SYNOPSIS OF THE THESIS SUBMITED TO SAMBALPUR UNIVERSITY

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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 activity) 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.