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Wound Healing Activity of Topical Herbal Gels Containing *Barringtonia acutangula* Fruit Extract: *In silico and In vivo* Studies

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The current study describes the efficacy of *B. acutangula* fruit extract in wound healing *via* incorporation within topical gels. *B. acutangula* fruit extract was produced by solvent extraction method. The bioactive extract was incorporated within Carbopol 940-based topical gels, which were applied topically over the excision and incision wounds. The change in healing process was observed till 20 days. The percentages of closure of excision wound area were 92.89% and 93.43%, when treated with topical herbal gels containing *B. acutangula* fruit extract of 5% and 10%, respectively. The tensile strengths of incision area in rats treated with topical herbal gels containing 5% and 10% methanol extract of *B. acutangula* fruits were found to be 25 ± 5.12 g and 30 ± 4.10 g, respectively. The wound healing activity of topical herbal gels containing *B. acutangula* fruit extract in rats was found to be significant when compared with that of the reference standard and untreated groups. In addition, *in silico* studies suggested about good skin permeability and binding to the proteins responsible for delaying wound healing. It can be concluded that this topical herbal gels containing *B. acutangula* fruit extract could be used clinically for the treatment of wounds.

1. Introduction

Cutaneous wound healing is an intricate progress which ends up in formation of scars and disfiguring.[1] Wound denotes to damage to the skin or underlying organs or tissues caused by any of the reasons may be due to diseases or chemicals or trauma or surgery.[2,3] The common symptoms of wound are characterized by pain, redness, bleeding and swelling. Healing or repairing process of wound is the reconstitution of skin as well as its related soft tissues. The process is divided in to three phase.[4,5] The first one is the inflammation phase, the second one is the proliferation phase, and the third one is the remodeling phase. An inflammatory reaction is initiated after the injury followed by production of more and more collagen below the dermis. Regeneration of epithelial tissue is the next process. Proliferative phase is characterized by angiogenesis, deposition of collagen, granulation, tissue development, and epithelialization followed by wound contraction.[5] The later stage is called remodeling stage. The potential for enhancing the healing process, mitigating scar formation, and optimizing the characteristics of regenerated skin may be attainable through the utilization of diverse skin care products and therapeutic interventions.[6] The appropriate healing process leads the wound to enter in to a chronic state followed by increased risk of infection and affecting patient's quality of life.[4] According to the recent literature review, most of the prescribed modern medications especially antibiotics causes side effects and produces antibiotic resistance. Biologically active compounds obtained from the plant sources can be considered safe and provide better patient compliances.[7] Also, wound healing involves various pharmacological targets for slowing the release of inflammatory cytokines and arresting the inflammatory transduction cascades.[8] Application of bioinformatics for screening of active ligands present in the phytoconstituents has increased the application of phytomedicine as conventional pharmaceuticals.[9,10]

Barringtonia acutangula is an evergreen tree of Lecythidaceae family and Ericales order. This tree grows to about 8–15 m high and found throughout India, plentifully in the plains of Odisha and Bengal. *B. acutangula* plants possess with simple, alternate leaves, dark scarlet flowers with 2-celled ovary and 4-lobed ovate calyx. Its fruit is oval-shaped. *B. acutangula* plant is traditionally used for the treatment and cure of various ailments like liver disorders, splenic disorders, stomach disorders as well as leprosy.[11–13] Various researchers already have mentioned its antioxidant properties, which can be useful in cytoprotection and tissue recovery. Cytoprotection and tissue recovery is the prime motto in wound healing. However, in the reported

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literature, no research endeavor has been carried out to evaluate the effectiveness of *B. acutangula* fruit extract. Therefore, current study was intended to evaluate the efficacy of *B. acutangula* fruit extract in quickening wound healing process in experimental animal model via incorporation within topical gels. *B. acutangula* fruit extract was produced by hot percolation method. The bioactive extract was transformed in to topical gel formulations using Carbopol 940, propylene glycol, triethylamine, methyl paraben and propyl paraben. These prepared novel herbal gel formulations were applied topically over the excision and incision wounds done on the Wister Albino rat's dorsal area. The change in healing process was observed, positively.

2. Material and Methods

2.1. Collection and Authentication

Unripe fruit of *B. acutangula* was collected from local area of Kendrapara, Odisha (Lat 20.619947°, long 86. 434705°) and identified by Dr. Gyana Ranjan Mahalik, Associate Professor, Department of Botany, Centurion University of Technology and Management (CUTM), Bhubaneswar, Odisha. The sample specimen (voucher no. CUTM/BOT/2023/02) has been submitted in the herbarium of CUTM for future reference. Rutin (Sigma-Aldrich, India), gallic acid (Sigma-Aldrich, India), Carbopol 940 (Hi-Media Laboratories Pvt. Ltd., India), propylene glycol (Loba Chemie Pvt. Ltd., India), triethylamine (Hi-Media Laboratories Pvt. Ltd., India), methyl paraben (SD Fine Chemicals, India), propyl paraben (SD Fine Chemicals, India), sodium nitrite (SRL Chemicals Pvt. Ltd., India), sodium hydroxide (SRL Chemicals Pvt. Ltd., India) and aluminum chloride (Merck Specialties Pvt. Ltd., India) were used. All other chemicals and reagents were of analytical grade and commercially available.

2.2. Preparation of B. Acutangula Fruit Extract by Solvent Extraction

The fresh unripe fruits were washed thoroughly 2–3 times with running tap water and once with sterile water. Then fruits were shade dried without any contamination. The dried fruits were powdered and passed through the mesh no. 30 to get the uniform size. The coarse powder of *B. acutangula* fruit was subjected to continuous hot extraction with solvents- petroleum ether, chloroform, ethyl acetate and methanol successively by using a Soxhlet apparatus for 24 h.[14] The solvents were removed under pressure and the extracts were concentrated under vacuum at 40–60 °C. After extraction, the extracts of *B. acutangula* fruits were collected in a beaker and filtered through filter papers to remove any insoluble materials. The filtrates were evaporated under reduced pressure to obtain the concentrated extracts. The extracts of *B. acutangula* fruits were then dried in an oven at a suitable temperature to remove any residual solvent. The resulting extract was stored in a cool and dry place until further study.

2.3. Preliminary Phytochemical Screening

The petroleum ether, chloroform, ethyl acetate and methanol extracts of *B. acutangula* fruits was subjected to different qualitative chemical tests using accepted techniques to identify the presence of phytoconstituents. These chemical tests were: Mayer's Test, Wagner's Test and Hager's Test for presence of alkaloids; $FeCl_3$ test, lead acetate test and $K_2Cr_2O_7$ test for presence of phenolics/tannins; Shinoda test for presence of flavonoids; Ninhydrin test for presence of proteins/amino acids; Molisch's test and Felhings test for presence of carbohydrates; foam test for presence of saponins; Knollar's test for presence of terpenoids; and Borantrager's test for presence of anthraquinone.

2.4. Biochemical Estimations

2.4.1. Determination of Total Phenolic Content

To determine the total phenolic content in the extracts of *B. acutangula* fruits, spectrophotometric assay was used. Sample (1 mg/mL) was mixed with 1 mL Folin and Ciocalteu's phenol reagent. After duration of 3 min, 1 mL solution containing saturated sodium carbonate was introduced to the mixture, and the total volume was adjusted to 10 mL using distilled water. The reaction was kept in the dark for 90 min, and then, the absorbance was measured at 725 nm by a UV-VIS Spectrophotometer (LABINDIA 3200). Gallic acid (20–100 μg/ml) was used as standard to prepare the calibration curve ($y = 0.0073\ x - 0.0276$; $R^2 = 0.9940$).[15]

2.4.2. Determination of Total Flavonoid Content

To determine the flavonoid contents in the extracts of *B. acutangula* fruits, spectrophotometric assay was used. Sample (250 μL, 1 mg/mL) was mixed with 1.25 mL of distilled water and 75 μL of a 5% sodium nitrite solution. After 5 min, 150 μL of 10% aluminum chloride solution was added, followed by 500 μL of 1 M sodium hydroxide and 275 μL of distilled water. The solution was mixed well and the absorbance was measured at 510 nm by a UV-VIS Spectrophotometer (LABINDIA 3200). Rutin (20–100 μg/ml) was used as standard to prepare the calibration curve ($y = 0.0075\ x - 0.011$; $R^2 = 0.9812$).[15]

2.5. In Vitro Antioxidant Activity

2.5.1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The assay involved the addition of 1 ml of different extracts (10–100 μg/ml) to 0.1 ml of solution of DPPH (3 mM) in ethanol. The reaction mixture was kept in dark for 20 min. The decrease in absorbance of DPPH radical was measured at 517 nm using a UV-VIS Spectrophotometer (LABINDIA 3200). Different concentrations of ascorbic acid were used as a standard drug.

The scavenging activity was determined by utilizing the formula:

$$\text{Scavenging activity } (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

In this equation, $A_{control}$ represents the absorbance value of the control, and A_{sample} refers to the absorbance value of the extract being tested.

2.5.2. Hydrogen Peroxide (H_2O_2) Assay

This assay involved the measurement of the scavenging ability of H2O2 by the extract. Different concentrations of 1 ml of extract was added to 100 µl of hydrogen peroxide (1 mM), 360 µl of deoxyribose (28 mM) and 1 ml of iron-EDTA solution (200 µM ferrous ammonium sulfate and 1 mM EDTA). To the reaction mixture, 0.22% of 100 µL of ascorbic acid was added and incubated at 80–90 °C for 15 min. Then, 1 ml of 10% TCA solution was added to stop the reaction. Finally, 3 ml of Nash reagent was added and kept at room temp for 15 min and absorbance was measured at 412 nm using a UV-VIS Spectrophotometer (LABINDIA 3200). Different concentrations of ascorbic acid were used as reference drug.

The scavenging activity percentage was determined by using the formula:

$$\text{Scavenging activity } (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

In this equation, $A_{control}$ represents the absorbance value of the control, and A_{sample} refers to the absorbance value of the extract being tested.

2.6. Antimicrobial Assay

2.6.1. Evaluation of Minimum Inhibitory Concentration (MIC)

By using the micro dilution method and Clinical & Laboratory Standards Institute (CLSI) guideline, the MIC of methanol extract of unripe fruits of *B. acutangula* against *E. coli* was calculated. The bacterial culture was grew in Luria broth (LB) medium for 18 h and then adjusted to 0.5 McFarland. A stock solution of 100 mg/mL of the extract was prepared in Millipore water and serial diluted to get different concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 mg/mL), which were treated to the bacterial culture and incubated overnight at 37 ± 1 °C. After the incubation period, the absorbance was measured at 600 nm, LB medium was used as blank and LB medium with bacterial culture was regarded as a negative control.

2.6.2. Antibacterial Activity

The antibacterial activity of methanol extract *of B. acutangula* fruits was performed by agar well diffusion assay using Kirby-Bauer method on sterile and solidified Mueller-Hinton agar (MHA) plates. The wells (6 mm) were prepared by using sterile borer on solidified agar medium and MIC of the extract was added to it in which methanol was taken as control. The MHA plates were incubated at 37 ± 1 °C for 18–24 h and the hollow zone around the well was considered as zone of inhibition (ZOI) and measure of antibacterial activity.

2.7. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of methanol extract *of B. acutangula* fruits was performed in a GC-MS instrument (SCION 436-GC, Bruker) equipped with triple quadruple-MS and fused capillary column BR-5MS (5% diphenyl/95% dimethyl polysiloxane; 30 m ×0.25 mm ×0.25 µm). The ionization energy of 70 eV was used with EIS detector (+ Ve ionization mode). The carrier gas He (99.999%) was used at constant flow rate (1 mL/min) and sample was injected (2 µL, split ratio 10:1). The source and injector temperature were set at 250 °C and 280 °C, respectively. The oven was isothermally programmed for 3.5 min at 110 °C (increasing rate of 10 °C/min up to 200 °C and then at rate of 5 °C/min up to 280 °C for 12 min) with total run time of 40.5 min. Mass spectra and chromatogram were programmed in MS station 8 and NIST database (version 11) was used for identification of chemical compounds.[16]

2.8. In Silico Molecular Docking Studies

Based on the percentage (%) of compounds in GC-MS analysis report, the ligands were selected and downloaded in Structured Data File (SDF) format from the Pubchem database. The PDB format of ligands was converted to Autodock format for docking. The cofactors, ligand molecule and water molecules were removed from the target site of the receptor and saved as macromolecule in Pymol virtual screening tool. The molecular docking was performed by using of Pymol software. The binding energy was noted for ligand-protein interaction and the complex was visualized by using Discovery Studio software program.

2.9. Preparation of Herbal Gels

In brief, 1.5% Carbopol 940 gel was prepared using via progressively pouring into a known volume of distilled water. Using a magnetic stirrer (Remi Motors, India), the mixture was constantly stirred at 800 rpm for 1 h to avoid formation of lump. While stirring, drop wise addition of triethanolamine and propylene glycol was done. Lastly, the methanol extract *of B. acutangula* fruits (5% and 10%) was added with methyl paraben-propyl paraben to the gel formulation with constant stirring. The final gel formulations were stored in small mouth plastic containers with a plastic lid and kept at room temperature.

2.10. Characterization of Herbal Gels

2.10.1. Organoleptic Tests

Organoleptic properties such as appearance, color and odor of these prepared gel formulations were observed and reported.[17]

2.10.2. Determination of pH

The prepared gel formulations were tested for pH determination using a pH meter (Elico Ltd., India).[18]

2.10.3. Determination of Viscosity

Viscosities of these prepared gel formulations were measured using a viscometer (DV-II + Pro, Brookfield). The gel samples were placed in a container, individually. Afterward, the spindle was set to a specific speed, and the test gel was stirred using the spindle for a certain amount of time. The spindle was then lifted out of the gel, and viscosity reading was taken from the scale on the viscometer.

2.10.4. Spreadability

The gel formulations were individually placed in-between two glass slides and a standardized weight (100 g) was applied for a fixed period of time. The diameter of the resulting gel sample was then measured, and the spreadability factor was determined by using the formula:[19]

$$\text{Spreadability factor} = \text{Maximum spread area } (\text{cm}^2)/\text{Total weight stressed}$$

2.10.5. Gelling Strength

Gelling strengths of these prepared gel formulations (20 g) were measured using a texture analyzer (Brookfield CT3) and the appropriate probe with a 21 mm diameter was attached to the surface of the gel. The force required to penetrate the gel to 5 mm depth was measured. The higher the force required, the greater the gelling strength of the gel. The gelling strength was determined by using the formula:

$$\text{Gelling strength} = \text{Weight } (\text{g}) \times \text{Diameter } (\text{cm})/\text{Time } (\text{sec})$$

2.11. In Vivo Wound Healing Activity

2.11.1. Experimental Animals

Wister Albino rats (160-180 g), of either sex, were purchased from the National Institute of Science Education and Research (Regd. No. 1634/GO/ReRcBiBt/S/12/CPCSEA). They were acclimatized in the departmental animal house at 26 ± 2 °C and relative humidity of 44–56 %, light and dark cycles of 12 h, respectively, for one week before and during the experiment. Animals were given a regular rat pellet diet and unlimited access to water. Prior to the experiment, the protocols were approved from Institutional Animal Ethical Committee (2024/PO/Re/S/18/CPCSEA).

2.11.2. Dose Administration

To study the effect of prepared gel formulations in incision and excision models, the Wister Albino rats were randomly divided into 4 groups containing 6 animals in each group:

Group-I: Carbopol 940 (1.5 %, 100 mg, once daily)-based gel without methanol extract of *B. acutangula* fruits as control applied topically,

Group-II: Povidone iodine ointment (5 % w/w, 100 mg, once daily) treated positive control applied topically,

Group-III: Carbopol 940 (1.5 %, 100 mg, once daily)-based gel containing methanol extract of *B. acutangula* fruits (5 % w/w) applied topically, and

Group-IV: Carbopol 940 (1.5 %, 100 mg, once daily)-based gel containing methanol extract of *B. acutangula* fruits (10 % w/w) applied topically.

To stop the rats from licking the solution of the wound and to prevent a major infection, they were kept apart. Throughout the experiment, digital photographs of the wound area were taken (up to 20 days).

2.11.3. Excision Wound Model

Ketamine (80 mg/Kg) was used to put rats in anesthetic condition, and a standard ring was used to mark a 400 mm^2 area on the back of each rat. The marked skin was then meticulously sliced throughout. On 1 mm^2 graph paper, wounds were traced on the day of injury, then every other day until 20^{th} day and on the final day of healing. Every so often, the size of the wound was measured, and the formula below was used to compute the rate of wound contraction. By comparing the healed wound area on the corresponding days with the healed wound area of the control group, significance in wound healing of the test groups is determined. Also, epithelization period was noted.

$$\% \text{ Wound contraction} = (\text{Initial wound area} - \text{Wound area on a specific day}) / \text{Initial wound area}$$

2.11.4. Incision Wound Model

After administering anesthetics (ketamine; 80 mg/Kg), two parallel 6 cm paravertebral incisions were made, 1 cm laterally to the midline of the spinal column. Surgical sutures were used to close the wounds. On 7th post-injury day, the sutures were taken out. Wound breaking strength (WBS) was measured on the 10th post wounding day in anaesthetized rats. According to the method described by Murthy et al., 2013, Alice forceps was firmly applied to both side of the wound and free weight was suspended to one of the forceps.[20] The weight was added gradually until the incision site began to break apart. The weight was ceased as soon as the wound first opened and noted.

2.11.5. Histological Analysis

The histopathological examination aimed to study the process of epithelialization on the excised wound and to identify any evidence of granuloma, dysplasia, edema, or malignancy in the skin under examination. The skin, which had newly formed on the wounds, was subjected to histological examination on the 10th, 14th, 20th day post-wounding. The skin was fixed in 10% formalin and paraffin sections (5–10 μ) were prepared. These sections were stained with hematoxylin and eosin and mounted in di-n-butyle phthalate in xylene medium.

2.12. Statistical Analysis

All measured data are presented as mean ± S.D. (standard deviation) and processed in Microsoft Excel.

3. Results and Discussion

3.1. Preliminary Phytochemical Screening

Phytochemical screening of different extracts of *of B. acutangula* fruits was performed and the results revealed the presence of a number of secondary metabolites (Table 1). The preliminary phytochemical screening of petroleum ether, chloroform, ethyl acetate and methanol extracts were performed to identify the presence of different secondary metabolites, such as phenolic, flavonoid, saponin and terpenes, *etc.* Literatures suggested about the improving of immune system, reducing the blood clot and lowering of blood sugar level of phenolic.[21] Moreover saponins promote the production of skin fibroblasts.[22] As like saponins, flavonoids also increase collagen secretion and promote wound healing.[23] Tanin content in the extract acts as an astringent which helps in reduction in mucosal permeability leading to strengthening the bond between the mucosa and inhibits the entry of harmful chemicals and microorganisms.[24] Also, terpenoids present in the phytoconstituents promotes the wound healing.[25]

3.2. Biochemical Estimations

3.2.1. Total phenolic and Total Flavonoid Contents

Total phenolic contents of methanol, ethyl acetate, chloroform, petroleum ether and extracts of *B. acutangula* fruits were measured. The results indicated that the methanol extract possessed highest total phenolic content at 79.71 μg/mg of extract. In contrast, ethyl acetate, chloroform and petroleum

Table 1. Phytochemical screening of fruit extract of *B. acutangular.*

Phytochemical constituents	Tests	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Alkaloids	Mayer's Test	–	+	+	+
	Wagner's Test	–	+	+	+
	Hager's Test	–	+	+	+
Phenolics/Tannin	$FeCl_3$	+	++	++	+++
	Lead acetate test	+	++	++	+++
	$K_2Cr_2O_7$ test	+	++	++	+++
Flavonoids	Shinoda Test	+	++	+++	+++
Proteins/amino acids	Ninhydrin Test	+	+	+	+
Carbohydrates	Molishs Test	+	++	++	++
	Felhings Test				
Saponins	Foam Test	+	+	+	+
Terpenoids	Knollar's Test	–	+	+	+
Anthraquinine	Borantrager's Test	+	+	+	+

ether extracts contained 41.76, 32.75 and 18.77 μg/mg of *B. acutangula* fruits extract, respectively.

Total flavonoid contents of different extracts, such as petroleum ether, chloroform, ethyl acetate and methanol were measured, and the results indicated that the ethyl acetate extract contained highest flavonoid content of approximately 109.52 μg/mg of *B. acutangula* fruits extract. Even methanol, chloroform and petroleum ether extracts possessed lower phenolic content of approximately 79.35, 32.08 13.21 μg/mg of extract, respectively.

3.3. In vitro Antioxidant Activity

3.3.1. DPPH Assay

The DPPH radical scavenging activity of *B. acutangula* fruits extracts was measured. The results showed that the radical scavenging activity increased uniformly with the concentration of the extracts. The IC50 values were calculated to determine the antioxidant activity of the different extracts. The results showed that the methanol extract of *B. acutangula* fruits had the highest antioxidant activity, with an IC50 value of 0.70 μg/mL. The ethyl acetate extract presented an IC50 value of 0.88 μg/mL while chloroform extract presented 29.607 μg/mL and the petroleum ether extract of *B. acutangula* fruits presented the lowest IC50 value of 35.609 μg/mL. In comparison, the positive control (ascorbic acid) presented an IC50 value of 1.11 μg/mL, indicating that the methanol extract had the closest antioxidant activity.

3.3.2. H_2O_2 Assay

The H_2O_2 assay results showed the increased scavenging activity with increasing concentration for all extracts of *B. acutangula* fruits. The highest activity was exhibited by the methanol extract of *B. acutangula* fruits, with an IC50 value of 38.637 μg/mL. In contrast, the chloroform and petroleum ether extracts had IC50 values of 0.31 μg/mL and 1.02 μg/mL, respectively, and lower absorption values, indicating their lower reducing ability compared to the methanol extract.

3.4. Antimicrobial Activity

In vitro antimicrobial activities of different extracts of *B. acutangula* fruits against *E. coli* were performed and highest MIC was exhibited by methanol extract and its ZOI was determined. The MIC value of methanol extracts of *B. acutangula* fruits was found 12.5 mg/mL; whereas MIC values of petroleum ether, chloroform, and ethyl acetate extracts of *B. acutangula* fruits was found to be 17.46, 9.18, and 6.25 mg/mL, respectively (Figure 1). The ZOI of methanol extract was found to be 18.66 ± 0.83 mm (Figure 2). This finding indicated the good antimicrobial action of methanol extracts of *B. acutangula* fruits.

3.5. Selection of Extract for Further Herbal Gel Formulations

The aim of this research was to develop novel herbal topical gel formulations containing *B. acutangula* fruit extract for effective wound management. The highest total phenolic and flavonoid contents were recorded in case of methanol extract of *B. acutangula* fruits indicated its wound healing potential.

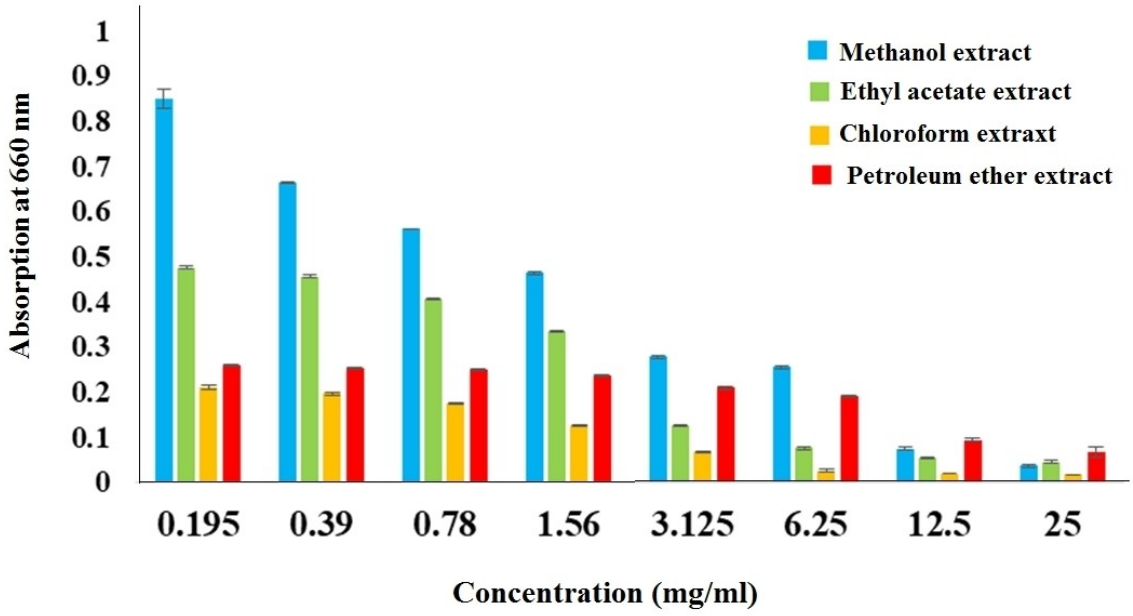

Figure 1. MIC values of petroleum ether, chloroform, ethyl acetate and methanol extracts of *B. acutangula* fruits at different concentrations against *E. coli.*

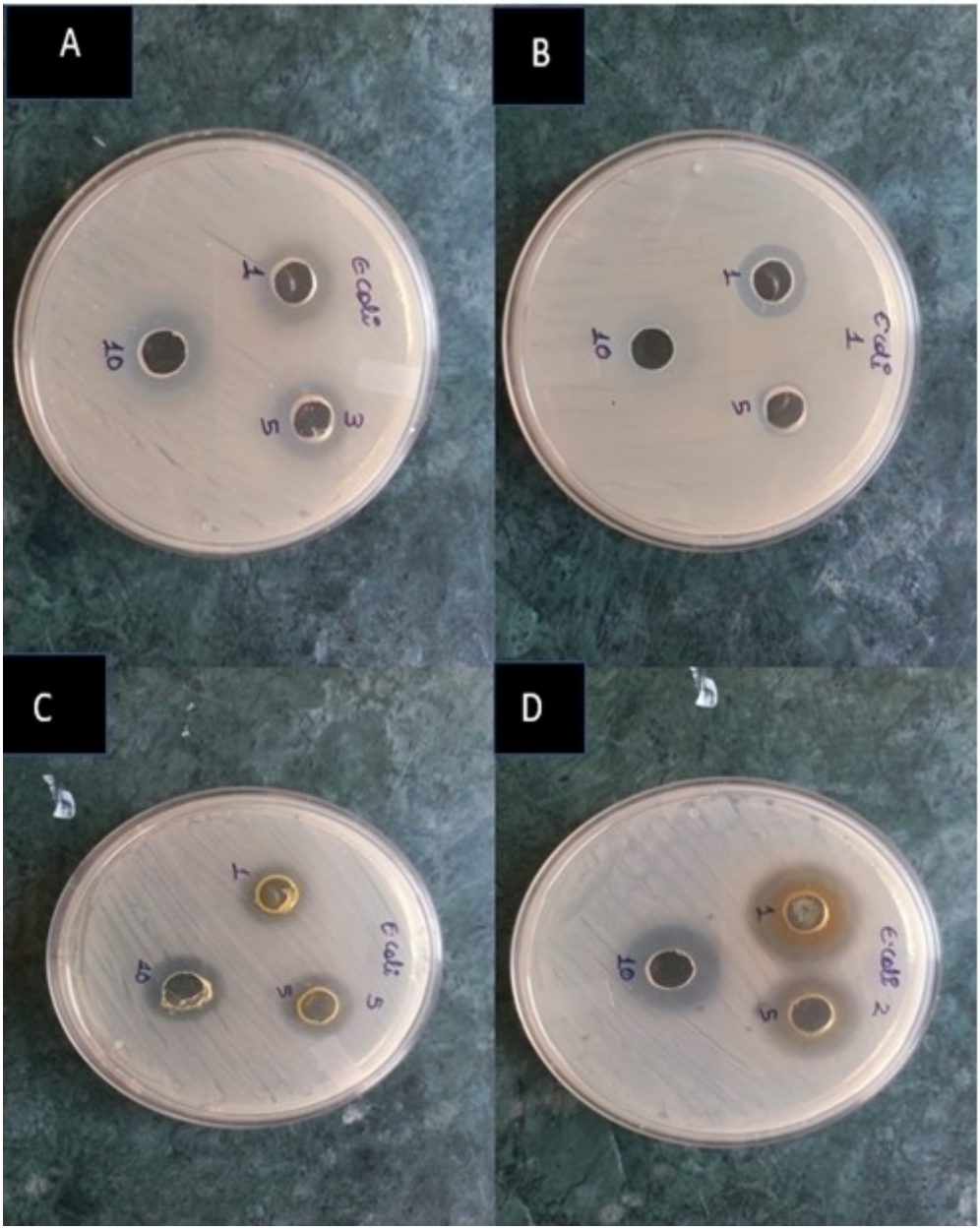

Figure 2. ZOI produced by: (A) petroleum ether, (B) chloroform, (C) ethyl acetate, and (D) methanol extract of *B. Acutangula* fruits at different concentrations against *E. coli.*

Amongst different extracts obtained, methanol extract of *B. Acutangula* fruits exhibited good antioxidant and antibacterial activities in comparison to other extracts. In addition, the identified phytochemicals in the preliminary phytochemical screening were also found beneficial for wound healing.[26] On the basis of these prospective results, we have selected methanol extract of *B. acutangula* fruits for further research to develop novel herbal topical gel formulations for effective wound management

3.6. GC-MS Analysis of Methanol Extract of B. Acutangula Fruits

GC-MS analysis of methanol extract *of B. acutangula* fruits was performed and a total no. of 21 different phytochemicals was identified by using NIST library. The relative (%) of each compound was calculated by comparing the average peak area of each component to the overall area. The compounds were represented with their name, retention time, molecular formula, molecular mass, chemical class, percentage area (%) and skin permeability in Table 2. The results indicated that all the compounds followed Lipinski's rule of five and the skin permeability values of the compounds suggested their greater

Table 2. GC-MS analysis of methanol extract of *B. acutangula* fruits.

Sl. No.	RT	Name of the compound	Class of compound	Molecular formula	M.W (g/mol)	% area	Skin permeability ($logk_p$, cm/s)
1.	5.67	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	Ester	$C_{13}H_{21}NO_4$	255.31	5.02	−6.13
2.	6.16	4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester	Ester	$C_{11}H_{20}O_3$	200.27	0.90	−6.41
3.	6.93	D-Mannopyranose	Carbohydrate	$C_6H_{12}O_6$	180.16	10.85	−9.70
4.	7.14	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Heterocyclic	$C_6H_8O_4$	144.12	7.92	−7.44
5.	7.79	2-Cyclohexylpiperidine	Heterocyclic	$C_{11}H_{21}N$	167.29	0.88	−5.13
6.	8.72	2(3H)-Furanone, 5-heptyldihydro	Heterocyclic	$C_{11}H_{20}O_2$	184.27	1.86	−5.11
7.	9.06	5-Hydroxymethylfurfural	Heterocyclic	$C_6H_6O_3$	126.11	3.44	−7.48
8.	9.37	1,2,3-Propanetriol, 1-acetate	Aliphatic	$C_5H_{10}O_4$	134.13	5.95	−7.83
9.	10.24	5-(2-Hydroxyethyl)-4-methylthiazole	Heterocyclic	C_6H_9NOS	143.21	5.66	−6.57
10.	11.70	3-Deoxyglucose	Carbohydrate	$C_6H_{12}O_5$	162.14	1.49	−9.05
11.	12.67	4-Hexenoic acid, 6-(acetyloxy)-4-methyl	Aliphatic	$C_9H_{14}O_4$	186.20	2.18	−6.72
12.	13.26	4-Methyl(trimethylene)silyloxyoctane	Aliphatic	$C_{12}h_{26}osi$	214.42	2.32	−4.26
13.	14.36	Melezitose	Carbohydrate	$C_{18}H_{32}O_{16}$	504.4		−3.2
14.	15.66	2-Methyl-6-propylpiperidine	Heterocyclic	$C_9H_{19}N$	141.25	2.01	−5.46
15.	17.76	1-Nitro-β-d-arabinofuranose, tetraacetate	Heterocyclic	$C_{13}H_{17}NO_{11}$	363.27	0.93	−8.90
16.	18.08	3-Deoxy-d-mannoic lactone	Heterocyclic	$C_6H_{10}O_5$	162.14	1.06	−8.04
17.	18.34	Desulphosinigrin	Heterocyclic	$C_{10}H_{17}NO_6S$	279.31	8.70	−8.91
18.	21.25	Melibiose	Carbohydrate	$C_{12}H_{22}O_{11}$	342.30	0.25	−13.53
19.	25.86	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]	Cyclic hydrocarbon	$C_{25}H_{42}O_2$	374.6	0.47	−2.48
20.	26.74	N-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	386.8	1.57	−2.77
21.	30.91	Cis-Vaccenic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	1.24	−2.60

penetration ability through the skin. The prime compounds were identified as D-Alanine, N-propargyloxycarbonyl-, isohexyl ester (1), D-Mannopyranose (2), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (3), 1,2,3-Propanetriol, 1-acetate (4), 5-(2-Hydroxyethyl)-4-methylthiazole (5), and desulphosinigrin (6) (Figure 3).

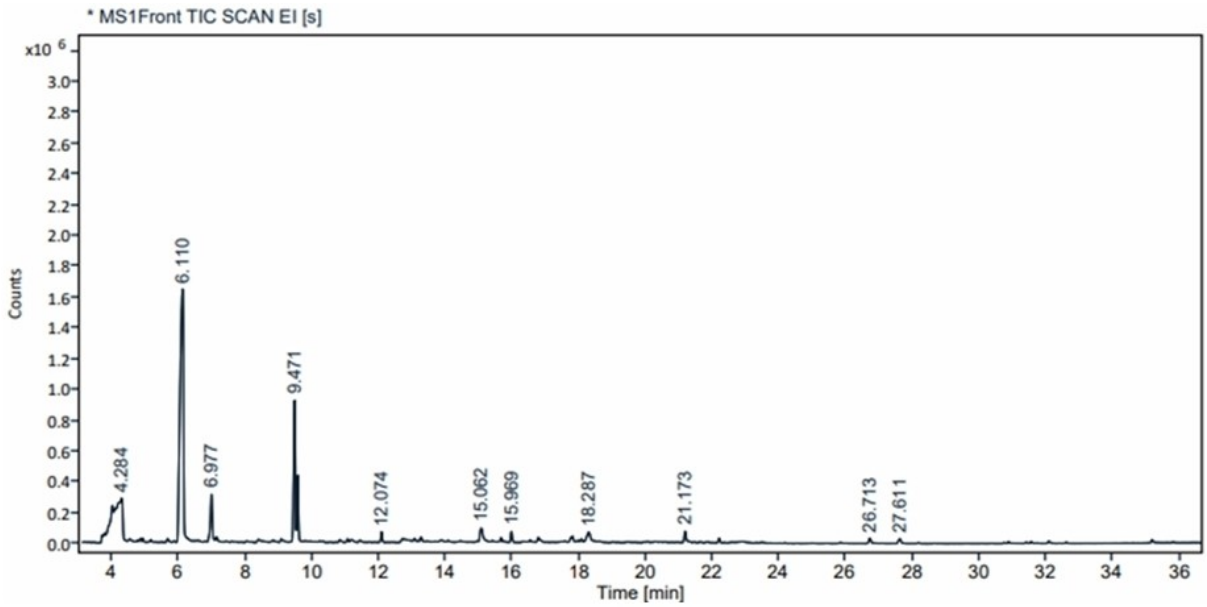

Figure 3. GC-MS spectrum of methanol extract of *B. Acutangula* fruits.

3.7. In Silico Molecular Docking

The selected compounds of GC-MS analysis were docked with β-catechin, protein related migration of dermal fibroblasts; Myc-Max, transcriptional factor in wound healing and TGF-β, signaling protein of epithelialization (Figure 4, 5 and 6). The ligands were formed conventional hydrogen and pi-alkyl bond with the proteins. The compounds such as D-Alanine, N-propargyloxycarbonyl-, isohexyl ester; desulphosinigrin and 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl exhibited greater binding affinity towards β-catechin, Myc-Max and TGF-β respectively. Thus, the results suggested that ethyl acetate extract possess significant wound healing activity due to the greater binding affinity of the compounds present in the ethyl acetate extract. Amongst all the ligands, the highest binding energy (−5.7 kcal/M) towards 1JDH was shown by D-Alanine, N-propargyloxycarbonyl-, isohexyl ester. The ligand desulphosinigrin exhibited highest binding energy of −6.2 kcal/M towards 1NKP whereas the interaction energy was found to be highest (−6.7 kcal/M) by 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl and D-Alanine, N-propargyloxycarbonyl-, isohexyl ester towards 5E8W. The ligands formed conventional hydrogen bond, pi-alkyl interaction, alkyl interaction to amino acid residues of the proteins. Binding affinities (in kcal/M) of compounds present in the methanol extract of fruit of *B. acutangula* are presented in Table 4.

3.8. Characterization of Herbal Gels

3.8.1. Organoleptic Properties

In the current research, the methanol extracts of *B. acutangula* fruits (5% and 10%) was incorporated within 1.5% Carbopol 940-based gels. To prepare the Carbopol 940-based gel-base, other ingredients were triethanolamine, propylene glycol, methyl paraben and propyl paraben. The formulation chart of topical herbal gels is presented in Table 2. Different organoleptic properties of the prepared gels, such as appearance, color and odor of these prepared gel formulations were observed and reported in Table 3. The color of both the prepared herbal gels containing methanol extract of *B. acutangula* fruits (5% and 10%) was yellowish white; whereas the gel base exhibited its clear appearance (Figure 7). A typical odor of *B. acutangula* fruits extract from the prepared herbal gels was noticed. In addition, pH, viscosity, spreadability and gelling strength of these herbal gels were evaluated in comparison to the gel-base and the povidone iodine ointment (Table 3).

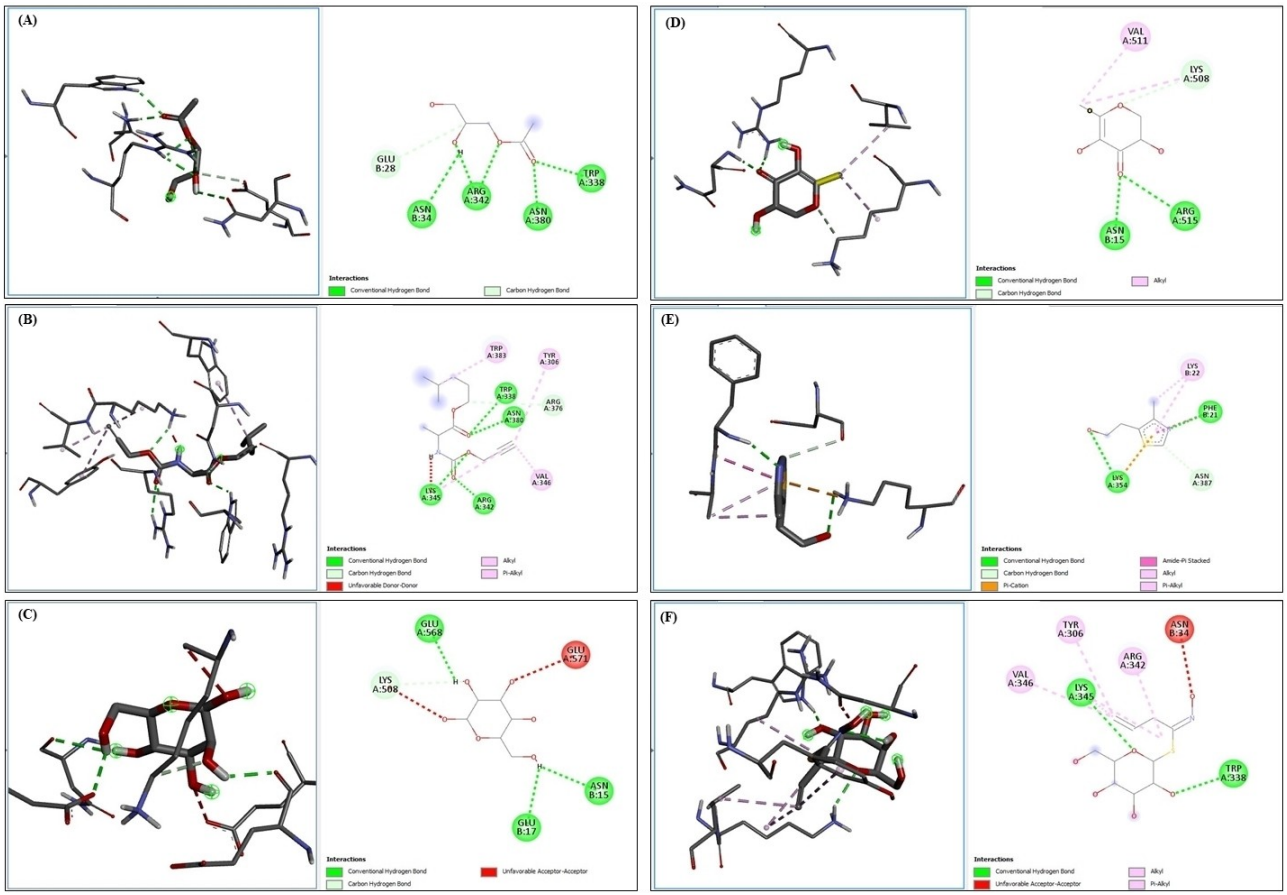

Figure 4. Representation of interactions: (A) 1,2,3-Propanetriol, 1-acetate; (B) D-alanine, N-propargyloxycarbonyl-isohexyl ester; (C) D-Mannopyranose; (D) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; (E) 5-(2-Hydroxyethyl)-4-methylthiazole and (F) desulphosinigrin with β-catenin (PBD ID: 1JDH).

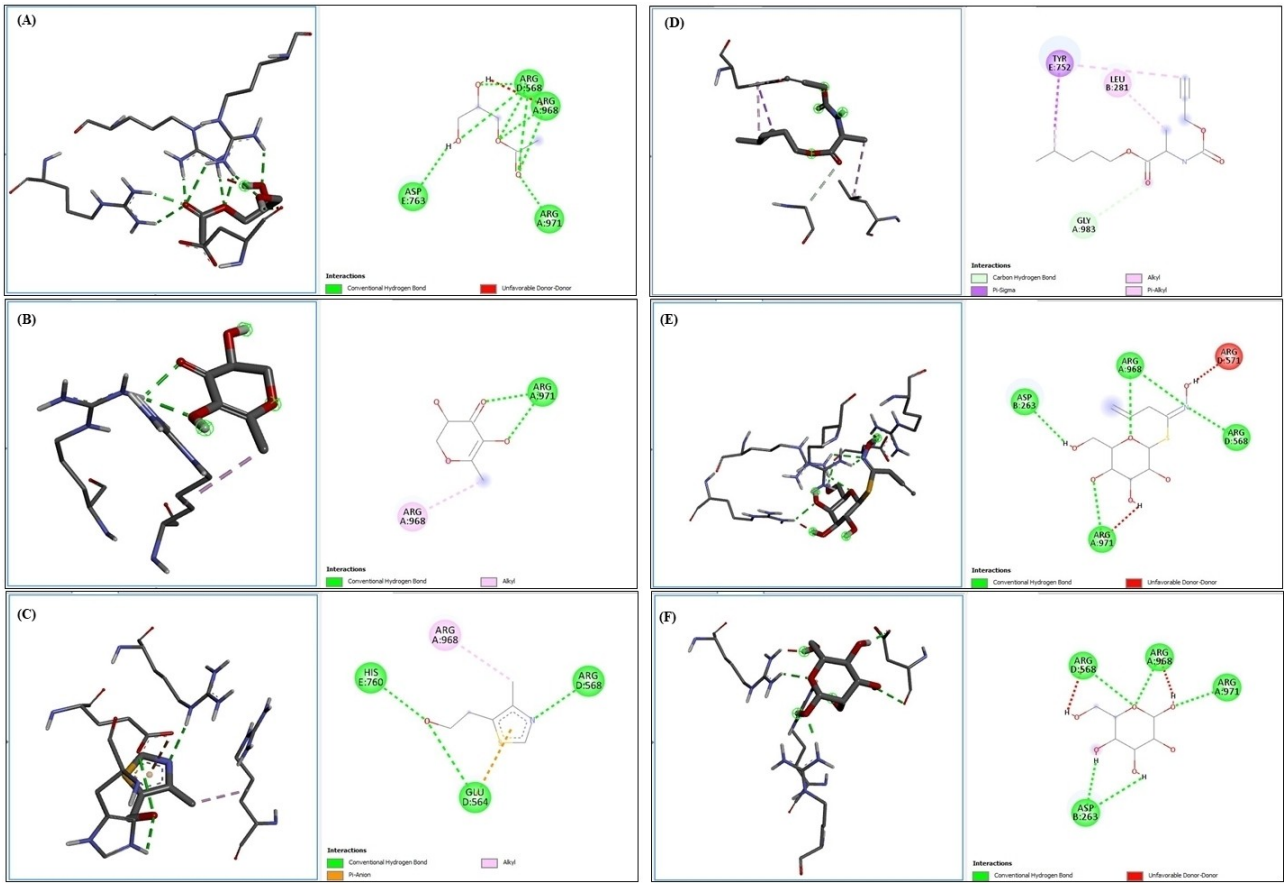

Figure 5. Representation of interactions: (A) 1,2,3-Propanetriol, 1-acetate; (B) D-alanine, N-propargyloxycarbonyl-isohexyl ester; (C) D-Mannopyranose; (D) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; (E) 5-(2-Hydroxyethyl)-4-methylthiazole and (F) desulphosinigrin with c-myc (PBD ID: 1NKP).

Table 3. Organoleptic properties with pH, viscosity, spreadability, and gelling strength.

Group	Color	Odor	Consistency	pH	Viscosity (cP)	Spreadability (cm^2/g)	Gelling strength (%)
Simple base	Clear	Distinctive smell	Thick	6.02	191.6	1.04 ± 0.89	2.2 ± 0.84
Povidone iodine ointment	Yellow-brown	No	Thick	6.05	347.5	1.12 ± 0.59	5.2 ± 0.08
Gel containing 5% herbal extract	Yellowish-white	Typical odor of fruit extract	Thick	6.12	198.5	1.08 ± 0.38	4.1 ± 0.34
Gel containing 10% herbal extract	Yellowish-white	Typical odor of fruit extract	Thick	6.09	248.6	1.09 ± 0.78	4.6 ± 0.69

3.8.2. pH

The pH values of herbal gels containing 5% and 10% methanol extract of *B. acutangula* fruits were found 6.12 and 6.09, respectively (Table 3). The ideal pH range for wound healing gels varies depending on the specific product, but typically falls within the range of pH 5.0 to 7.0.[27] This range is generally considered to be optimal for supporting the natural healing processes of the skin and promoting tissue regeneration.

3.8.3. Viscosity

The viscosities of herbal gels containing 5% and 10% methanol extract of *B. acutangula* fruits were found 198.5 cP and 248.6 cP, respectively (Table 3). The gel base (without methanol extract of *B. acutangula* fruits) exhibited viscosity of 191.6 cP. A high viscous gel may provide better adherence to the wound surface and may be more effective in creating a barrier against bacteria and other contaminants.[28] However, a gel with very high viscosity may be difficult to apply and may not spread evenly over the wound surface.

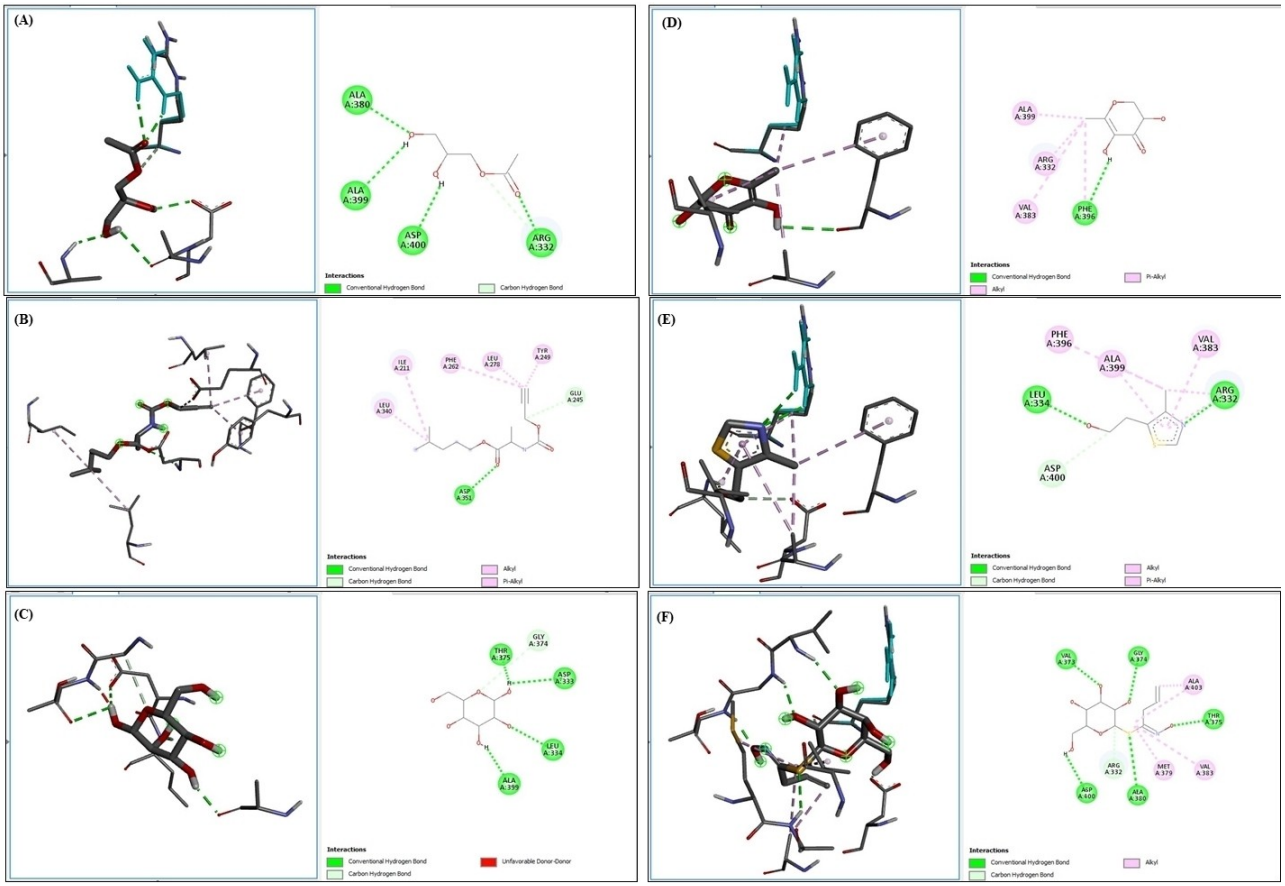

Figure 6. Representation of interactions: (A) 1,2,3-Propanetriol, 1-acetate; (B) D-alanine, N-propargyloxycarbonyl-isohexyl ester; (C) D-Mannopyranose; (D) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; (E) 5-(2-Hydroxyethyl)-4-methylthiazole and (F) desulphosinigrin with TGF-β (PBD ID: 5E8W).

3.8.4. Spreadability

The spreadability values of herbal gels containing 5% and 10% methanol extract of *B. acutangula* fruits were found 1.08 ± 0.38 cm^2/g and 1.09 ± 0.78 cm^2/g, respectively (Table 3). The gel base (without methanol extract of *B. acutangula* fruits) exhibited 1.04 ± 0.89 cm^2/g spreadability. A gel having high spreadability can be easily applied and spread evenly, while a gel having low spreadability may be more difficult to apply and spread.[29]

3.8.5. Gelling Strength

Gelling strength refers to the ability of a gel to maintain its shape and structure under mechanical stress or deformation.[30] It is a measure of the gel's firmness or hardness, and is typically determined by the amount of force required to penetrate or deform the gel. The gelling strengths of herbal gels containing 5% and 10% methanol extract of *B. acutangula* fruits were found 4.1 ± 0.34% and 4.6 ± 0.69%, respectively (Table 3). These gelling strength values were found higher than that of the gel base (without methanol extract of *B. acutangula* fruits). The gel base exhibited 2.2 ± 0.84% gelling strength. The increased gelling strength in cases of herbal gels compared to gel base can be explained by the fact of viscosity increment with the incorporation of extract within it. In general, a gel with a higher gelling strength may provide better support and protection for the wound bed, while a gel with a lower gelling strength may be easier to apply and remove.[31]

3.9. In Vivo Wound Healing

The wound healing process takes place by itself and does not require much help, but various risk factors such as infection and delay in healing brought attention to promote this process. Topical application of herbal gels containing methanol extract of *B. acutangula* fruits at the wound site produced significant wound healing activity.

The wound closure area in case of excision wound after the completion of the treatment by gel base, povidone iodine ointment, and two topical herbal gels containing methanol extract of *B. acutangula* fruits (5% and 10%) are presented in Figure 8a. The percentage wound closure values of excision wound area after the completion of the experimentation was found to be 92.89% and 93.43% for topical herbal gels containing 5% and 10% methanol extract of *B. acutangula* fruits, respectively; whereas the povidone iodine solution exhibited the percentage closure of 95.64% (Table 4). The

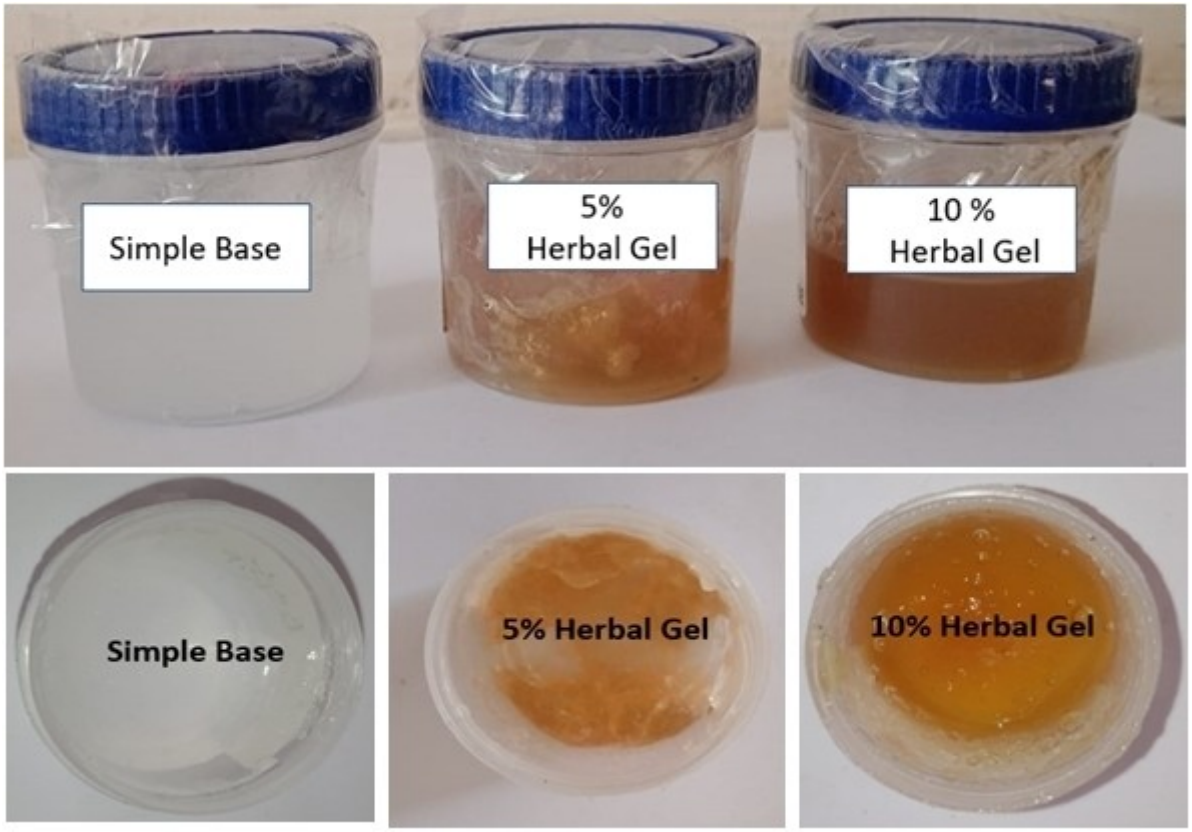

Figure 7. The color of gel base and both the prepared herbal gels containing methanol extract of *B. acutangula* fruits (5% and 10%).

Table 4. *In vivo* wound healing activity results (wound area ± SD) of topical herbal gels containing 5% methanol extract of *B. acutangula* fruits and topical herbal gels containing 10% methanol extract of *B. acutangula* fruits.

Wound area in mm^2/rat										Epithelization period (days)	Scar area (mm^2)
Treatment (topical)	0 day	4th day	8th day	12th day	14th day	16th day	18th day	20th day	22nd day		
Control	417.80 ±0.72	410.53 ±0.50	409.40 ±0.36	401.26 ±0.25	389.76 ±0.68	379.46 ±1.28	376.50 ±0.50	365.90 ±1.01	363.26 ±0.46	12.66 ±0.57	98
Povidone iodine ointment	412.16 ±0.28	390.16 ±0.28	339.83 ±0.76	309.83 ±0.76	279.83 ±0.76	250.16 ±0.28	199.83 ±0.76	189.83 ±0.76	159.83 ±0.76	9.66 ±1.15	75
5% Herbal gel	410.83 ±0.76	358.83 ±0.76	216.83 ±0.76	76.83 ±0.76	42.50 ±0.50	21.83 ±0.76	4.13 ±0.32	0.00	0.00	9 (1.00)	62
10% Herbal gel	415.83 ±0.76	370.83 ±0.76	228.83 ±0.76	67.83 ±0.76	41.83 ±0.76	19.13 ±0.32	3.03 ±0.15	0.00	0.00	8.33 (1.15)	59

current study on the effectiveness of wound healing using excision wound model revealed that the prepared topical herbal gels containing methanol extract of *B. acutangula* fruits increased the wound healing performances in dose dependent healing pattern on 14th post wounding days. The topical herbal gel containing 10% methanol extract of *B. acutangula* fruits showed comparatively higher wound healing activity in comparison to that topical herbal gel containing 5% methanol extract of *B. acutangula* fruits.

The histology of excision biopsy of skin from the group of animals taking 10% gel at 20th day showed healed skin structures with normal epithelization as compared to control, positive control, gel containing 5% herbal extract groups (Figure 9).

The wound closure area in case of incision wound after the completion of the treatment by gel base, povidone iodine ointment, and two topical herbal gels containing methanol extract of *B. acutangula* fruits (5% and 10%) are presented in Figure 8b. Tensile strength of wound (the force required to open the healing skin) is used to measure the completeness of the wound healing. Usually wound healing agents promote the gaining of tensile strength. The tensile strengths of incision area

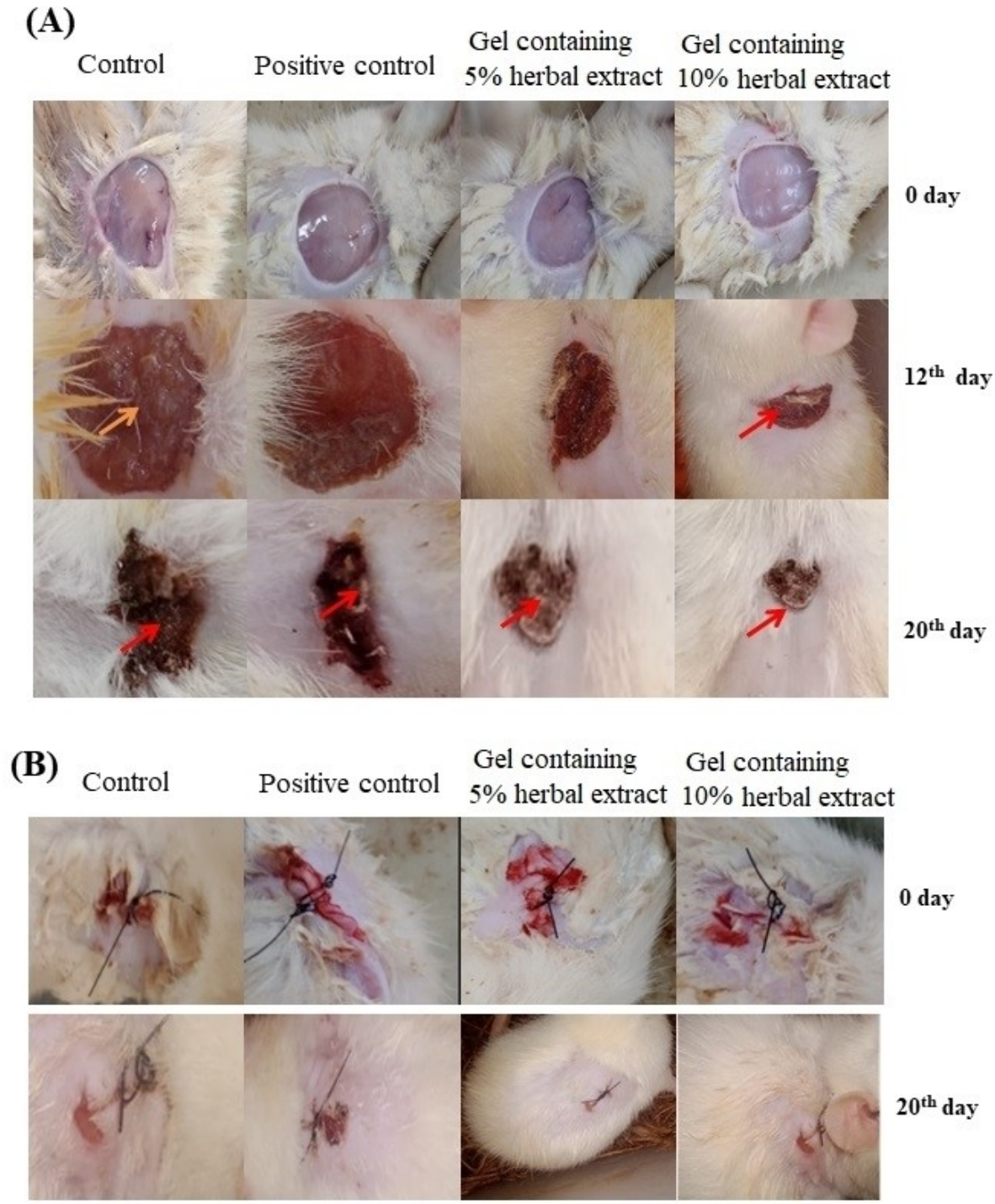

Figure 8. A: Photographic representation showing wound contraction area in excision model in different post excision days of control, positive control, topical herbal gels containing 5% methanol extract of *B. acutangula* fruits and topical herbal gels containing 10% methanol extract of *B. acutangula* fruits. B: Photographic representation showing wound contraction area on different post incision. [Red arrow indicated epithelization. Yellow arrow indicated deep granulation in the wound.]

in rats treated with for topical herbal gels containing 5% and 10% methanol extract of *B. acutangula* fruits were found to be 25 ± 5.12 g and 30 ± 4.10 g, respectively; whereas the povidone iodine ointment exhibited the tensile strength of 15 ± 3.20 g on 20th post wound day (Table 4).

The topical application of herbal gels containing methanol extract of *B. acutangula* fruits in the wounded section promoted healthy granulation in the wounded area. Controlled group animals showed unhealthy granulation, which was dark red in colour, often bleed on contact, and may indicate the presence of wound infection.[20] Promotion of healthy granulation might

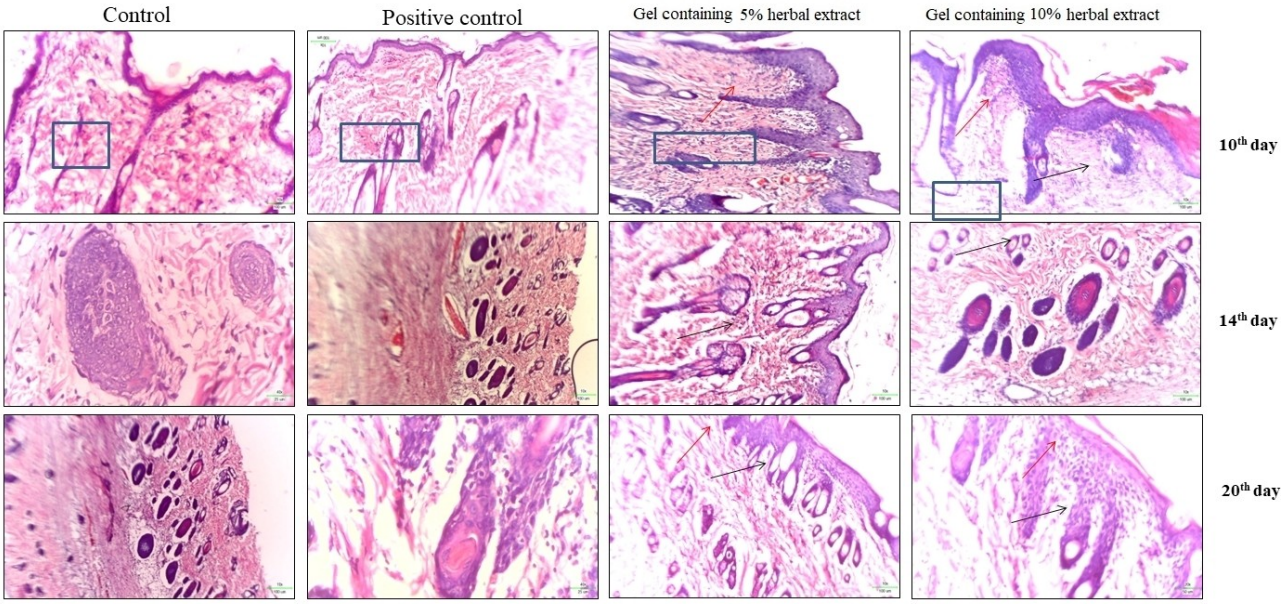

Figure 9. Histopathology of skin at different time frame [Rectangular areas are showing granular formation; whereas black areas are showing the formation of blood vessel.]

be due to the increase in skin permeation capacity of the active constituents present in the extract. The fruit extract's active ingredients might be bound to the proteins c-myc, tgfb, and β-catenin, which are crucial for wound healing. The β-catenin pathway is a conventional network of routes that hasn't changed throughout time. This pathway's major goal is to adjust or regulate the healing process as well as newborn growth and development. This pathway is activated with any type of injury and is in charge of causing fibrosis and encouraging regeneration.[32] The transcriptional alterations that occur, when β-catenin is activated are crucial for wound healing and scar size control.[33] Additionally, it is important for the proliferative stage of wound healing and repairs.[34–36] Studies have also shown that c-myc and β-catenin are abundant in non-healing wounds may be leading to formation of scars. Another element, such as tgfb expression, is essential for maintaining skin homeostasis and promoting angiogenesis and granulation.[37] Additionally, numerous studies have shown that inflammation is brought on by excessive tgfb expression when a wound first develops. The active ingredients in this fruit extract successfully suppressed proteins and controlled the healing process. The effectiveness of the developed topical herbal gels containing methanol extract of *B. acutangula* fruits might be due to the increase in contact time, which might have increased the skin permeability.

4. Conclusions

The current study was intended to evaluate the efficacy of *B. acutangula* fruit extract in quickening healing process in experimental animal model via incorporation within topical gels. *B. acutangula* fruit extract was produced by solvent extraction method. The bioactive extract was transformed into 1.5% Carbopol 940-based topical gel formulations. The *in silico* approach used in this study supported the wound healing potential of the topical herbal gel, as it showed the presence of several bioactive compounds that are known to promote wound healing. The wound healing activity of topical herbal gels containing *B. acutangula* fruit extract in rats was found effectual for the management of the excision and incision wounds. These prepared herbal gel formulations were applied topically over the excision and incision wounds. The results showed a significant improvement in the wound closure rates and histological parameters, indicating its potential to promote wound healing. Therefore, the outcome of this research suggests that these topical herbal gels containing methanol extract of *B. acutangula* fruits could be used clinically for the treatment of wounds. The use of natural products, such as herbal extracts could offer a safe and cost-effective alternative to conventional therapies for wound healing. Furthermore, the use of such kinds of topical herbal formulations could provide a sustainable approach to wound healing management, especially in resource-limited settings. The development of topical herbal gels containing *B. acutangula* fruit extract could have broader implications for the treatment of various skin conditions and diseases.

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Author's contributions

KP conceived and designed the study. AN, SK, AS and CD conducted the experiments. RNS wrote the manuscript. PKN and AKN conceptualized and supervision. All authors have read and approved the final manuscript.

Declartion of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Herbal products · Topical gels · *Barringtonia acutangula* fruit extract · Wound healing, *In silico*

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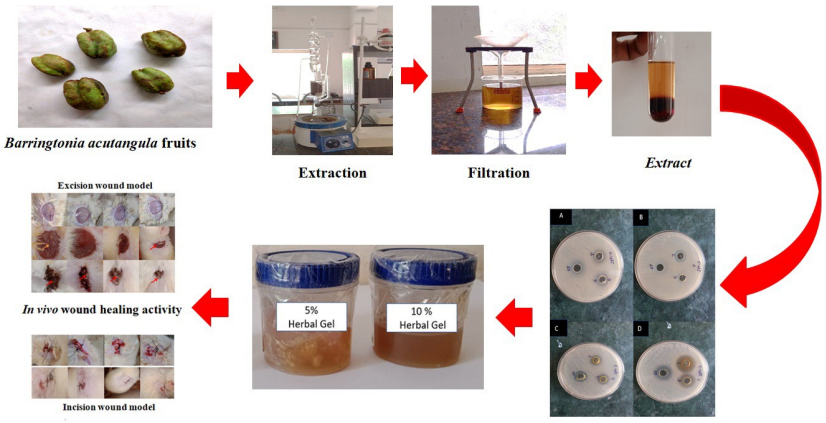

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Wound Healing Activity of Topical Herbal Gels Containing *Barringtonia acutangula* Fruit Extract: *In silico and In vivo* Studies

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Rudra Narayan Sahoo: Conceptualization:Equal; Formal analysis:Lead; Methodology:Lead; Supervision:Lead; Writing – original draft:Supporting
Ashirbad Nanda: Conceptualization:Equal; Formal analysis:Lead; Funding acquisition:Equal; Investigation:Equal; Methodology:Supporting; Resources:Lead; Writing – original draft:Supporting

Satish Kanhar: Investigation:Equal; Methodology:Equal; Resources:Supporting; Software:Lead
Chandan Das: Formal analysis:Equal; Funding acquisition:Supporting; Investigation:Supporting; Methodology: Supporting; Writing – original draft:Supporting
Abhijit Sahu: Formal analysis:Equal; Investigation:Lead; Methodology:Equal; Validation:Equal; Visualization:Lead
Pradeep Kumar Naik: Resources:Lead; Supervision:Equal; Writing – review & editing:Lead
AMIT KUMAR NAYAK: Supervision:Lead; Writing – review & editing:Lead