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Wound healing activity of polyherbal (gel) formulation of *Cissampelos pareira* and *Vaccinium macrocarpon* extracts

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ABSTRACT

Wound healing is a typical biological process. It involves four intricate steps: homeostasis/coagulation; inflammation, migration, and proliferation; reepithelialization; and restoration. A number of mediators, including platelets and cytokines, inflammatory cells, cellular and extracellular matrix, proteinases, growth factors, and inhibitors, have an impact on each stage of the healing process for wounds. In the present work, a polyherbal gel formulation for wound healing activity was developed using methanolic extract of *Vaccinium macrocarpon* and *Cissampelos pareira*. The developed formulation was evaluated for organoleptic parameters like colour, odour and physicochemical parameters like pH, viscosity, spreadability, skin irritation study, etc. *In-vitro* antibacterial activity of both the extracts carried out against *Escherichia coli* by well diffusion assay. Apart from that, the wound healing activity of a polyherbal formulation was evaluated by an incision model on wistar rats. The results showed that the polyherbal formulation exhibited slightly yellowish grey colour with a smooth texture and Semisolid gel. The gel showed pH ranged from 6.1- 6.5, viscosity was F1-1453±0.70, F2-1436±0.27 and F3-1489±0.83cps with spindle number 63, and spreadability was found to be F1-23.99, F2-24.19 and F3-25.20 (g.cm/s), and no irritation was found on the healthy volunteer's skin. In the wound healing activity, polyherbal did not affect the normal feed and water uptake. The wound did not show any pus formation throughout the treatment period. In the Planimetry assessment, wounds treated with polyherbal formulation showed a good score at the end of the treatment. Results showed that polyherbal formulation of *Vaccinium macrocarpon* and *Cissampelos pareira* extracts have good wound healing activity.

Keywords: Wound healing activity, *Vaccinium macrocarpon, Cissampelos pareira,* Polyherbal gel, *Escherichia coli*

Introduction

In many parts of the world, wounds have a major role in morbidity and death. According to studies, there are 10,000 microbial infections related mortality for every million wound patients [1, 2]. In addition to causing pain, loss of function and mobility, depression, distress, and anxiety, embarrassment, and social isolation, chronic morbidity, and even death due to the low rate of complete healing, chronic wounds have a significant

negative impact on the health and quality of life of patients and their families [2]. Debridement, irrigation, the use of antiseptics, antibiotic and corticosteroid therapy, and tissue grafts are some of the current methods for treating wounds. These therapeutic methods come with undesirable side effects, too, including bleeding, tissue damage, contact dermatitis, a delay in wound healing, and the possibility of bacterial resistance [3]. Just 1-3 percent of the medications listed in western pharmacopoeias are intended for use on wounds, despite the enormous advancements made in the pharmaceutical drug industry [4]. More so in developing nations, infection-related morbidity and mortality have grown due to a rise in resistance bacteria, high costs, and a lack of next generation medications [5]. In order to create nontoxic and efficient wound healing agents, there is a huge need for scientific study of medicinal plants. Strong bioactive chemicals produced by plants enable them to communicate with other creatures in their surroundings. These bioactive substances play a crucial role in defence systems and help people avoid sickness. The bioactivity of plant extracts and their constituent parts against harmful pathogenic organisms has been assessed by numerous researchers [6]. *Cissampelos pareira* Linn. (Menispermaceae) is a climbing shrub distributed throughout warm parts of Asia, East Africa, and America. The roots are used as a diuretic and febrifuge, as a remedy for heart trouble, dysentery and soares [7]. Furthermore, the roots are also used to prevent a threatened miscarriage and the herb is used to stop uterine hemorrhage [8]. A novel tropoloisoquinoline alkaloid named pareirubrine A was reported for antileukemic activity [9]. Pradhan et al carried out pharmacological and clinical studies on hayatin methiodide from C. pareira for its muscle relaxant properties [10]. Basu et al reported curare like activity of hyatinin methochloride from C. pareira [11]. Cissamperine and other four bisbenzylisoquinoline alkaloids isolated from C. pareira were found to show significant and reproducible inhibitory activity against human carcinoma of the nasopharynx cell culture (KB) [12]. The roots of this plant are mainly incorporated into many traditional Ayurvedic formulation prescribed for diseases like rheumatism, ulcers, fevers etc. Cranberry (*Vaccinium macrocarpon* L., Ericaceae) has become the subject of interest of the food industry in the last two decades due to the increased awareness of consumers about functional food and its preventive and positive effects on human health. Cranberry is a rich source of valuable phytochemicals, including vitamins and phenolic compounds (e.g. anthocyanins, proanthocyanidins, phenolic acids and flavonols). The profile of bioactive compounds in cranberry differs from other types of berries, as it is rich in proanthocyanidins type A, contrary to the majority of fruits in which proanthocyanidins type B are predominate. Cranberry is recommended as the best source of flavonols among 30 flavonols-containing plants. Different positive health effects of polyphenols are reported, including antioxidant, antimicrobial, anti-cancer, and anti-inflammatory activities, as well as the potential of reducing the risk of cardiovascular diseases. Due to these positive effects attributed to phenolic compounds, which are also found in cranberries, this fruit is recommended for daily use. Cranberries are considered as excellent raw material for the production of juices and other products. Cranberries are mostly consumed in processed form (juices, jams, syrups, or dried) since the sour taste of fresh cranberries is widely unacceptable for consumers. However, during processing their polyphenol content decreases. Also, a significant amount of sugar is added to these products in order to improve the taste, which also reduces the activity of polyphenolic compounds. Six yellow flavonol pigments, namely quercetin-3-galactoside, quercetin-3-ramnoside, quercetin-3-arabinoside, quercetin, myricetin-3-arabinoside and myricetin-3-digalactoside also contribute to the color of cranberry juice p [13].

Present study concluded that *Vaccinium macrocarpon* and *Cissampelos pareira* species is wonderful herb for both antimicrobial and wound healing activity.

Materials and methods

Collection and authentication of plant material

The medicinal plant *Vaccinium macrocarpon* (Leaves) and *Cissampelos pareira* (Leaves) was collected. After cleaning, were dry under shade at room temperature till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration. Authentication of seeds of medicinal plant *Vaccinium macrocarpon* and *Cissampelos pareira* was performed by a plant taxonomist in order to confirm its identity and purity. Authentification no. of *Vaccinium macrocarpon* is AC/037/23 and Authentification no. of *Cissampelos pareira* is AC/038/23.

Chemical reagents

The HiMedia Labs Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. provided all the chemicals used in this study (Mumbai, India). The investigation only employed analytical-grade compounds.

Extraction of plant by soxhlet extraction method

Coarsely powered plant parts of *Vaccinium macrocarpon* and *Cissampelos pareira* (300 gm) was then extracted by successive extraction using different organic solvents, defatted with petroleum ether and successively extracted with methanol for 36 hrs using soxhlet apparatus. To ensure complete extraction each extract was evaporated to dryness under reduced pressure by rotary evaporator and the resulted dried residue was stored in air-tight container for further use [14].

Formula;

$$\% \, yield = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{x} 100$$

Qualitative phytochemical estimation of extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents of *Vaccinium macrocarpon* and *Cissampelos pareira* leaf extracts by using standard procedures**.** The extracts prepared in Ethyl acetate and Methanol was subjected to chemical tests [15, 16].

Quantitative phytochemical estimation

TPC

The total phenolic content of *Vaccinium macrocarpon* and *Cissampelos pareira* extracts was determined using the Folin-Ciocalteu Assay. The *Vaccinium macrocarpon* and *Cissampelos pareira* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically [17].

TFC

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Vaccinium macrocarpon* and *Cissampelos pareira* extracts solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight [18].

Acute toxicity study

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight [19, 20].

Formulation of topical gel

Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container carboxymethyl cellulose and methyl paraben was added into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then triethanolamine (Drop wise) was added to neutralize the pH and Formulations I, II, were 1% of each concentration of extract and formulation III was 2% concentration (i.e. 1% of each extract) were incorporated into the dispersion to obtained gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps.

Table 1: Composition of prepared herbal gel

Name of Ingredient	Formulation I	Formulation II	Formulation III
Carbopol 940	1 gm	1 gm	1 gm
Carboxymethyl cellulose	1 gm	1 gm	1 gm
Propylene glycol	0.5 ml	0.5 ml	0.5 ml
Methyl paraben	0.2 ml	0.2 ml	0.2 ml
Vaccinium macrocarpon	1 gm	----	1 gm
Cissampelos pareira	----	1 gm	1 gm
Triethanolamine	q.s	q.s	q.s
Water	100 ml	100 ml	100 ml

Characterization of extracts loaded gel formulation [21,22]

Physical appearance

The prepared gel formulations were evaluated for appearance, Colour, Odour, and homogeneity by visual observation.

pH determination

pH of the formulation was determined by using Digital pH meter (EI).

Viscosity determination

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 63 at 100 rpm at the temperature of 25^0C.

Spreadability

An ideal topical gel should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was evaluated by placing about 1 g of formulation on a glass slide. Another glass slide of the same length was placed above that, and a mass of 50 mg was put on the glass slide so that the gel gets sandwiched between the two glass slides and spreads at a certain distance. The time taken for the gel to travel the distance from the place of its position was noted down. Spreadability was determined by the following formula

$$S= M*L/T$$

Where, S-Spreadability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T-Time for spreading gel in sec.

Skin irritation test

The intact skin of Wistar rats of either sex with average weight 150– 200 g was used. The hairs were removed from the rat 2-3 days before the experiment. The gel was applied on the properly shaven skin of rat. The animals were treated daily for 2-3 days, and undesirable skin changes, i.e., change in color, change in skin morphology was checked for a period of 24 h and erythema and edema on the treated skin were examined.

Wound healing studies

A partial thickness burn wound model was employed as per [23]. The rats were anaesthetized with diethyl ether and the hair on the back was shaved with a sterile blade. The shaved area was disinfected with 70% (v/v) ethanol. Then burn wound was created by pouring hot molten wax (2 gm) at (80 °C). The wax was allowed to remain on the skin till it gets solidified. Immediately after injury and on subsequent days, all the gel was daily applied topically for 21 d or till complete epitheliazation which ever will earlier. After animal recovered completely from anesthesia, they were kept in individual cages and followed all norms of good laboratory practice in carrying the animals. The animals were randomly divided into 5 groups and each group containing 6 animals. The treatments of each gel (500 mg/rats) were applied topically once a day.

Group I: Control group.

Group II: Test group treated with *Vaccinium macrocarpon* gel. (Formulation I)

Group III: Test group treated with *Vaccinium macrocarpon* gel. (Formulation II)

Group IV: Test group treated with (*Vaccinium macrocarpon* and *Cissampelos pareira*) (Formulation III)

Group V: Gentamicin gel (Reference Standard Marketed Preparation).

Wound contraction was monitored by measuring the progressive changes which the wound surface area was evaluated. The tracing was then transferred to 1 mm^2 graph sheet, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 300 mm^2, as 100% by using the following equation.

$$\text{Percentage of wound contraction} = \frac{Initialwoundarea - Specificdaywoundarea}{\text{Initial wound area}} \times 100$$

Antimicrobial activity (Well Diffusion Assay)

Anti-bacterial Activity

Preparation of dilutions of the samples

The dilutions of the samples were made for the concentration as 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml respectively of the sample, after that volume makeup was done with distilled water till 1ml.

Preparation of nutrient agar media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121 o C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

Well diffusion assay

Culture of bacterial strains (*E. coli*) was spread on the Nutrient agar media (NAM). The wells were then formed for the inoculation of the samples (Formulation 1, Formulation 2 and Formulation 3) given in the different concentrations, volume make-up was donetill 1 ml. 100 µl of the sample was loaded. The plates were allowed to incubate at 370 C for 48-72 hours for the best results. The bacterial suspension was standardized to10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculum from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, over the entire sterile agar surface. Four wells of 6 mm were bored in the inoculated mediawith the help of sterile cork-borer. Each well was filled with different concentration (100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) of samples. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37 o C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well [24, 25].

Results

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Vaccinium macrocarpon* and *Cissampelos pareira* is shown in Table 2. The results of qualitative phytochemical analysis of the crude powder of leaf of *Vaccinium macrocarpon* and *Cissampelos pareira* were shown in Table 3, 4. Methanolic and Pet. Ether of *Vaccinium macrocarpon* and *Cissampelos pareira* showed the presence of alkaloids, glycosides, flavonoids, saponins, phenols, proteins and amino acids

and carbohydrate ect. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.011X+0.011, R^2= 0.998,** where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as rutin equivalent (mg/100mg) using the equation based on the calibration curve: **Y=0.001X + 0.131, R^2=0.955**, where X is the rutin equivalent (QE) and Y is the absorbance Table 5, 6 & Figure 1,2. An evaluation of the gel, including colour, appearance and homogeneity, was conducted. Gel was discovered to have a slightly yellowish grey colour to it when tested. Gel exhibited the same colour, and Appearance as the I.P. requirements for these characteristics and the results were listed in Table 7. The pH of all prepared formulation ranged from 6.1- 6.5. The pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin. Viscosity is an important property of fluids which describes a liquids resistance to flow and is related to the internal friction within the fluid. This rheological property helps in determining consistency and also the diffusion rate of drug from gel. The measurement of viscosity of the prepared gel was done with Brookfield viscometer with spindle no: 7. Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. Spreadability of different gel formulation was studied. The formulations produced good spreadability. Results of skin irritation test indicate that prepared gels were not produce irritation, redness, or edema on application and free from dermatological reaction Table 8. Wound contraction is another parameter used to assess wound healing. Significant wound contraction was shown in Table 9 & Figure 3, 4. The *in vitro* antibacterial activities of the extracts of Formulation 1, Formulation 2 and Formulation 3 samples have been investigated. Antibacterial activity was performed against *E. coli* by well diffusion assay with concentration ranging 100µg/ml. Formulation1 extract showed best zones of inhibition of 20 mm in diameters at 100µg/ml concentration against *E. coli*. Similarly, Formulation 2 extract showed best zones of inhibition of 19 mm in diameters at 100µg/ml concentrations against *E. coli*. Formulation 3 showed best zones of inhibition of 25 mm in diameters at 100µg/ml concentration against *E. coli* Table 10.

Discussion

Qualitative phytochemical screening of *Vaccinium macrocarpon* is showed the presence of active metabolites such as alkaloids, flavonoids, proteins, saponin, glycosides, phenols and steroids is presented and *Cissampelos pareira* showed the presence of active metabolites such as alkaloids, glycosides, flavonoids, saponin, phenols, proteins and amino acid. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 500 mg/kg have been fixed for study. The pH of all prepared formulation ranged from 6.1- 6.5. The pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin. The measurement of viscosity of the prepared gel was done with Brookfield viscometer with spindle no: 63. the results were found to be Formulation 1- 1453, Formulation 2 -1436 and Formulation -1489 cps. Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. Spreadability of different gel formulation was studied. The formulations produced good spreadability. Skin irritation test indicate that prepared gels were not produce irritation, redness, or edema on application and free

from dermatological reaction. The *in vitro* antibacterial activities of the extracts of Formulation 1, Formulation 2and Formulation 3have been investigated. Antibacterial activity was performed against *E. coli* by well diffusion assay with concentration ranging 100µg/ml. Formulation 3showed best zones of inhibition of 25 mm in diameters at 100µg/ml concentration against *E. coli*. Further we were performed the wound healing studies like contraction of wound model for 21 days. For wound healing activity, the extracts were loaded in the gel. The development wound curing activity by excision model was evaluated by wound shrinkage of the excision wound of different groups Group I: Control group, Group II: Test group treated with *Vaccinium macrocarpon* gel (Formulation I), Group III: Test group treated with *Cissampelos pareira* gel (Formulation II), Group IV: Test group treated with (Polyherbal gel) (Formulation III), Group V: Gentamicin gel (Reference Standard Marketed Preparation). The wound contraction studies revealed that the wound contraction increases on increasing the concentration of herbal extract. The study also reveals that the better activity of polyherbal formulation may be due to the synergistic action of the plants constituents present in the formulation. Thus, the prepared topical gels possess a versatile approach in healing the wound contraction.

Conclusion

Herbal extracts gel containing the extracts of *Vaccinium macrocarpon* and *Cissampelos pareira* promotes wound healing in albino Wistar rats with a comparison of the synthetic formulation. The wound contraction rate was higher in the treatment of gel containing the combination of *Vaccinium macrocarpon* and *Cissampelos pareira.*

Table 2: Percentage yield of crude extracts of *Vaccinium macrocarpon* and *Cissampelos pareira* extracts

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Vaccinium macrocarpon*	Pet ether	300	1.56	0.52%
2		Methanol	350	5.58	1.86%
S. No	**Plant name**	**Solvent**	**Theoretical weight**	**Yield(gm)**	**% yield**
1	*Cissampelos pareira*	Pet ether	300	1.30	0.43%
2		Methanol	367	5.95	1.62%

Table 3: Phytochemical testing of *Vaccinium macrocarpon*

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		

3.1	Molish's test	Absent	Absent
3.2	Fehling's test	Absent	Absent
3.3	Benedict's test	Absent	Absent
3.4	Barfoed's test	Absent	Absent
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Present
8.2	Libbermann-Burchard's test	Present	Present

Table 4: Phytochemical testing of *Cissampelos pareira*

S. No.	**Experiment**	**Presence or absence of phytochemical test**	
		Pet. Ether extract	**Methanolic extract**
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Absent
3.2	Fehling's test	Absent	Absent
3.3	Benedict's test	Absent	Absent
3.4	Barfoed's test	Absent	Absent
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Present
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present

5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Absent	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Absent
8.2	Libbermann-Burchard's test	Absent	Absent

Table 5: Total phenolic content in extracts

	Total phenolic content (mg/gm equivalent to gallic acid)	
Extracts	***Vaccinium macrocarpon***	***Cissampelos pareira***
Absorbance (mean±SD)	0.179±0.07	0.196±0.09
TPC	76	93

Table 6: Total flavonoid content in extracts

	Total flavonoid content (mg/gm equivalent to rutin)	
Extracts	***Vaccinium macrocarpon***	***Cissampelos pareira***
Absorbance (mean±SD)	0.156±0.010	0.170±0.009
TFC	25	39

Table 1: Organoleptic properties

S. No	**Parameters**	**Results**
1.	Appearance	Semisolid gel
2.	Colour	Slightly yellowish grey gel
3.	Homogeneity	Absence of aggregates

Table 8: Results of measurement of pH, viscosity, spreadability and skin irritation

S. No	**Formulation**	**pH**	**Viscosity(cps)**	**Spreadability (gm.cm/sec)**	**Skin irritation**
1.	Formulation1	6.1	1453±0.70	23.99	Not irritant
2.	Formulation 2	6.2	1436±0.27	24.19	Not irritant
3.	Formulation 3	6.5	1489±0.83	25.20	Not irritant

Table 9: Percentage wound closure in various treatment groups

Sr. No.	**Formulation**	**Area of wound during different days of observation (%)**				
		4 day	8 day	12 day	16 day	21day
1	Control	8.31±0.7120	8.45±0.8143	8.49±0.7820	8.39±0.8848	8.42±0.9819
2	Formulation I	8.98±0.4048	25.02±0.4355	39.19±0.7070	59.99±0.5421	69.95±0.5532
3	Formulation II	8.39±0.7823	20.12±0.5239	51.45±0.5519	68.41±0.5532	75.87±0.5824
4	Formulation III	11.08±0.8599	28.13±0.9523	55.44±0.6679	89.11±0.3359	91.99±0.3826
5	Reference Standard(Gentamicin gel)	12.42±0.7437	32.09±0.7279	61.03±0.6429	90.99±0.6431	93.75±0.2523

Table 10: Antimicrobial activity

Concentration (µg/ml)	**Zone of Inhibition (in mm)**		
	Formulation 1	**Formulation 2**	**Formulation 3**
100	20 mm	19 mm	25 mm

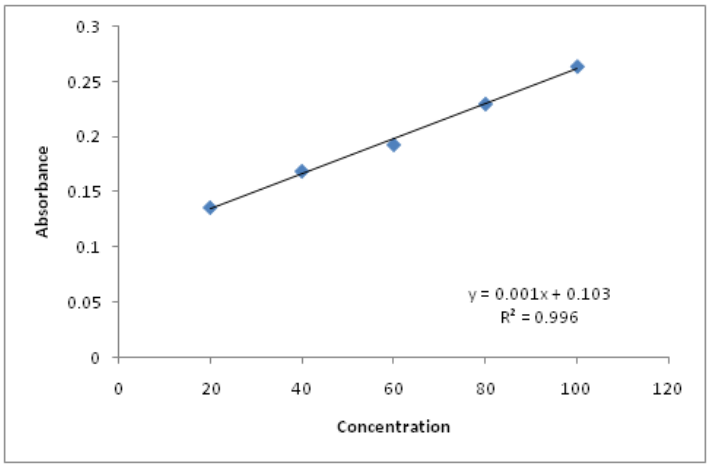


Figure1: Standard curve of gallic acid

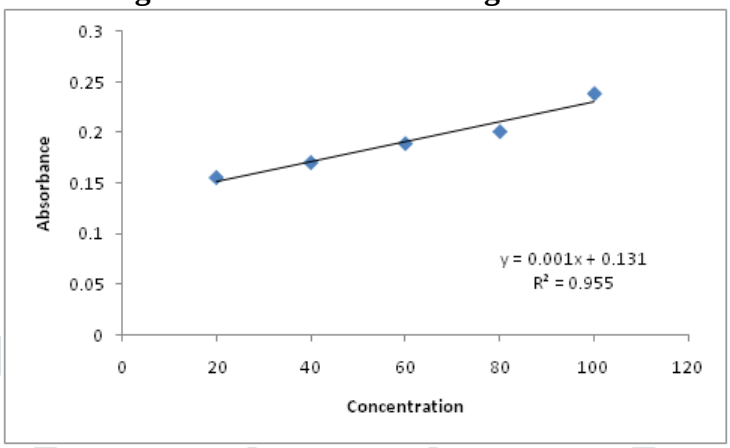


Figure 2: Standard curve of rutin

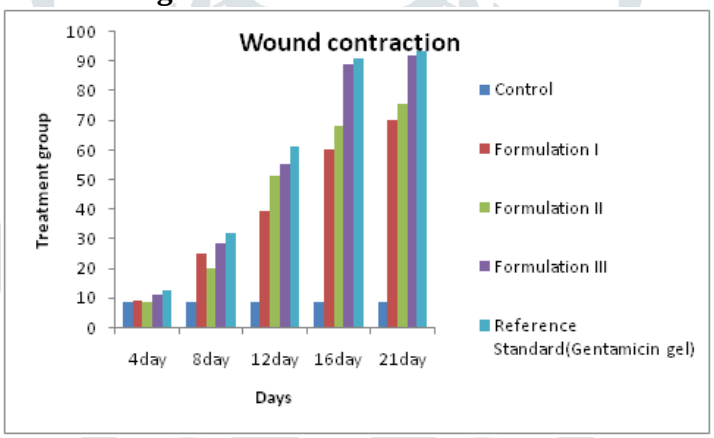
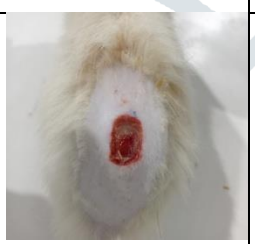
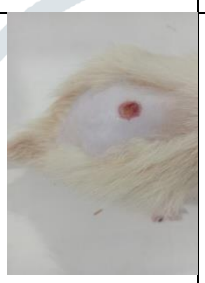
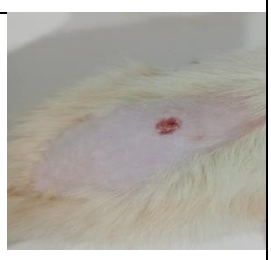
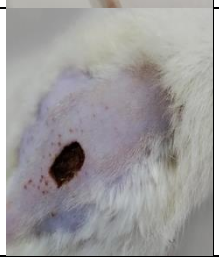
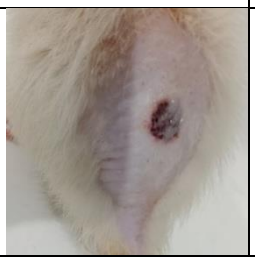
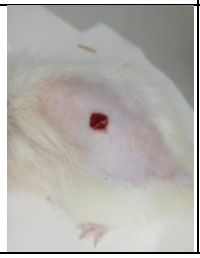
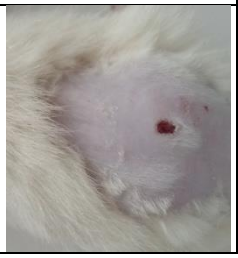
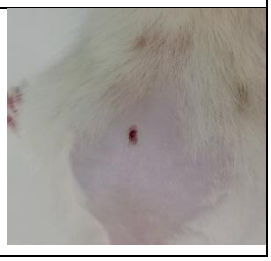


Figure 3: Evaluation of wound healing activity

Group	4 Day	8 Day	12 Day	16 Day	21 Day
Control					
Formulation I					

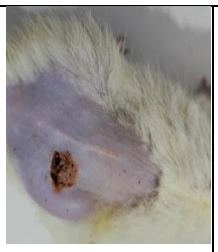
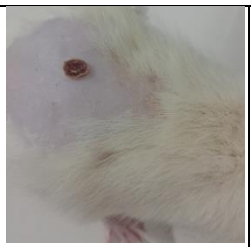
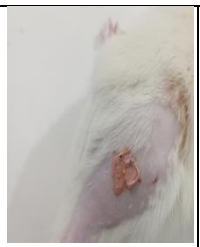
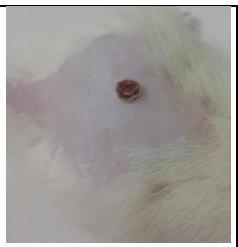
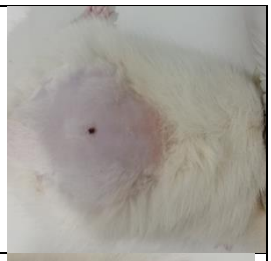
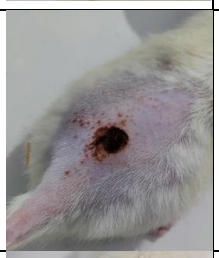
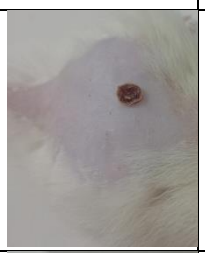
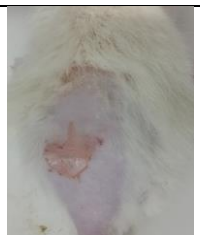
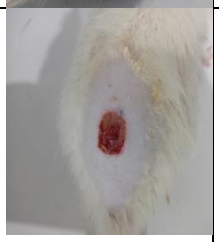
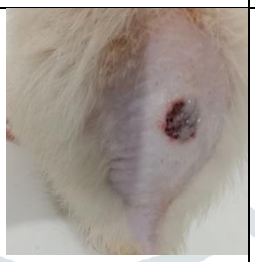
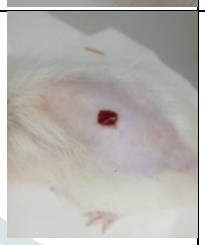
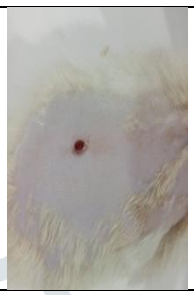
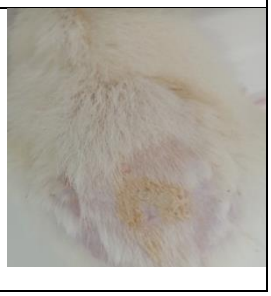
Formulation II					
Formulation III Polyherbal gel					
Standard(Gentamicin gel)					

Figure 4: Images of wound closure in various treatment groups

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