parameters between treated and untreated groups, indicating no side effect to animals. In contrast, Ma,Y *et al*. (2014) observed an increased level of ALT and AST in mice treated with 32 mg/kg and 64 mg/kg of *D. bulbifera* tuber extract compared to control where livers lost normal architecture with extensive acute hemorrhage, swelling hepatocytes and widespread necrosis. In another study, Wang, LL., *et al*. (2017) observed a significant increase in ALT and AST levels in both 2.10 g/kg and 8.40 g/kg body weight treated groups with *D. bulbifera* extract compared to the control group and confirmed hepatotoxicity in rats. Zhao *et al*. (2018) found an increased level of AST in the treated groups with the administration of 1.8 g/kg body weight and 18 g/kg body weight of *D. bulbifera* tuber aqueous extract.

Table 5.2. Blood biochemical parameters between the control and treated groups of animal with raw *D. bulbifera* tuber aqueous extract.

Parameters	**Group-I (control)**	**Group-II 200mg/kg body weight**	**Group-III 400mg/kg body weight**	**Group-IV 800mg/Kg body weight**	**Normal range**
Glucose(mg/dl)	85.06±0.66	85.53±0.41	84.70±0.41	85.25±0.36	70-110
Urea(mg/dl)	31.99±0.61	32.73±0.33	32.00±0.26	31.82±0.61	15-45
Creatinine(mg/dl)	0.94±0.02	0.93±0.02	0.90±0.01	0.94±0.03	0.5-1.5
Total protein(mg/dl)	6.84±0.02	6.93±0.12	6.84±0.02	6.88±0.11	6.0-8.0
Total cholesterol (mg/dl)	128.0±0.03	128.1±0.06	128.3±0.19	128.3±0.18	140-250
Tri glycerides(mg/dl)	92.05±0.03	91.93±0.36	92.15±0.28	92.25±0.35	25-160
(AST)(IU/L)	32.19±0.24	32.22±0.22	31.98±0.54	32.13±0.39	Up to 46
(ALT)(IU/L)	27.72±0.32	28.26±0.31	28.43±0.31	28.53±0.34	Up to 40

The values are mean±standard deviation. The differences in various blood biochemical parameters are statistically not significant using one away anova test among control and treated groups of the animal at p≤0.05). Group-I (Control), Group-II (200 mg/kg bodyweight), Group-III (400 mg/kg body weight), Group-IV (800 mg/Kg body weight), AST (Asparate aminotransferase), ALT (Alanine aminotransferase).

Table 5.3. Blood biochemical parameters between the control and treated groups of animals with boiled *D. bulbifera* tuber aqueous extract.

Parameters	**Group-I (control)**	**Group-II 200mg/kg body weight**	**Group-III 400mg/kg body weight**	**Group-IV 800mg/Kg body weight**	**Normal range**
Glucose(mg/dl)	78.46±0.46	75.24±0.12	76.10±0.05	75.24±0.08	70-110
Urea(mg/dl)	35.60±0.62	32.37±0.18	31.84±0.29	34.21±0.28	15-45
Creatinine(mg/dl)	0.86±0.05	0.85±0.03	0.83±0.01	0.83±0.007	0.5-1.5
Total protein(mg/dl)	6.56±0.11	6.81±0.099	6.83±0.05	6.92±0.10	6.0-8.0
Total cholesterol (mg/dl)	165.1±0.99	167.5±0.53	158.4±0.74	161.2±0.88	140-250
Tri glycerides(mg/dl)	92.12±0.83	113.5±1.77	113.1±0.83	115±1.06	25-160
(AST)(IU/L)	32.37±0.51	37.24±0.13	39.08±0.22	42.05±0.02	Up to 46
(ALT)(IU/L)	27.46±0.41	26.58±1.42	31.53±0.67	34.05±0.03	Up to 40

The values are mean±standard deviation. The differences in various blood biochemical parameters are statistically not significant using one away anova test among control and treated groups of the animal at p≤0.05). Group-I (Control), Group-II (2000 mg/kg body weight), Group-III (4000 mg/kg body weight), Group-IV (8000 mg/Kg body weight), AST (Aspartate aminotransferase), ALT (Alanine aminotransferase).

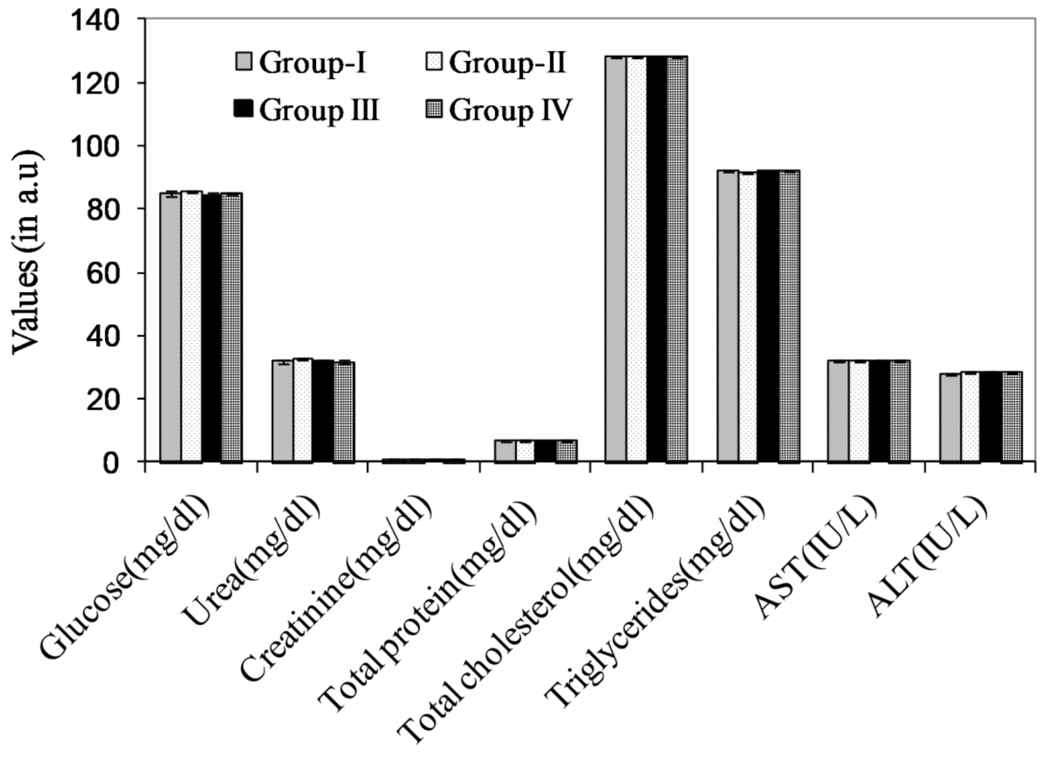


Figure 5.2. Comparison of blood biochemical parameters between the treated and untreated groups of animals with the administration of raw tuber extract.

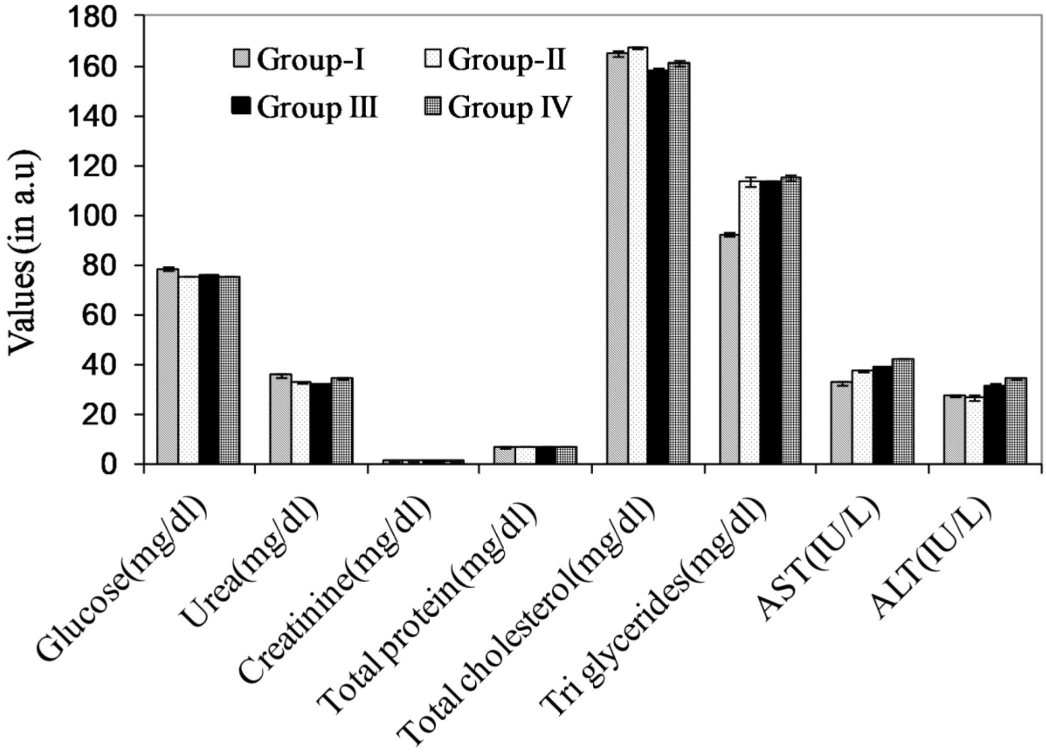
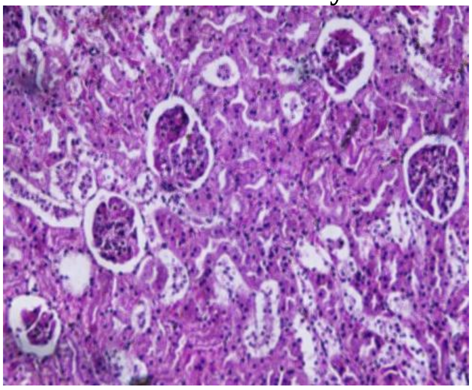


Figure 5.3. Comparison of blood biochemical parameters between the treated and untreated groups of animals with the administration of boiled tuber extract.

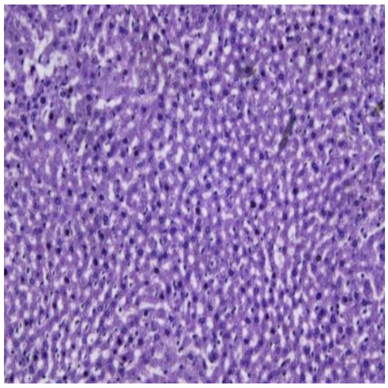
5.3.2.2. Histopathological studies

Effect of aqueous extract of both raw and boiled *D. bulbifera* tuber on the histological changes of kidney and liver tissues after 45 days of the treatment has been shown below. The treatment with daily doses of 200/2000, 400/4000 and 800/8000 mg/kg body weight for 45 days failed to reveal any major changes as compared to the control group. Necrosis, infiltration, oedema and conjunction, which are sign of hepatotoxicity, were not observed in the liver cells of the experimental group. The liver showed normal hepatic lobular architecture. The kidneys revealed normal glomeruli, proximal and distal tubules, interstitium, and blood vessels. The histopathological images of kidney and liver of control and treated with different doses are shown in Figure 5.4 and 5.5.

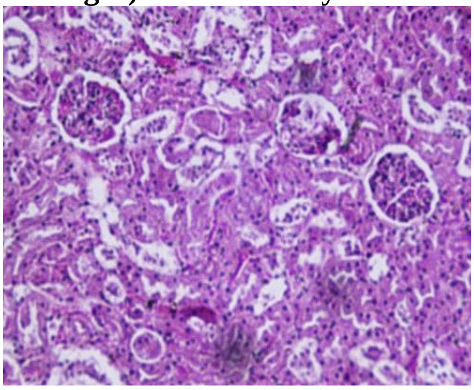
A. Control T.S. of Kidney



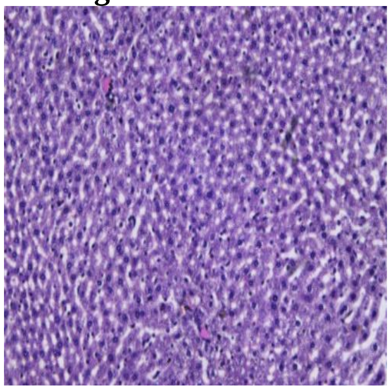
B. Control T.S. of liver



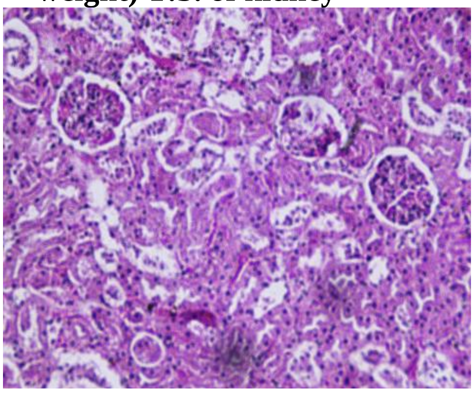
C. Treated (200 mg/kg body weight) T.S. of kidney



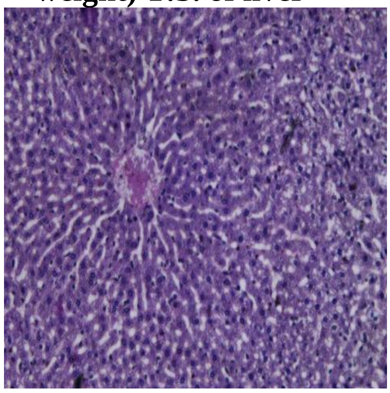
D. Treated (200 mg/kg body weight T.S. of liver



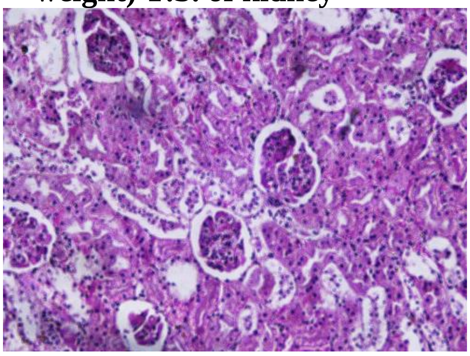
E. Treated (400 mg/kg body weight) T.S. of kidney



F.Treated (400 mg/kg body weight) T.S. of liver



G. Treated (800 mg/kg body weight) T.S. of kidney



H.Treated (800 mg/kg body weight) T.S. of liver

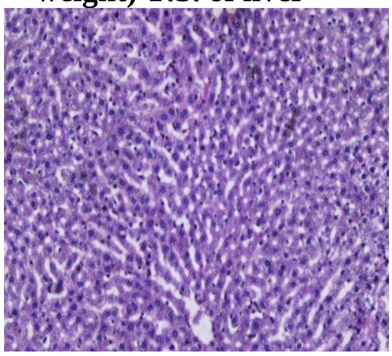


Figure 5.4. Panels represent H&E staining of paraffin-embedded 5 micron-thick sections of the kidney and liver at magnifications 200x of control and treated animals with increasing dose of raw *D. bulbifera* tuber aqueous extract. The liver showed normal hepatic lobular architecture. The kidneys revealed normal glomeruli, proximal and distal tubules, interstitium, and blood vessels.

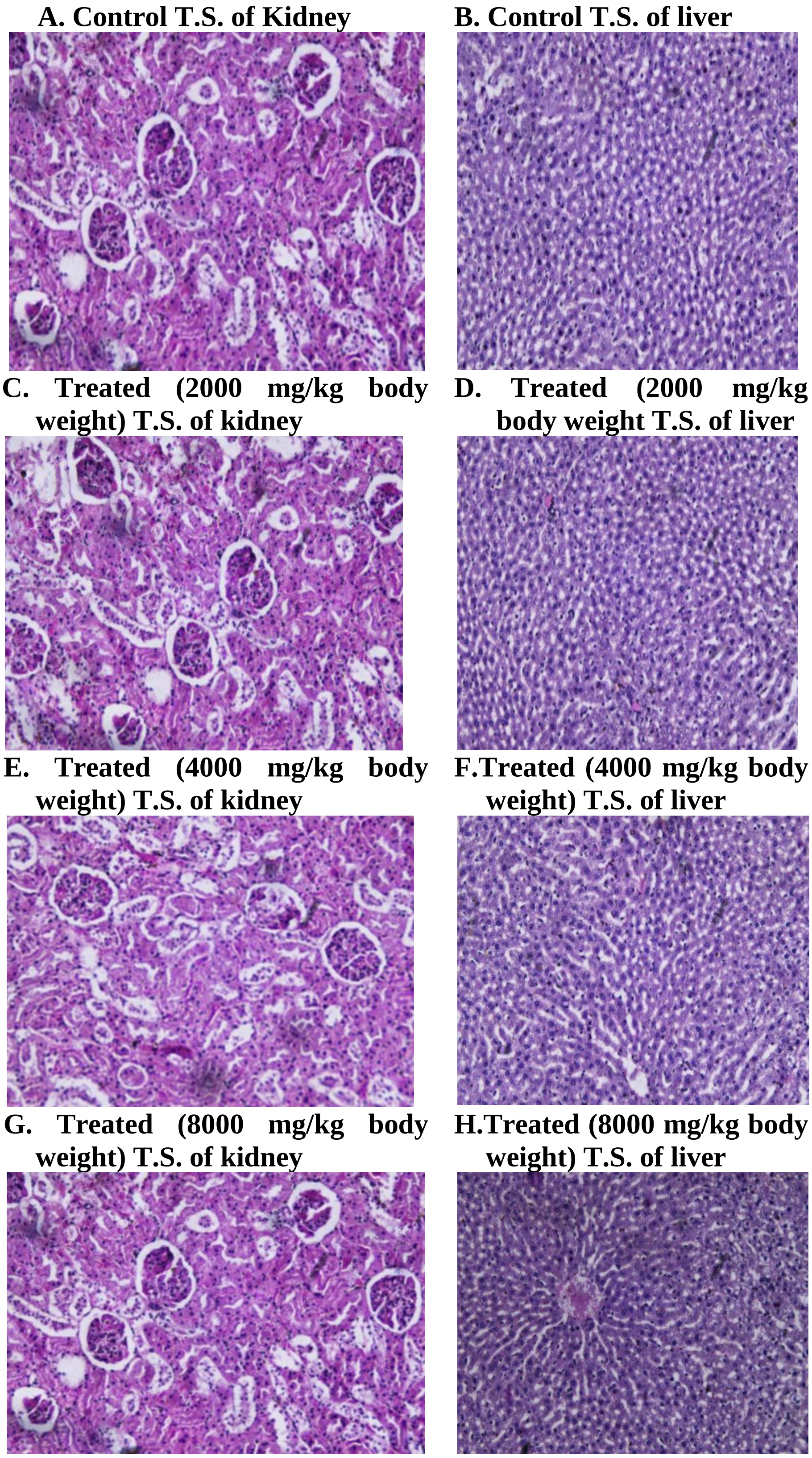


Figure 5.5. Panels represent H&E staining of paraffin-embedded 5 micron-thick sections of the kidney and liver at magnifications 200x of control and treated animals with increasing dose of boiled *D. bulbifera* tuber aqueous extract. The liver showed normal hepatic lobular architecture. The kidneys revealed normal glomeruli, proximal and distal tubules, interstitium, and blood vessels.

5.4. Conclusion

All the biochemical parameters were found to be within the normal range in all the treated groups and also there were no changes in the structure of the kidney and liver in all the treated groups. On the whole, it seems that the consumption of both raw and boiled *D.bulbifera* did not create any kind of injury.

CHAPTER VI

PRODUCT DEVELOPMENT FROM BOILED DIOSCOREA BULBIFERA TUBER FLOUR

6.1. Introduction

Cookies and cakes are ready-to-eat, convenient food products. Cookies are produced from unpalatable dough that is transformed into a light porous, readily digestible and appetizing product through heat. The principal ingredients are wheat, fat, sugar, water while other optional ingredients include milk, salt, flavouring agent, aerating agent and other food additives. They can be served with soft drinks or tea and taken between meals like any other snacks (Bansod *et al*., 2020). Cakes are sweet and often baked, usually prepared from wheat flour, sugar, shortening, baking powder and egg as principal ingredients (Olatunde *et al*., 2019). The cake is becoming a popular delicacy during celebration periods among little children and adult people (Olatunde *et al*., 2019). Papad is a popular and tasty food item in the Indian diet for many centuries. Papad is a traditional food item having a thin-cripsy wafer-like texture which is consumed as an accompaniment along with the meals and snacks (Bukya *et al*., 2018). The papad is generally prepared using cereal, legumes flour, or potato sago with minor quantities of spice, vegetable oil, salt and alkaline additive. It is widely consumed in India as an adjunct after frying or roasting (Gupta and Singh, 2019). There is an increasing trend among consumers for functional food products. This has greatly influenced the use of composite flours in which flours from locally grown crops replace a portion of wheat flour for use in snack production (Olatunde *et al*., 2019). Composite flour can be described as a mixture of several flours obtained from root, tuber, cereal and legume with or without the addition of wheat flour, which is created to satisfy specific functional characteristics and nutrient composition (Bansod *et al*., 2020). The use of composite flour promotes the use of locally available crops and also increases the nutritional content of food products. *Dioscorea bulbifera* is a good source of both macro and micronutrients still its utilization is limited. Therefore here an attempt has been taken to made cake, cookies and papad from composite flour containing *Dioscorea bulbifera* flour. Successful production of cake, cookies and papad from *Dioscorea bulbifera* composite flour will encourage improved cultivation of this crop as well as enhance the economic value of the crop.

6.2. Materials and methods

6.2.1. Preparation of composite flour

The flour was prepared from the boiled tuber of *Dioscorea bulbifera* flour (DBF) and wheat flour (WF). Different percentages (25%, 50% and 75%) of DBF and WF (75%, 50% and 25%) mixed well to make cake and cookies. Similaly different percentages of DBF (25%, 50% and 75%) and sagu (75%, 50% and 25%) combined to make papad. The outline for preparing the composite flour is mentioned in Figure 6.1.

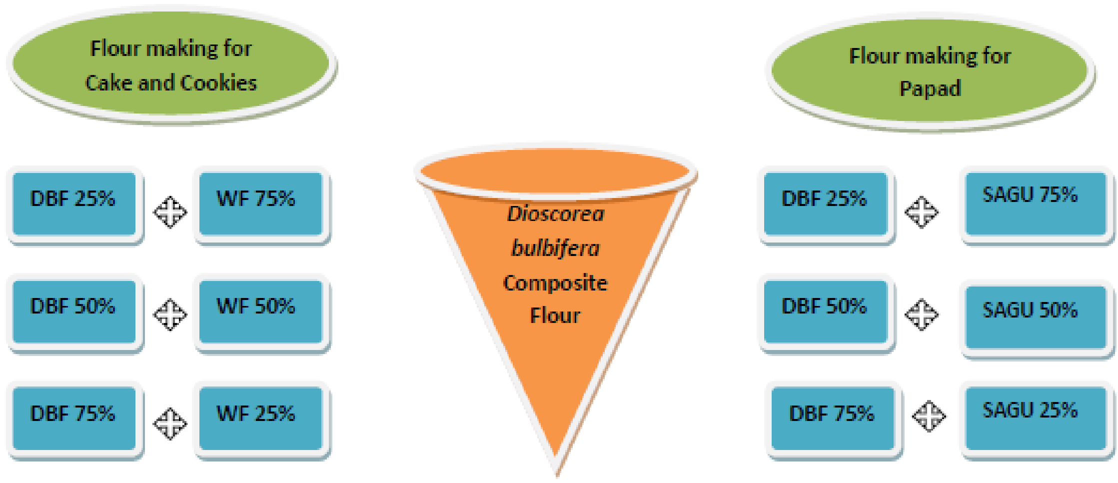


Figure 6.1. The flow chart demonstrates the preparation of composite flour of *Dioscorea bulbifera* tuber.

6.2.2. Determination of functional parameters of flours

6.2.2.1. Bulk density

The gravimetric method of Okezie *et al*. (1988) was used for the determination of bulk density of flour samples. Both loose volume and packed volume were recorded. About 10 g flour sample was taken in a 25 ml of measuring cylinder and volume was recorded as loose volume. The cylinder was tapped on a laboratory bench repeatedly until the constant volume was observed, which was recorded as packed volume. The ratio of sample weight to the volume of the sample before and after tapping was mentioned as loose bulk density (LBD) and packed bulk density (PBD).

6.2.2.2. Water and oil absorption capacity

Flour samples were analyzed for water and oil absorption capacity following the standard method (Abbey and Ibeh, 1988). A weight of 10% w/w of the sample was thoroughly mixed with water/oil using a warring mixer for 30s. After mixing at room temperature it was allowed to stand for 30 min then centrifuged at 5000 rpm for 30 min. The supernatant was directly read from the graduated centrifuge tube. The volume of water/oil absorbed by flours was converted to weight by multiplication of density of water (1 g/ml) and soyabean oil (0.924 g/ml). The water and oil absorption capacities (WAC/OAC) were expressed in grams of water/oil absorbed per gram of flour sample.

6.2.2.3. Emulsion activity

The emulsion activity of the flour sample was carried out following the standard method with slight modification (Okezie *et al*., 1988). At room temperature, 2 g of flour sample was blended with 25 ml of distilled water for 30s using a warring blender at 1600 rpm. About 25 ml soya oil was added to this gradually after complete dispersion and it was blended again for 30s. The blend was centrifuged for 5 min at 1600 rpm. After centrifugation separate volume of oil was read directly from the tube. Emulsion activity was calculated as the ratio of the height of emulsion to the total height of flour and was expressed as a percentage.

6.2.2.4. Cake, cookies and papad making process

Cake and cookies were prepared from composite flours of 25%, 50% and 75% DBF substituted to WF. Ingredients used for cake making were dry milk powder, refined oil, sugar, leavening agent and composite flours (Table 6.1). Cookies dough was made from butter, leavening agent, sugar and composite flours (Table 6.2). Small round shape cookies were prepared from the dough and backed in OTG for 20 minutes. For the cake the dough were backed in the baking oven. Besides, 100% WF made cake and cookies were taken as control. Papad was prepared from composite flour containing sagu and Dbf. Powered sagu was poured in hot water followed by the addition of Dbf, spices and salt (Table 6.3). The mixture was stirred to form a thick batter which was spread in papad like shapes and dried. The protocols used for making of cakes, cookies and papads are included in Figure 6.2, 6.3 and 6.4, respectively.

Table 6.1. Composition of cakes.

Ingredients	C0(1)	C1(2)	C2(3)	C3(4)
DBF	0gm	25gm	50gm	75gm
WF	100gm	75gm	50gm	25gm
Milk powder	25gm	25gm	25gm	25gm
Refined oil	25gm	25gm	25gm	25gm
Sugar	25gm	25gm	25gm	25gm
Leavening agent	2gm	2gm	2gm	2gm
Pineapple essence	5drops	5drops	5drops	5drops

Table 6.2. Composition of cookies.

Ingredients	CO0(5)	CO1(6)	CO2(7)	CO3(8)
DBF	0gm	25gm	50gm	75gm
WF	100gm	75gm	50gm	25gm
Butter	25gm	25gm	25gm	25gm
Sugar	25gm	25gm	25gm	25gm
Leavening agent	1gm	1gm	1gm	1gm

Table 6.3. Composition of papads.

Ingredients	P0(9)	P1(10)	P2(11)	P3(12)
DBF	0gm	25gm	50gm	75gm
Sagu	100gm	75gm	50gm	25gm
Salt	1tea spoon	1tea spoon	1tea spoon	1tea spoon
Asafoetida	0.25gm	0.25gm	0.25gm	0.25gm
Cumin seed	1gm	1gm	1gm	1gm
Black pepper	1gm	1gm	1gm	1gm

Composite flour was taken in a bowl

↓

Milk powder was mixed with a little amount of hot water in a cup

↓

Milk powder solution, refined oil, sugar, leavening agent, essence were added to the composite flour

↓

All the ingredients were mixed homogenously by blender

↓

Cake batter was prepared

↓

The batter was poured in cake making plate

↓

Cakes were baked in oven for ½ hour

↓

Cakes were prepared

↓

(C_0)

(C_1)

(C_2)

(C_3)

Figure 6.2. Flow chart for making of cakes.

Composite flour was taken in a bowl

↓

Butter, sugar, leavening agents were added to composite flour

↓

All the ingredients were mixed properly

↓

Dough was prepared from mixed ingredients

↓

Small round shaped cookies were made from dough

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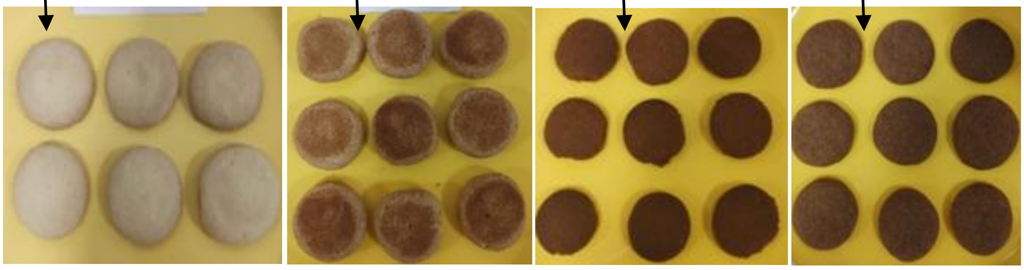
All cookies were baked in OTG for 20minutes at 120 ^{0}C

↓

Cookies were prepared

↓



(CO_0) **(CO_1)** **(CO_2)** **(CO_3)**

Figure 6.3 Flow chart for making of cookies.

Sagu was taken in a vessel

↓

Sagu was boiled for 10minutes

↓

During boiling Dbf was added slowly with continuous stirring

↓

Salt, cumin seed, asafetida, black peppers were added to this

↓

All the ingredients were mixed properly

↓

It was cooked until a thick consistency was not obtained

↓

Batter was applied in small round shaped on white cotton cloth

↓

Papads were prepared

↓

Figure 6.4. Flow chat for making of papads.

6.2.3. Analysis of products

6.2.3.1. Sensory evaluation of products

Cakes, cookies and papads made from *D. bulbifera* flour were evaluated for sensory parameters like appearance, flavor, taste, texture and overall acceptability by 30 untrained panelists. The evaluation process was based on a nine-point hedonic scale where 1 stands for dislike extremely and 9 for like extremely. Unsalted crackers and distill water were provided to the panelist to refresh their palate after tasting each sample.

6.2.3.2. Proximate analysis of products

Proximate compositions of products were analyzed by methods described in sections 2.2.4.1(Moisture), 2.2.4.2(Ash), 2.2.4.3(Carbohydrate), 2.2.4.6(Protein), 2.2.4.9(Fat). The fiber content of products was estimated by AOAC, 985.29(2016) standard method.

6.3. Result and discussion

6.3.1. Functional properties of Flours

Functional properties of flours are estimated to assume the behavior of flour constituents in products. Functional properties including loose bulk density (LBD), packed bulk density (PBD), water absorption capacity (WAC), oil absorption capacity (OAC) and emulsion stability (ES) are compared in between wheat flour and three composite flours having the ratio of 25%, 50% and 75% of *Dioscorea bulbifera* flour with wheat flour. The functional properties of flours were shown in Table 6.4. Loose bulk density (LBD) and packed bulk density (PBD) were found lowest in wheat flour (0.38 g/ml and 0.66 g/ml) while highest in *Dioscorea bulbifera* flour (0.51 g/ml and 0.78 g/ml). There was no significant difference in PBD between composite flours of (25%Dbf+75%Wf), (50%Dbf+50%Wf) and (75%Dbf+25%Wf). While LBD of (100% wf), (25% Dbf+75%wf), (50%Dbf+50%wf), (75%Dbf+25%wf) and (100% Dbf) were found to be 0.38, 0.44, 0.46, 0.49 and 0.51, respectively. The bulk density of flour depends on moisture content and particle size of flour. Bulk density refers to the porosity of flour. Flour having a high bulk density value may be used as thickeners in a food product. In contrast, the low density of flour is suitable for the making of nutrient-rich weaning food (Adebowale *et al*., 2012). Sanni *et al.* (2020) reported a higher value of LBD and PBD of wheat flour than the present findings. Amandikwa *et al.* (2015) reported a lower value of LBD and PBD for dbf than present findings while Ojinnaka *et al.* (2016) reported a range of 0.573-0.613 g/cm^3 of bulk density for two cultivars of *Dioscorea bulbifera* flour. The oil absorption capacity (OAC) of 100%Wf and

25%Dbf+75%Wf composite flour received similar value (0.92 ml/g), while the composite flour consisting of 50%Dbf+50%Wf and 75%Dbf+25%Wf obtained related OAC value (1.84 ml/g). Sanni *et al.* (2020) found a very similar OAC value for the wheat flour (0.96 ml/g), while Amandikwa *et al.* (2015) found a lower value of OAC (0.88 ml/g) for wheat flour but Toan *et al.* (2018) reported much higher value of OAC for wheat flour than present findings. Amandikwa *et al.* (2015) reported a higher value of water absorption capacity (WAC) for DBF than the present findings. DBF had lowest emulsion stability (ES) value (42.10%), while the composite flour (75%Dbf+25%Wf) obtained highest ES value (57.37%). There was no significant difference between ES value of 100%wf and 50%Dbf+50Wf.

Table 6.4. Functional properties of flours.

Sample	LBD(g/ml)	PBD(g/ml)	OAC(ml/g)	WAC(ml/g)	ES(%)
1(100% Wf)	$0.38±0.002^{a}$	$0.66±0.007^{a}$	$0.92±0.007^{ab}$	$1.42±0.07^{a}$	$54.01±0.6^{bc}$
2(25%Dbf+75%Wf)	$0.44±0.01^{b}$	$0.72±0.01^{bcd}$	$0.92±0.005^{ab}$	$1.80±0.09^{b}$	$56.01±0.76^{d}$
3(50%Dbf+50%Wf)	$0.46±0.01^{c}$	$0.71±0.004^{bcd}$	$1.84±0.004^{de}$	$2.46±0.05^{c}$	$53.72±0.84^{bc}$
4(75%Dbf+25%Wf)	$0.49±0.01^{de}$	$0.70±0.005^{bcd}$	$1.84±0.007^{de}$	$2.93±0.05^{d}$	$57.37±0.9^{e}$
5(100% Dbf)	$0.51±0.01^{de}$	$0.78±0.03^{e}$	$1.09±0.008^{c}$	$3.10±0.005^{e}$	$42.10±0.01^{a}$

The values are mean ± SD. The identical superscripts within the same column are not significantly different ($P \leq 0.05$). Wf, Wheat flour, Dbf, *D. bulbifera* flour.

6.3.2. Proximate composition of Cookies and Cake

6.3.2.1. Moisture content

The incorporation of *D.bulbifera* flour (Dbf) with wheat flour (Wf) significantly increased the moisture content of composite flours. CO3 (75%Dbf+25%Wf) contained highest moisture content (18.95%) followed by CO2 (50%Dbf+50%Wf) having moisture content 15.35%, CO1 (25%Dbf+75% Wf) with moisture content 11.21% and CO0 (100%Wf) with moisture content 8.03% (Table 6.5).Moisture content increased significantly with an increase in the level of Dbf in cake formulation in which C3(75%Dbf+25%Wf) has the maximum moisture content of 30.37%, followed by C2 (50%Dbf+50%Wf) with moisture content 22.72, C1 (25%Dbf+75% Wf) with moisture content 16.71 and C0 (100%Wf) with moisture content 10.40 (Table 6.5). The moisture content of food products is a prime aspect concerning the storage potential of the product. The lower the moisture content the better is the shelf life of the product (Sanni *et al*., 2006). The moisture content of wheat-made cookies was found to be 8.03% in the present study. Previously Sanful and Essuman (2016) reported a lower value of moisture content (4.82% and 5.5%) than the present findings for wheat cookies and biscuits. In contrast, the moisture content of composite flour-made cookies and cakes ranged between 11.21-18.95% and 10.40-30.37%, respectively which was higher than the previous findings (Sanful and Essuman, 2016; Nwosu, 2014). This variation is may be due to the use of different making procedures. In this study moisture content gradually increases with an increase in substitution of Dbf to Wf in both cookies and cakes formulations. Enhancement of moisture content with the increased level of Dbf in wheat:Dbf cookies, wheat:Dbf bread, and wheat: sweet potato cake were also reported earlier (Bansod *et al*.,2020; Amandikwa, 2015; Okorie and Onyeneke, 2012). Olatunde *et al.* (2019) and Sanni *et al.*,(2020) reported a decrease of moisture content with an increased level of pigeon pea:sweet potato and sorrel seed protein isolate:yellow cassava flour substitution to wheat flour in cake and cookies formulations, respectively. But Srivastava *et al.*(2012) found no significant difference in moisture content in biscuits due to the incorporation of sweat potato flour in treatment.

6.3.2.2. Ash content

Ash content was enhanced with an increase in the percentage of Dbf in composite flour such as CO3 (3.51%) > CO_2 (3.45%) > CO1 (3.36%) > CO0 (3.24%) (Table 6.5). Inclusion of Dbf with wheat flour showed an appreciable enhancement of ash in cakes such as C3 (3.2%) > C2 (3.12%) > C1 (3.06%) > C0 (2.84%) (Table 6.5). Ash content represents mineral constituents of products after the destruction of organic matter. Ash content of wheat cookies and cake was found to be 3.24% and 2.84%, respectively. Whereas ash content of Wheat-Dbf cookies and cakes ranged between 3.36-3.51% and 3.06-3.2%, respectively. The ash content increased gradually with the increased level of Dbf in the formulation in both cookies and cake. This corroborates the earlier findings for wheat-Dbf cookies and wheat-sorrel seed protein isolate-yellow cassava composite flour made cookies (Bansod *et al*., 2020; Sanni *et al*., 2020). An increase of ash content with an increased percentage of sweet potato flour in cake formulation was also reported (Okorie and Onyeneke, 2012; Olatunde *et al*., 2019). In contrast, Oluwamukomi *et al.* (2011) observed substitution of *Dioscorea bulbifera* flour and cassava flour to wheat flour decrease the ash content in cookies and biscuit.

6.3.2.3. Fiber content

Generally, cookies are poor sources of fiber but the addition of Dbf with wheat flour improves the fiber content of cookies. Fiber content also gradually increased with an increase in substitution of Dbf to wheat flour such as CO3 (0.28%) > CO2 (0.24%) > CO1 (0.20%) > CO0 (0.09%) (Table 6.5). Similarly fiber content of cakes considerably improved with increased percentage of Dbf in formulation such as C3 (0.32%) > C2 (0.31%) > C1 (0.29%) > C0 (0.11%) (Table 6.5). Cookies and cake were found to attain a very low amount of fiber content (0.09-0.32). The lowest amount of fiber was found in wheat cookies and cake. Increased level of Dbf in formulation results in the enhancement of fiber in both cookies and cake. Fiber content reported by Bansod *et al.* (2020) supports the present findings. Whereas, Sanful and Essuman (2016) found to be no significant variation in fiber content in cookies due to the addition of Dbf in the formulation. However, the whole wheat cake had the lowest fiber content, while fiber content increased in composite flour made cakes with the increase in the level of pigeon pea and sweet potato in formulations (Olatunde *et al*., 2019).

6.3.2.4. Fat content

The highest amount of fat was found in wheat flour cookies which was gradually declined at 25%, 50%, and 75% Dbf substituted cookies (CO0 (24.37%) > CO1 (22.97%) > CO2 (21.90%) > CO3 (19.40%) (Table 6.5). This might be due to Dbf being a poor source of fat. Wheat flour cake contained a significantly higher amount of fat than composite flour-made cakes (C0 (19.19%) > C1 (18.56%) > C2 (18.14%) > C3 (17.41%). Table 6.5 represents the proximate composition of the cake. The fat content of 100% wheat cookies and cake was found to be 24.37% and 19.19%, respectively. Bansod *et al.* (2020) reported a lower value (19.98%) while Sanni *et al.* (2020) found a higher quantity (26.67%) of fat for wheat cookies in comparison to the present findings. The inclusion of Dbf in the increased ratio in cookies and cake formulation caused the degradation of fat content gradually. In contrast, Bansod *et al.* (2020) reported an increase of fat with an increased proportion of Dbf in the formulation. Okorie and Onyeneke (2012) also found enhancement of fat content with an increasing percentage of sweet potato flour in the formulation of wheat-sweet potato cake. However, Oluwamukomi *et al.*(2011) did not find any significant difference in fat content in wheat-cassava flour composite biscuits. At the same time, Olatunde *et al.*(2019) found a significant difference in the fat value of cakes made by wheat-pigeon pea-sweet potato composite flours.

6.3.2.5. Carbohydrate content

The Carbohydrate content of cookies decreased gradually with an increased level of Dbf in cookies. Carbohydrate content was ranged between 51.08%-55.15%. Addition of Dbf at the rate of 25%, 50% and 75% with wheat flour gradually decrease carbohydrate content of cakes (C0 (58.62%) > C1 (53.37%) > C2 (48.66%) > C3 (42.84%) (Table 6.5). The carbohydrate content of cookies and cakes was ranged between 51.08-55.15% and 42.84-58.62%, respectively. Increased proportion of Dbf in formulation resulted in a reduction of total carbohydrate both in cookies and cakes. This conforms with the findings of Sanful and Essuman (2016). Sanni *et al.* (2020) found a decrease in carbohydrate content with increasing substitution of wheat flour with sorrel protein isolate and yellow cassava flour in cookies. Okorie and Onyeneke (2012) also found the same in the case of wheat-sweet potato blend cakes. In contrast, Olatunde *et al.* (2019) found a higher quantity of carbohydrates in a cake made from composite flour containing wheat, pigeon pea, and sweet potato than wheat-made cake.

6.3.2.6. Protein content

The protein content of cookies decreased gradually with an increase level of Dbf in cookies (Table 6.5). Protein content ranged in between 6.78%-9.12%. Protein content in Dbf substituted cakes gradually decreases with the increase in the ratio of Dbf in formulation (C0 (8.84%) > C1 (8.01%) > C2 (7.05%) > C3 (5.86%) (Table 6.5). The protein content of cookies and cakes ranged between 6.78-9.12% and 5.86-8.84%, respectively. Similarly, the protein content for wheat-Dbf flour made cookies and biscuits were reported between 7.13%-9.15%, 8.02-9.29% and 7.74-9.51% (Nwosu and Justina, 2014; Sanful and Essuman, 2016; Bansod *et al*., 2020). The increase in Dbf in formulation caused a decrease in protein content in both cookies and cakes. This was supported by Bansod *et al.* (2020). Okorie and Onyeneke (2012) also found a decrease in protein content of the wheat:sweet potato composite flour made cakes with an increase in potato flour. Oluwamukomi *et al.* (2011) also reported that an increase in the level of cassava flours with wheat flour decreases the protein content in biscuits. Sanful and Essuman (2016) found an increase in protein content with an increase in Dbf in the baked cookies.

Table 6.5. Proximate content of cookies and cakes.

Sample	Moisture (%)	Ash (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Protein (%)
(a) Cookies						
CO0	8.03± 0.01[a]	3.24± 0.03[a]	24.37± 0.24[d]	0.09± 0.01[a]	55.15± 0.35[d]	9.12± 0.008[d]
CO1	11.21± 0.007[b]	3.36± 0.02[b]	22.97± 0.007[c]	0.20± 0.008[b]	54.32± 0.01[c]	7.94± 0.02[c]
CO2	15.35± 0.009[c]	3.45± 0.04[c]	21.90± 0.08[b]	0.24± 0.005[c]	52.05± 0.02[b]	7.01± 0.01[b]
CO3	18.95[d]± 0.009[d]	3.51± 0.06[d]	19.40± 0.22[a]	0.28± 0.008[d]	51.08± 0.02[a]	6.78± 0.14[a]

(b) Cakes						
C0	10.40± 0.01[a]	2.84± 0.11[a]	19.19± 0.36[d]	0.11± 0.005[a]	58.62± 0.41[d]	8.84± 0.09[d]
C1	16.71± 0.02[b]	3.06± 0.10[bcd]	18.56± 0.26[c]	0.29± 0.01[b]	53.37± 0.58[c]	8.01± 0.26[c]
C2	22.72± 0.009[c]	3.12± 0.10[bcd]	18.14± 0.14[b]	0.31± 0.007[c]	48.66± 1.68[b]	7.05± 0.02[b]
C3	30.37± 0.01[d]	3.2± 0.11[bcd]	17.41± 0.17[a]	0.32± 0.008[d]	42.84± 0.10[a]	5.86± 0.05[a]

The values are mean±SD. The values bearing the same superscript within the same column are not significantly different (at P≤0.05). CO0 = 100% Wf, CO1=25%Dbf+75% WF, CO2=50%Dbf+50%Wf, CO3=75%Dbf+25%Wf. C0=100%Wf, C1=25%Dbf +75% Wf, C2=50%Dbf+50%Wf, C3=75%Dbf+25%Wf.

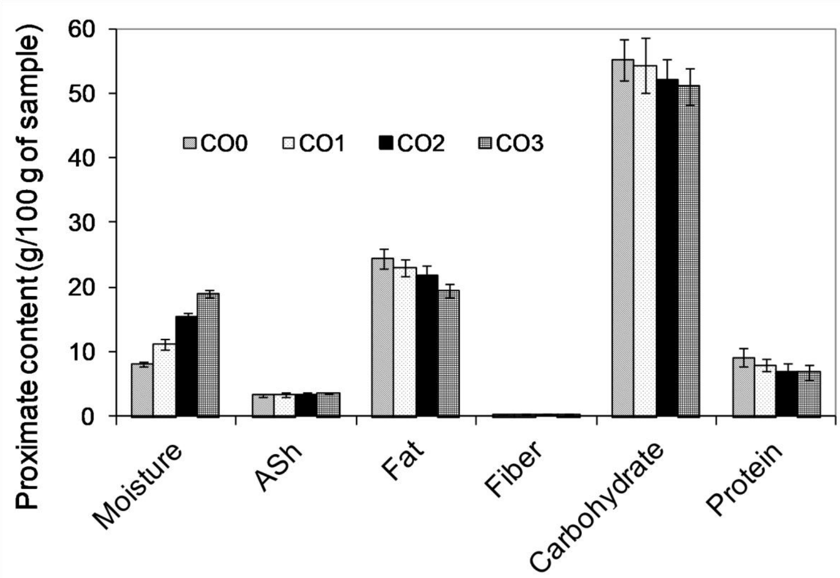


Figure 6.5. Proximate composition of cookies.

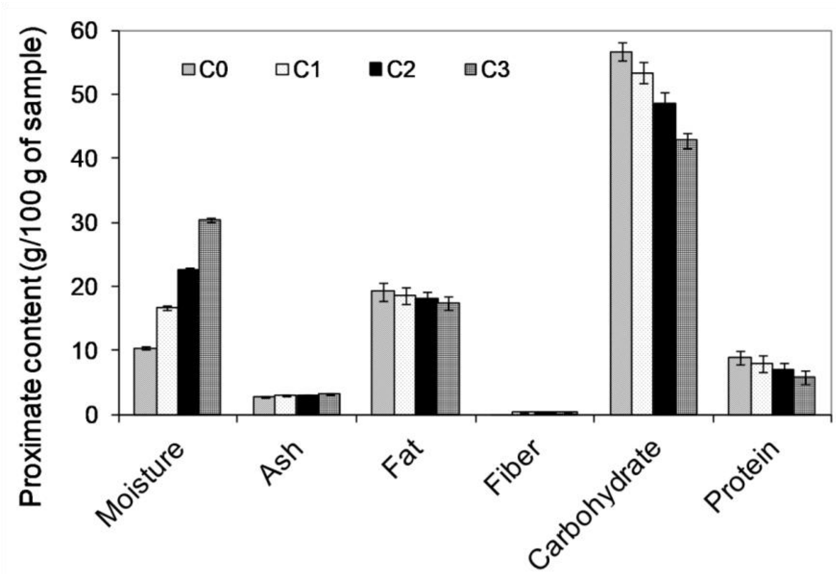


Figure 6.6. Proximate composition of cakes.

6.3.3. Sensory characteristics of cookies and cake sample

6.3.3.1. Appearance

The appearance of Ddf substituted cookies was found to be superior to wheat flour cookies**.** Cookies with 50% substituted Dbf scored highest (8.40) for appearance followed by substitution with 75% Dbf (7.93), 25% Dbf (6.93) and wheat flour cookies (6.80) (Table 6.6). Similarly, Dbf substituted cakes scored significantly higher scores compared to 100% wheat flour cake in appearance (Table 6.6). Cakes (C2) with 50% substituted Dbf scored the highest score for appearance compared to cakes (C3) with 75% substituted Dbf. The acceptability of a product directly depends on its appearance. Thus, cookies and cakes with 50% substituted Dbf is more acceptable. This finding was corroborated with the previous report for wheat-Dbf cookies and wheat-sweet potato cake (Bansod *et al*., 2020; Okorie and Onyeneke, 2012). In contrast, Sanful and Essuman (2016) reported that the addition of Dbf does not significantly affect the product appearance. Nwosu and Justina (2014) found the highest score of appearance for wheat flour biscuit which was reduced due to the addition of Dbf in the formulation. Etudaiye *et al.* (2011) also found a reduction of colour when the substitution level of cassava flour and sweet potato flour to wheat flour increases in biscuit and cake formulation.

6.3.3.2. Taste

Taste of 25% Dbf substituted cookies was not found statistically different from wheat flour cookies (Table 6.6). At the same time, 50% and 75% Dbf substituted cookies obtained statistically similar scores for taste. Taste of the cake notably increased due to the addition of Dbf in the formulation. Wheat flour cake had the lowest score (3.33) for taste, while taste of 25%, 50% and 75% Dbf substituted cakes were not statistically different. Increased percentage of Dbf in product formulation significantly increased the taste of both cookies and cakes. Wheat flour cookies and cakes obtained the lowest score, while 50% Dbf substituted cookies and cakes obtained the highest score for taste by panelists. Bansod *et al.* (2020) reported enhancement of taste due to the addition of an increased level of Dbf in cookies formulation. Sanful and Essuman (2016) also found a statistically similar score of taste for wheat flour cookies, 50% and 80% Dbf substituted cookies. Nwosu and Justina (2014) reported the highest score of taste for wheat flour cookies, while 10% and 20% Dbf substituted cookies obtained closest score to the wheat flour cookies. Etudaiye *et al.* (2016) found sweet potato and cassava flour substituted cake and biscuit had a lower score of taste in comparison of wheat cake and biscuit.

6.3.3.3. Texture

Wheat flour cookies had the lowest score (4.80) for texture. In contrast, the addition of Dbf in cookies formulation enhanced the texture of cookies. However, the addition of Dbf in formulation resulted in the decline of the texture of cakes (Table 6.6). Bansod *et al.* (2020) found enhancement of cookies texture, while Nwosu and Justina (2014) reported a decrease in cookies and biscuit texture due to the addition of Dbf in formulations. However, Sanni *et al.* (2020) found no significant difference in the texture of wheat and wheat:sorrel seed protein isolate: yellow cassava flour made cookies. Srivastava *et al.* (2012) found the highest score of texture for 40% sweet potato flour substituted biscuit in comparison to 20%, 40%, 60%, 80%, 100% sweet potato substituted biscuits and 100% wheat flour biscuit.

6.3.3.4. Flavour

Flavour of cookies made up of 100% wheat flour as well as 25% and 50% substituted Dbf was not found significantly different. In contrast, cookies made up of 50% substituted Dbf has the highest score for flavor (8.33) by the panelist (Table 6.6). The score for cookies greatly increased due to the addition of Dbf in the formulation. Bansod *et al.* (2020) reported that the flavour of cookies gradually increased after substitution of 20%-60% of Dbf with wheat flour, while substitution of 80% Dbf degrades the flavor of cookies. Sanful and Essuman (2016) found a score of 7.15-7.88 for the flavour of cookies made up of 50%-100% substituted Dbf. In the present study, a score of 6.93-8.26 for the flavour for both cookies and cakes was recorded. Nwosu and Justina (2014) reported a decrease in flavor for the biscuits prepared with increasing substitution of Dbf.

6.3.3.5. Overall acceptability

The cookies prepared with 50% substitution of Dbf were highly accepted in respect to appearance, taste, texture and flavor (Table 6.6)**.** The scores of overall acceptability for cookies were 8.46, 7.95, 7.03 and 6.11 for 50%, 75%, 25% substituted Dbf. Similarly, the cake with 50% substituted Dbf had the highest overall acceptability score (8.15) compared to 25%, 75% substituted Dbf and 100% wheat flour. Overall acceptabilities of wheat flour made cookies and cakes were found to be lowest in comparison to wheat:Dbf composite flours. Bansod *et al.,* (2020) reported that cookies made up of 20%-60% substitution of Dbf increase the acceptance in comparison to wheat flour cookies but substitution of 80% Dbf decreased the acceptance score. Further, Nwosu and Justina (2014) found that biscuits prepared with 10%-50% substitution of Dbf were acceptable but above 50% substitution was not suitable for biscuits making.

6.3.4. Proximate compositions of papad

Results of the study revealed that the replacement of sagu with Dbf increased the moisture and fiber content of papad (Table 6.7). Enhancement of moisture may be due to the higher fiber content of Dbf. Protein and carbohydrate content gradually decreased with an increased level of Dbf to sagu. Protein content ranged between 6.22-9.45%, which was reasonable. In contrast, carbohydrate content was found to be 51.30-55.91%, which was much higher than other papads (Bukya *et al.*, 2018). There was no significant difference in fat and ash content of P2 and P3 at $p \leq 0.05$.

Table 6.6. Sensory characteristics of cookies and cakes prepared.

Sample	Appearance	Taste	Texture	Flavour	Overall Acceptability
(a) Cookies					
CO0 (100%)	6.80 ± 1.01^{ab}	5.66 ± 1.29^{ab}	4.80 ± 1.14^{a}	7.20 ± 1.14^{abc}	6.11 ± 0.58^{a}
CO1 (25Dbf:75Wf)	6.93 ± 0.88^{ab}	6.66 ± 1.63^{ab}	7.20 ± 1.20^{b}	7.33 ± 0.81^{abc}	7.03 ± 0.61^{b}
CO2 (50Dbf:50Wf)	8.40 ± 0.63^{cd}	8.33 ± 0.81^{cd}	8.80 ± 0.41^{cd}	8.33 ± 0.61^{cd}	8.46 ± 0.33^{d}
CO3 (75Dbf:25Wf)	7.93 ± 0.59^{cd}	7.93 ± 0.96^{cd}	8.13 ± 0.51^{cd}	7.80 ± 1.14^{abc}	7.95 ± 0.47^{c}
(b) Cakes					
C0 (100%Wf)	3.26 ± 1.09^{a}	3.33 ± 1.24^{a}	8.20 ± 0.77^{cd}	2.33 ± 1.23^{a}	4.28 ± 0.59^{a}
C1 (25Dbf:75Wf)	7.20 ± 0.77^{b}	7.66 ± 0.72^{bcd}	6.53 ± 0.83^{ab}	6.93 ± 1.03^{bc}	7.08 ± 0.45^{bc}
C2 (50Dbf:50Wf)	8.53 ± 0.51^{cd}	8.33 ± 0.81^{bcd}	7.46 ± 1.12^{bc}	8.26 ± 1.09^{cd}	8.15 ± 0.48^{d}
C3 (75Dbf:25Wf)	8.06 ± 0.96^{cd}	7.60 ± 0.82^{bcd}	6.60 ± 0.82^{bc}	7.80 ± 1.14^{cd}	7.51 ± 0.46^{bc}

The values are mean±SD. The values bearing same superscript within the same column are not significantly different ($P \leq 0.05$). Dbf: *D. bulbifera* tuber flour, Wf: Wheat flour

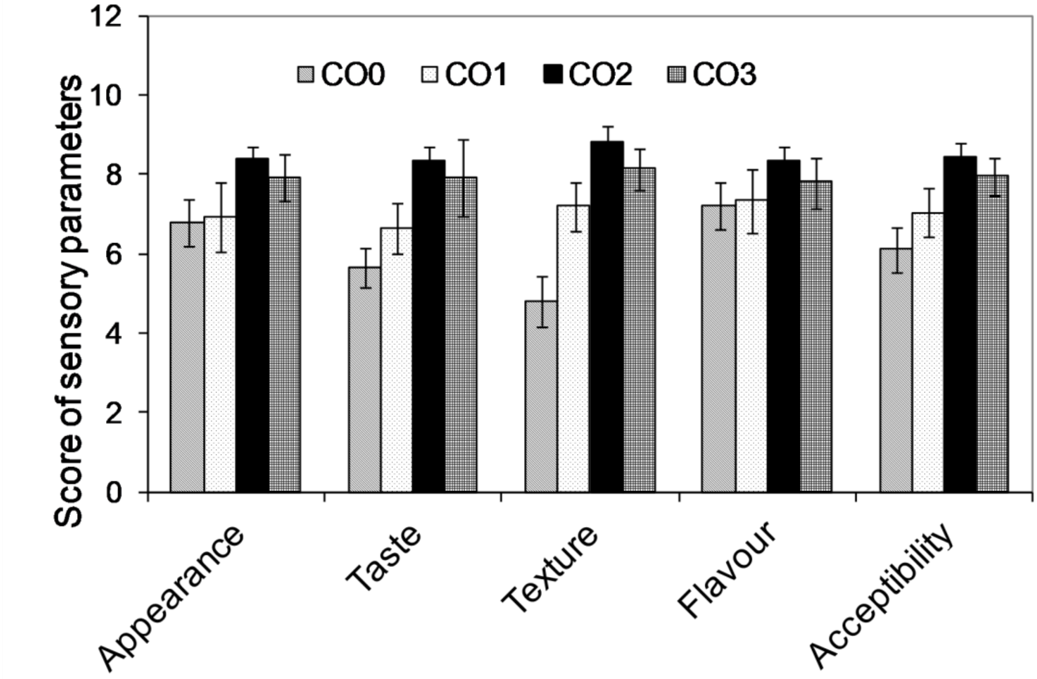


Figure 6.7. Sensory parameters of cookies made from composite flour of *D. bulbifera* tuber and wheat flour.

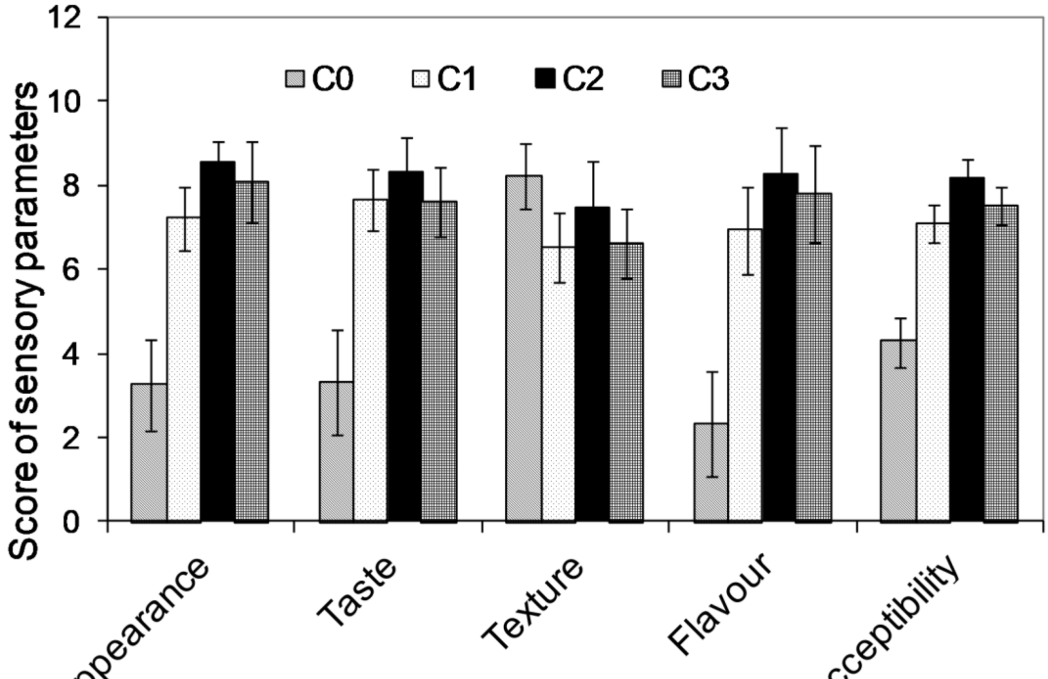


Figure 6.8. Sensory parameters of cakes made from composite flour of *D. bulbifera* tuber and wheat flour.

Table 6.7. Proximate composition of papad

Parameters	P0	P1	P2	P3
Moisture	13.26±0.04[a]	14.06±0.03[b]	15.06±0.01[c]	16.05±0.02[d]
Protein	9.45±0.24[d]	8. 35±0.24[c]	7.05±0.03[b]	6.22±0.16[a]
Carbohydrate	55.91±0.02[d]	54.04±0.28[c]	52.07±0.03[b]	51.30±0.48[a]
Fat	2.5±0.18[d]	2.08±0.03[c]	1.83±0.05[ab]	1.73±0.05[ab]
Ash	5.98±0.09[a]	6.26±0.09[b]	6.51±0.08[cd]	6.67±0.35[cd]
Fiber	10.24±0.005[a]	11.07±0.28[b]	12.03±0.009[c]	14.07±0.01[d]

The values are mean±SD. The values bearing the same superscript within the same row are not significantly different ($P \leq 0.05$).

6.3.5. Sensory properties of papad

Substitution of Dbf to sagu in different proportions in papad making improves the appearance of the product. There was no statistically significant difference in the appearance of P1 and P3, whereas, P2 secured the highest score for appearance. Substitution with Dbf (25%) was found to be most suitable for taste and flavor (Table 6.8). The score for texture of P1 was statistically not different from $P_{0.}$ However, 75% substitution was found to be least accepted with respect to texture. The papad, P1 has the highest score of overall acceptability. Therefore 25% substitution was most suitable compared to 50% and 75% substitution.

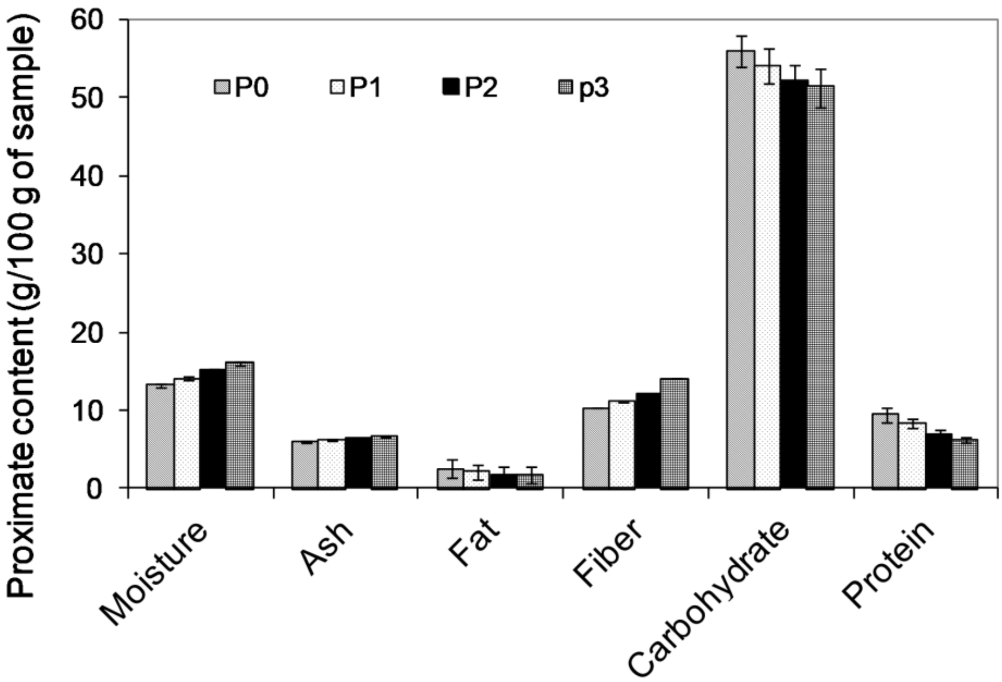


Figure 6.9. Proximate analysis of papad made from the composite flour of *D. bulbifera* tuber and sagu flour.

Table 6.8. Sensory characteristics of papad prepared.

Parameter	P_0	P_1	P_2	P_3
Appearance	$6.4±0.13^{a}$	$7.\ 18±0.008^{bd}$	$7.48±0.08^{c}$	$7.18±0.08^{bd}$
Taste	$8.01±0.008^{c}$	$8.23±0.09^{d}$	$7.78±0.08^{b}$	$7.58±0.08^{a}$
Flavour	$7.78±0.03^{c}$	$8.00±0.09^{d}$	$7.37±0.07^{b}$	$7.07±0.07^{a}$
Texture	$7.86±0.05^{cd}$	$7.83±0.05^{cd}$	$7.15±0.05^{b}$	$6.72±0.08^{a}$
OA	$7.51±0.03^{c}$	$7.81±0.04^{d}$	$7.45±0.02^{b}$	$7.14±0.04^{a}$

The values are mean±SD. The values bearing the same superscript within the same row are not significantly different ($P \leq 0.05$).

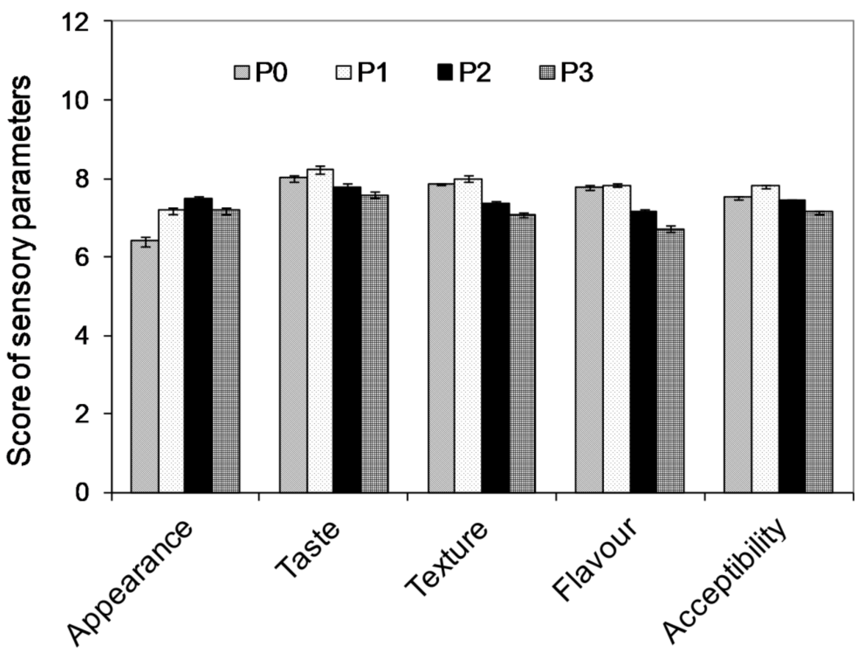


Figure 6.10. Sensory parameters of papad made from the composite flour of *D. bulbifera* tuber and sagu flour.

6.4. Conclusion

The study revealed that the replacement of wheat flour with Dbf significantly affects the nutritional and sensory parameters of cookies, cakes and papad. Moisture, ash and fiber content increases, while fat, carbohydrate and protein content decreases with the increased level of Dbf substitution to wheat flour in the formulation. Wheat cookies and cake obtained lowest score for all the sensory attributes in comparison to Dbf substituted cookies and cakes. Substitution of 50% Dbf with wheat flour was found to be the most acceptable one in terms of appearance, taste, flavor and texture followed by 75% substitution. According to the result of the present study Wheat-Dbf composite flour can be used to make nutritious and appealing cookies and cake. In the case of papad 25% level of substitution was most suitable concerning sensory and proximate parameters.

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APPENDIX

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
SCHOOL OF PHARMACEUTICAL SCIENCES
SIKSHA 'O' ANUSANDHAN (DEEMED TO BE UNIVERSITY)
(Declared U/S 3 of the UGC Act, 1956)
Bhubaneswar, Odisha, India
Regd. No. : 1171/PO/RE/S/08/CPCSEA

Date: 29/06/2019

CERTIFICATE

This is to certify that project entitled **"Phytochemicals, nutritional and pharmacological characterization of dioscorea bulbifera tuber."**bearing protocol number **IAEC/SPS/SOA/19/2019** has been approved in the Institutional Animal Ethics Committee meeting held on 29.06.2019.

Chairman
Prof. (Dr.) S.C.Si
Dean, SPS

Chairman
IAEC SPS, SOAU
Bhubaneswar-03

Member Secretary
Dr. Durga Madhab Kar
Professor & HOD
Dept. of Pharmacology, SPS

Member Secretary
IAEC, SPS, SOAU
Bhubaneswar-03

CPCSEA Nominee
Dr. Saurabh Chawla
Scientific Officer
School of Biological Sciences
NISER, Bhubaneswar.

Nominee, CPCSEA
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Bhubaneswar-03

Chapter V

1.Biochemical Parameters for raw aqueous *D.bulbifera* extract

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	168.000	28	6.000		
	Total	168.000	31			
glucose	Between Groups	2.952	3	.984	4.292	.013
	Within Groups	6.420	28	.229		
	Total	9.373	31			
urea	Between Groups	3.939	3	1.313	7.558	.001
	Within Groups	4.863	28	.174		
	Total	8.802	31			
cretinine	Between Groups	.009	3	.003	4.225	.014
	Within Groups	.020	28	.001		
	Total	.029	31			
protein	Between Groups	.041	3	.014	1.730	.184
	Within Groups	.224	28	.008		
	Total	.265	31			
cholesterol	Between Groups	.429	3	.143	6.632	.002
	Within Groups	.604	28	.022		
	Total	1.033	31			

2.Biochemical Parameters for raw aqueous *D.bulbifera* extract

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replica	Between Groups	.000	3	.000	.000	1.000
	Within Groups	168.000	28	6.000		
	Total	168.000	31			
Triglycerides	Between Groups	.436	3	.145	1.693	.191
	Within Groups	2.401	28	.086		
	Total	2.837	31			
AST	Between Groups	.269	3	.090	.546	.655
	Within Groups	4.606	28	.165		
	Total	4.876	31			
ALT	Between Groups	3.159	3	1.053	9.990	.000
	Within Groups	2.951	28	.105		
	Total	6.110	31			

3.Biochemical parameters of boiled *D.bulbifera* extract

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	168.000	28	6.000		
	Total	168.000	31			
glucose	Between Groups	70.839	3	23.613	382.579	.000
	Within Groups	1.728	28	.062		
	Total	72.567	31			
Urea	Between Groups	71.460	3	23.820	160.870	.000
	Within Groups	4.146	28	.148		
	Total	75.606	31			
Creatinine	Between Groups	.005	3	.002	1.515	.232
	Within Groups	.028	28	.001		
	Total	.033	31			
Protein	Between Groups	.590	3	.197	21.037	.000
	Within Groups	.262	28	.009		
	Total	.852	31			
Cholesterol	Between Groups	393.625	3	131.208	201.306	.000
	Within Groups	18.250	28	.652		
	Total	411.875	31			

4.Biochemical parameters of boiled *D.bulbifera* extract

Triglycerides	Between Groups	2854.125	3	951.375	670.151	.000
	Within Groups	39.750	28	1.420		
	Total	2893.875	31			
AST	Between Groups	395.319	3	131.773	1835.758	.000
	Within Groups	2.010	28	.072		
	Total	397.329	31			
ALT	Between Groups	294.612	3	98.204	147.926	.000
	Within Groups	18.588	28	.664		
	Total	313.200	31			

Chapter VI

1.Funcational properties of flours

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	4	.000	.000	1.000
	Within Groups	210.000	35	6.000		
	Total	210.000	39			
LBD	Between Groups	.081	4	.020	202.950	.000
	Within Groups	.003	35	.000		
	Total	.084	39			
PBD	Between Groups	.064	4	.016	47.607	.000
	Within Groups	.012	35	.000		
	Total	.075	39			
OAC	Between Groups	7.247	4	1.812	33593.093	.000
	Within Groups	.002	35	.000		
	Total	7.248	39			
WAC	Between Groups	16.672	4	4.168	1099.418	.000
	Within Groups	.133	35	.004		
	Total	16.805	39			
ES	Between Groups	1182.306	4	295.577	575.748	.000
	Within Groups	17.968	35	.513		
	Total	1200.275	39			

LBD

			Subset for alpha = 0.05			
	Sample	N	1	2	3	4
Tukey HSD[a]	5	8	.3825			
	2	8		.4425		
	3	8			.4600	
	4	8				.4962
	1	8				.5100
	Sig.		1.000	1.000	1.000	.066

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

PBD

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	5	8	.6625		
	4	8		.7050	
	3	8		.7175	
	2	8		.7250	
	1	8			.7862
	Sig.		1.000	.208	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

OAC

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	5	8	.9250		
	2	8	.9250		
	1	8		1.0925	
	3	8			1.8375
	4	8			1.8438
	Sig.		1.000	1.000	.446

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

WAC

	Sample	N	Subset for alpha = 0.05				
			1	2	3	4	5
Tukey HSD[a]	5	8	1.4250				
	2	8		1.8000			
	3	8			2.4625		
	4	8				2.9375	
	1	8					3.1038
	Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

ES

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	1	8	42.1075			
	3	8		53.7250		
	5	8		54.0125		
	2	8			56.0125	
	4	8				57.3750
	Sig.		1.000	.928	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

2. Proximate compositions of Cookies

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	168.000	28	6.000		
	Total	168.000	31			
Carbohydrate	Between Groups	86.268	3	28.756	919.800	.000
	Within Groups	.875	28	.031		
	Total	87.144	31			
Protein	Between Groups	26.916	3	8.972	1732.808	.000
	Within Groups	.145	28	.005		
	Total	27.061	31			
Fat	Between Groups	106.089	3	35.363	1136.930	.000
	Within Groups	.871	28	.031		
	Total	106.960	31			
Ash	Between Groups	.335	3	.112	57.425	.000
	Within Groups	.055	28	.002		
	Total	.390	31			
Fiber	Between Groups	.163	3	.054	774.890	.000
	Within Groups	.002	28	.000		
	Total	.165	31			
Moisture	Between Groups	541.651	3	180.550	1898744.797	.000
	Within Groups	.003	28	.000		
	Total	541.654	31			

Carbohydrate

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	8	8	51.1038			
	7	8		52.0550		
	6	8			54.3238	
	5	8				55.1550
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Protein

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	8	8	6.7837			
	7	8		7.0325		
	6	8			7.9475	
	5	8				9.1213
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Fat

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	8	8	19.4013			
	7	8		21.9013		
	6	8			22.9763	
	5	8				24.3775
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Ash

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	5	8	3.2450			
	6	8		3.3638		
	7	8			3.4537	
	8	8				3.5175
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Moisture

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	5	8	8.0663			
	6	8		11.2463		
	7	8			15.3500	
	8	8				18.9513
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Fiber

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	5	8	.0962			
	6	8		.2088		
	7	8			.2450	
	8	8				.2888
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Proximate compositions of cakes

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	168.000	28	6.000		
	Total	168.000	31			
Carbohydrate	Between Groups	1084.656	3	361.552	428.480	.000
	Within Groups	23.626	28	.844		
	Total	1108.282	31			
Protein	Between Groups	39.474	3	13.158	641.645	.000
	Within Groups	.574	28	.021		
	Total	40.048	31			
Fat	Between Groups	13.388	3	4.463	71.111	.000
	Within Groups	1.757	28	.063		
	Total	15.145	31			
Ash	Between Groups	.573	3	.191	15.688	.000
	Within Groups	.341	28	.012		
	Total	.914	31			
Crudefiber	Between Groups	.238	3	.079	998.195	.000
	Within Groups	.002	28	.000		
	Total	.240	31			
Moisture	Between Groups	1742.043	3	580.681	2132336.267	.000
	Within Groups	.008	28	.000		
	Total	1742.050	31			

Carbohydrate

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	4	8	42.8488			
	3	8		48.6612		
	2	8			53.3763	
	1	8				58.6212
	Sig.		1.000	1.000	1.000	1.000
Tukey B[a]	4	8	42.8488			
	3	8		48.6612		
	2	8			53.3763	
	1	8				58.6212

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Protein

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	4	8	5.8600			
	3	8		7.0562		
	2	8			8.0150	
	1	8				8.8400
	Sig.		1.000	1.000	1.000	1.000
Tukey B[a]	4	8	5.8600			
	3	8		7.0562		
	2	8			8.0150	
	1	8				8.8400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Fat

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	4	8	17.4150			
	3	8		18.1463		
	2	8			18.5675	
	1	8				19.1938
	Sig.		1.000	1.000	1.000	1.000
Tukey B[a]	4	8	17.4150			
	3	8		18.1463		
	2	8			18.5675	
	1	8				19.1938

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Ash

	Sample	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1	8	2.8413	
	2	8		3.0613
	3	8		3.1250
	4	8		3.2000
	Sig.		1.000	.079
Tukey B[a]	1	8	2.8413	
	2	8		3.0613
	3	8		3.1250
	4	8		3.2000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

Crudefiber

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	1	8	.1162			
	2	8		.2975		
	3	8			.3150	
	4	8				.3288
	Sig.		1.000	1.000	1.000	1.000
Tukey B[a]	1	8	.1162			
	2	8		.2975		
	3	8			.3150	
	4	8				.3288

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

Moisture

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	1	8	10.4275			
	2	8		16.7500		
	3	8			22.7488	
	4	8				30.3937
	Sig.		1.000	1.000	1.000	1.000
Tukey B[a]	1	8	10.4275			
	2	8		16.7500		
	3	8			22.7488	
	4	8				30.3937

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

Proximate compositions of Papad

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	168.000	28	6.000		
	Total	168.000	31			
Moisture	Between Groups	35.218	3	11.739	11963.792	.000
	Within Groups	.027	28	.001		
	Total	35.246	31			
Protein	Between Groups	48.442	3	16.147	433.908	.000
	Within Groups	1.042	28	.037		
	Total	49.484	31			
Carb	Between Groups	103.097	3	34.366	583.731	.000
	Within Groups	1.648	28	.059		
	Total	104.745	31			
Fat	Between Groups	2.771	3	.924	90.348	.000
	Within Groups	.286	28	.010		
	Total	3.057	31			
Ash	Between Groups	2.179	3	.726	19.395	.000
	Within Groups	1.048	28	.037		
	Total	3.227	31			
Fiber	Between Groups	65.262	3	21.754	78342.650	.000
	Within Groups	.008	28	.000		
	Total	65.270	31			

Protein

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	12.00	8	6.2250			
	11.00	8		7.0563		
	10.00	8			8.3500	
	9.00	8				9.4500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Moisture

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	9.00	8	13.2675			
	10.00	8		14.0613		
	11.00	8			15.0613	
	12.00	8				16.0575

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Carbohydrate content

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	12.00	8	51.3037			
	11.00	8		52.0713		
	10.00	8			54.0475	
	9.00	8				55.9150

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Fat

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	12.00	8	1.7375		
	11.00	8	1.8375		
	10.00	8		2.0875	
	9.00	8			2.5000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Ash

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	9.00	8	5.9875		
	10.00	8		6.2625	
	11.00	8			6.5125
	12.00	8			6.6775

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Fiber

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	9.00	8	10.2463			
	10.00	8		11.0700		
	11.00	8			12.0300	
	12.00	8				14.0738

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Sensory parameters of cookies

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	1120.000	56	20.000		
	Total	1120.000	59			
Appearance	Between Groups	258.733	3	86.244	114.993	.000
	Within Groups	42.000	56	.750		
	Total	300.733	59			
Taste	Between Groups	236.133	3	78.711	92.601	.000
	Within Groups	47.600	56	.850		
	Total	283.733	59			
Flavour	Between Groups	333.733	3	111.244	87.007	.000
	Within Groups	71.600	56	1.279		
	Total	405.333	59			
Texture	Between Groups	28.133	3	9.378	11.550	.000
	Within Groups	45.467	56	.812		
	Total	73.600	59			
OA	Between Groups	131.146	3	43.715	170.596	.000
	Within Groups	14.350	56	.256		
	Total	145.496	59			

Appearance

			Subset for alpha = 0.05	
	Sample	N	1	2
Tukey HSD[a]	5	15	6.8000	
	6	15	6.9333	
	8	15		7.9333
	7	15		8.4000
	Sig.		.968	.389

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

taste

	Sample	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	5	15	5.6667	
	6	15	6.6667	
	8	15		7.9333
	7	15		8.3333
	Sig.		.122	.805

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

Texture

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	5	15	4.8000		
	6	15		7.2000	
	8	15			8.1333
	7	15			8.8000
	Sig.		1.000	1.000	.186

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

Flavour

	Sample	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	5	15	7.2000	
	6	15	7.3333	
	8	15	7.8000	7.8000
	7	15		8.3333
	Sig.		.326	.431

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

OA

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	5	15	6.1167			
	6	15		7.0333		
	8	15			7.9500	
	7	15				8.4667
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

Sensory parameters of Cakes

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	1120.000	56	20.000		
	Total	1120.000	59			
Appearance	Between Groups	27.117	3	9.039	14.113	.000
	Within Groups	35.867	56	.640		
	Total	62.983	59			
taste	Between Groups	66.717	3	22.239	15.017	.000
	Within Groups	82.933	56	1.481		
	Total	149.650	59			
Texture	Between Groups	137.800	3	45.933	57.246	.000
	Within Groups	44.933	56	.802		
	Total	182.733	59			
Flavour	Between Groups	11.867	3	3.956	4.304	.008
	Within Groups	51.467	56	.919		
	Total	63.333	59			
OA	Between Groups	48.321	3	16.107	60.233	.000
	Within Groups	14.975	56	.267		
	Total	63.296	59			

Appearance

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1	15	3.2667		
	2	15		7.2000	
	4	15			8.0667
	3	15			8.5333
	Sig.		1.000	1.000	.459

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

Taste

	Sample	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1	15	3.3333	
	4	15		7.6000
	2	15		7.6667
	3	15		8.3333
	Sig.		1.000	.142

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

Flavour

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1	15	2.3333		
	2	15		6.9333	
	4	15		7.8000	7.8000
	3	15			8.2667
	Sig.		1.000	.166	.673

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

Texture

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	2	15	6.5333		
	4	15	6.6000	6.6000	
	3	15		7.4667	7.4667
	1	15			8.2000
	Sig.		.997	.052	.128

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

OA

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1	15	4.2833		
	2	15		7.0833	
	4	15		7.5167	
	3	15			8.1500
	Sig.		1.000	.100	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

Sensory parameters of papad

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Replication	Between Groups	.000	3	.000	.000	1.000
	Within Groups	168.000	28	6.000		
	Total	168.000	31			
Appearance	Between Groups	5.206	3	1.735	182.493	.000
	Within Groups	.266	28	.010		
	Total	5.472	31			
Taste	Between Groups	1.894	3	.631	86.220	.000
	Within Groups	.205	28	.007		
	Total	2.099	31			
Flavour	Between Groups	4.118	3	1.373	277.036	.000
	Within Groups	.139	28	.005		
	Total	4.257	31			
Texture	Between Groups	7.386	3	2.462	612.785	.000
	Within Groups	.113	28	.004		
	Total	7.499	31			
OA	Between Groups	1.823	3	.608	371.645	.000
	Within Groups	.046	28	.002		
	Total	1.869	31			

Appearance

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	9.00	8	6.4000		
	10.00	8		7.1875	
	12.00	8		7.1875	
	11.00	8			7.4875

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Taste

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	12.00	8	7.5875			
	11.00	8		7.7875		
	9.00	8			8.0125	
	10.00	8				8.2375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Flavour

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	12.00	8	7.0750			
	11.00	8		7.3750		
	9.00	8			7.7875	
	10.00	8				8.0000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

Texture

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	12.00	8	6.7250		
	11.00	8		7.1500	
	10.00	8			7.8375
	9.00	8			7.8625

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

OA

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	12.00	8	7.1438			
	11.00	8		7.4500		
	9.00	8			7.5156	
	10.00	8				7.8156

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.000.

List of Publication

Sadhni Induar, Bikash chndra Behera, Debasmita dubey, Pradeep kumar Nik (2021). Proximate and Sensory Properties of *Dioscorea Bulbifera* Composite Flour Made Cookies and Cake**.** Asian journal of biological and life sciences, 10(2):-476-483. DOI: 10.5530/ajbls.2021.10.63

Sadhni Induar, Bikash chndra Behera, Debasmita dubey, Pradeep kumar Nik (2022). Assessment of nutritional, bioactive compound and DPPH scavenging activity of tuber of *Dioscorea bulbifera* collected from tribal forest area of Sundargarh district, Odisha, India. Carpathian journal of food science and technology.(Accepted)

List of Conference

Sadhni Induar and Pradeep Kumar Naik, International Conference on 'Food processing and Analysis' Oral presentation held in Hotel Shivalikview, Chandigarh on August 20-21**,** 2016 organised by Selected Biosciences India private Limited.

Sadhni Induar and Pradeep Kumar Naik Oral presentation, "Nutritional and Anti nutritional composition of both raw and boiled D.bulbifera" on 30th Annual Conference of Orissa Chemical Society, held from 24th – 25th December, 2016 organised by Department of Chemistry, Schoool of Applied Sciences, KIIT University Bhubaneswar, Odisha.

Sadhni Induar and Pradeep Kumar Naik Oral presentation, Two-Day Workshop on "Tribal Intellectuals and Inclusion: A Need Based Assessment" held from 28th – 29th February, 2020 at Kalinga Institue of Social Sciences (KISS), Deemed to be University, Bhubaneswar, Odisha. Jointly organized by Kalinga Institure of Social Sciences (KISS), Deemed to be University and Indian Institute of Public Administration (IIPA), supported by Ministry of Tribal Affairs, Government of India.

Proximate and Sensory Properties of *Dioscorea bulbifera* Composite Flour Made Cookies and Cake

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ABSTRACT

Dioscorea bulbifera is a traditional wild tuber belongs to family Dioscoreaceae. It is not only rich in nutrients but also possesses medicinal properties. In this study an attempt has been made to produce cookies and cake from combination of wheat and *Dioscorea bulbifera* flour. *Dioscorea bulbifera* flour was incorporated with wheat flour in ratio of 25%, 50% and 75%. Composite flour made cookies and cake were examined for acceptability on the basis of biochemical and sensory parameters. Result of the study revealed that moisture, ash and fiber content gradually increases with increase in incorporation level of *Dioscorea bulbifera* flour (Dbf) with wheat flour at the same time as fat, carbohydrate and protein content steadily decreased. According to panelist 50% substituted cake and cookies received highest score for all the attributes. But all cookies and cake

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INTRODUCTION

Cake and cookies are inexpensive suitable ready to eat food for all age groups in many countries. Wheat flour is used as main ingredient for cake and cookies production along with other ingredients such as sweeteners (sugar), leavening agents, milk, flavors, and butter. But in the last years, there is an increasing research interest in potential of composite flour in snacks preparation. Addition of locally available cereals, pulses and tuber flours with refined wheat flour enhances the quality of refined wheat flour. *Dioscorea bulbifera* is a traditional wild tuber belongs to family Dioscoreaceae. It is being consumed as staple food by rural and tribal people. Available literature reported that *Dioscorea bulbifera* is a good source of nutrients with appreciable levels of minerals[1,2] and has good quality of protein with 17 amino-acids.[3] The main use of *Dioscorea bulbifera* involves its tuber can be prepared and processed into edible food by boiling, frying or roasting or eaten as mixed with other vegetables.[4-6] Previous studies have shown the incorporation of *Dioscorea bulbifera* flour (Dbf) for the making of various products.[7] Prepared bread from wheat flour substituted with 25%, was found to be suitable while more than 25% substitution were not acceptable for this purpose. Composite flours containing blanched fermented sun dried aerial yam (AY) and Cassava flour(CS) in different ratio(AY_{100}, AY_{80} CS_{20}, AY_{60} CS_{40}, AY_{40} CS_{60} and AY_{20} CS_{80}) were also used to make paste. On the basis of sensory characteristics, out of the above different ratio, the 60-80% aerial yam incorporate composite flour found to be more suitable for the paste.[8] Aerial yam flour at different levels (10%, 20%, 30%, 40%, 50% up to 100%) with wheat flour were used for biscuit production. Biscuits made from 10% to 50% aerial yam substituted wheat flour were found to be acceptable while more than 50% substitution were not appreciable by panelists.[9]

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