



The Preliminary Phytochemical Screening and Evaluation of Herbal Antibacterial Gel of *Ipomoea Carnea*

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Abstract

Herbal medicine has become an item of global importance both medicinal and economical. Herbal remedies are increasing patient compliance as they are devoid of typical side effects of allopathic medicine. In this experimental study, herbal gel containing *Ipomoea carnea* methanolic flower extract was formulated and evaluated for antibacterial activity. The gel formulations were prepared by using Carbapol 940, *Ipomoea carnea* flower extract, propylene glycol, methyl paraben, propyl paraben and required amount of distilled water. The skin pH (6.8-7) was maintained by drop wise addition of Tri-ethanolamine. The physical parameters of formulated gel like colour, appearance, homogeneity, pH, viscosity and spreadability were evaluated. The gel were evaluated for antibacterial efficiency by agar diffusion method against some bacterial agents. The herbal gel shows that the formulation containing *Ipomoea carnea* flower extract has better antibacterial activity.

Keywords

Ipomoea carnea; Herbal gel; antibacterial; Carbopol 940; Spreadability; Viscosity.

INTRODUCTION

Throughout the ages, medicinal plants have been widely used in traditional medicine because of viability, safety, low toxicity, and pharmacological potential. (1) *Ipomoea carnea* which is generally known as bush morning glory. It is well distributed in India and found particularly in Chhattisgarh and Madhya Pradesh. In India, it has become a naturalized species invading the wetlands, canals, drain banks, waste lands, field edges and road sides. *Ipomoea carnea* grows up to a height of 6 m on terrestrial land but acquires a shorter height in the aquatic habitats. The species is used as folk medicine in traditional medicinal system including ayurveda, siddha and unani. Leaves are used as purgative. Leaves paste is applied on 'Haja' (a kind of sore between toes and finger due to fungal infection). It is reported to have stimulatory allelopathic effects. Roots are boiled to

use as laxative and to provoke menstruation. Traditional healers for treatment of skin diseases have used it. The milky juice of plant has been used for treatment of leukoderma and other related skin diseases. Only external applications have been recommended due to the poisonous nature of the plant. It has a depressant effect on the central nervous system. Also shows muscle relaxant property. The different parts of these plants have been reported to be used in folk medicine for the treatment of several disorders such as venereal and skin diseases, immunodeficiency, dysentery, gout, rheumatism, and hypertension. Moreover, several biological evaluations of the different extracts of *Ipomoea carnea* have been documented, including antimicrobial, anticancer, free radicals scavenging, antidiabetic, immunomodulatory, wound healing, anticonvulsant, anxiolytic, anti-inflammatory,

sedative, and hepatoprotective. (2) The chemical analysis of *Ipomoea carnea* plants has shown that they have several metabolites, including terpenes, flavonoids, coumarins, lignans, alkaloids. Also, tannins, amino acids, proteins, carbohydrates, sterols, and saponins. Swains nine and calystegines have been documented as the main components of *Ipomoea carnea*. (3)

Profile of *Ipomoea carnea* plant:

Scientific classification:

Table no. 1. Scientific classification of *Ipomoea carnea* Plant.

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Solanales
Family	Convolvulaceae
Genus	<i>Ipomoea</i>
Species	<i>Ipomoea carnea</i>

Fig.1 *Ipomoea carnea* flower



International Common Names:

- English: Bush morning-glory; tree morning glory
- India: Behaya, Besharam, Pink morning glory, Shrubby morning glory

Other Scientific Names:

- *Batatas crassicaulis* Benth.
- *Convolvulus batatilla* Kunth
- *Ipomoea fistulosa* Mart. ex Choisy

MATERIAL AND METHODS:

Chemicals and reagents:

The various chemicals used throughout experimental work are summarized.

Table No.2. List of chemicals used in experimental work.

Sr. No.	Chemicals
1	Methanol
2	Ethanol
3	Carbopol 940
4	Methyl paraben
5	Propyl paraben
6	Propylene glycol
7	Triethanolamine

8	Distilled water
9	Rose water
10	Streptomycin
11	Ethyl alcohol
12	Acetic acid
13	Agar
14	Peptone
15	Sodium chloride
16	Beef extract
17	Dimethyl sulfoxide

Instruments

The various instruments used throughout experimentation are given in Table no.3 Table no.3. Instrument used in experimental work.

Sr. No.	Name of instrument	Brand name
1	Analytical Balance	Contech
2	Digital Balance	Shimadzu, AU×220
3	Hot Air Oven	Tempo
4	Digital Autoclave	ASI-254
5	B.O.D Incubator	HMG

- Calibrated glassware used during experimentation.

Collection and Authentication

The *Ipomoea carnea* flowers were collected from the wetlands, canals, drain banks and from roadside, the collected flower were shade dried for 1 week then powder by mechanical manner and packed in airtight container. The sample was identified, and authentication done from Dhote Bandhu Science college.

Preparation of methanolic extract of *Ipomoea carnea*

Plant flowers were shade dried at room temperature for 10 d. The sample preparation was powdered by using a mechanical grinder. For methanol extract 10 gram of powdered material were taken in five hundred capacity thimbles of Soxhlet apparatus and refluxed with methanol and water separately until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a dark color. The extract was used for phytochemical analysis and antibacterial assay.

Concentrate the methanol extract of *Ipomoea carnea*

The Methanolic extract of *Ipomoea carnea* is concentrate on the rectangular water bath for 48hrs at 100°C.

Preparation of gels:

Firstly Carbopol 940 was dispersed in distilled water and purified water kept the beaker aside to swell the Carbopol 940 for half and hrs. Stirring should be done to mix the Carbopol 940 to form gel. Then in another beaker weight and transfer the required quantity of extracted drug and dissolved in propylene glycol and

the solution was added and mixed to the first solution. 10 ml of distilled water was taken, and the required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath and cooling the solution. Finally, all mixed ingredients were mixed properly to the Carbopol 940 gel with continuous stirring and lastly add triethanolamine drop wise to the formulation of adjustment of required skin pH (4.7-5) and to obtain the gel as required consistency.

Procedure for antibacterial activity by pour plate method:

The agar well diffusion method describes by Zaria, (1995) was adopted for the antimicrobial sensitivity test. For antibacterial studies, the microbial strains of *Escherichia coli* and *Streptococcus aureus* were collected from Manoharbai Patel Institute of Bachelor of Pharmacy, Kudwa, Gondia.

Prepare nutrient agar Petri plates for the growth of bacterial cultures. Pour the cultures in agar media. The test cultures used such as *Streptococcus aureus* and *Escherichia coli*. Prepare well seeded plates by using cork borer that is sterile by burning with absolute ethanol. Plant extract 1 ml of (0.1 mg/ml) is added in the labeled well and incubated. One well is prepared as control using streptomycin having 1 ml of (0.1 mg/ml) of pure solvent Dimethyl sulfoxide (DMSO). Bacterial test culture plates are incubated at 32-37° c for 48 hrs.

Minimum Inhibitory Concentration (MIC):

MIC by turbidity method:

Prepare nutrient broth test tubes and label. In the first tube (UT), inoculums is not added which issued for checking the sterility of medium and as a negative control. Other all test tubes, inoculums (three to four drops) is added to reach the final concentration of microorganism is 10⁶ cell/ml. In all test tubes, test antimicrobial compound is added about 0.1 to 1.0 ml except uninoculated (negative control) and control (positive) tube. The positive control tube is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculums.

Adjust the final volume (10 ml) in all test tubes by using sterile water. All test tubes are properly shaken and then incubated at 37°c for two days.

Thin Layer chromatography:

The pharmacologically active methanolic extract obtained from flowers of *Ipomoea carnea*. was subject to thin layer chromatography to find out number of compound present in it.

EVALUATION OF ANTIBACTERIAL GEL:

1. Physical appearance:

The prepared gel formulations containing *Ipomoea carnea* were inspected visually for their color, homogeneity, consistency, and phase separation.

2. Measurement of pH:

The pH of developed gel formulations was determined using digital pH meter. 1 gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

3. Spread ability:

Spread ability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spread ability was measured on the basis on slip and drag characteristics of gels. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weight was placed on the top of the two slides for 5 min. to expel air and to provide a uniform film of the gel between the slides. Excess gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spread ability.

Spread ability was calculated using the following formula:

$$S = M \times L / T$$

4. Determination of viscosity:

The Brookfield viscometer was used to test the viscosity, with the proper number of spindles selected. The 50 ml beaker was used to hold 50gm of preparation until the spindle groove was dipped and the rpm was set. Gel viscosity was measured at 5,10,20,50, and 100 rpm. The viscosity was computed using the factor obtained from the reading.

5. Washability:

This test is carried out by simply washing applied gel on the skin surface with water.

6. Homogeneity:

The formulation was tested for homogeneity by visual appearance and touch.

7. Irritancy Test:

Mark an area (one sq. cm) on the left-hand dorsal surface. The gel was applied to the specified area and time was noted. Irritancy erythema, edema was checked for regular intervals up to 24 hrs. and reported.

8. Stability Testing:

Stability testing of formulation was conducted at room temp, studied for 7 days. And then the formulation studied at $45 \pm 1^\circ\text{C}$ for 20 days. The formulation was kept both at room and elevated temperature and observed on 0th, 5th, 10th, 15th, and 20th day for all the evaluation parameters.

RESULT AND DISCUSSION

- **Phytochemical Screening:** The phytochemical Screening of herbal gel is done for identification of active chemical ingredients present in the flower of *Ipomoea carnea*.

Table no.5. Phytochemical screening of *Ipomoea carnea* flower extract.

Sr. no.	Tests	Observation	Result
Test for alkaloid:			
1	a. Dragendorffs test	Orange ppt Pale yellow ppt	+ve
	b. Mayers test		
	c. Wagners test		
	d. Hagers test		
Test for carbohydrates			
2	a. Molish test	Purple ring at junction Brick red ppt.	+ve
	b. Fehling test		
Test for flavonoids:			
3	a. Lead acetate test	Yellow Color observed. Pale yellow ppt.	+ve
	b. Shinoda test		
Test for Tannins			
4	a. Ferric chloride test	Dark greenish black color observed.	+ve
Test for Saponin:			
5	a. Foam test	Foam formation.	-ve
	b. Libermann-Bruchard test	Colour change from red to green reveals.	
Test for Amino acids:			
6	a. Ninhydrin test	Purple bluish color	+ve
Test for Glycosides:			
7	a. Killar-killani test	Formation of two layers	+ve
	b. Baljet test	Colour is change from yellow to orange.	
Test for Triterpenoids:			
8	a. Salkowaski test	Yellow color observes.	+ve
	b. Libermann-burchard test	Formation of brown ring at the junctionof two liquids.	
Test for Protein:			
9	a. Biuret test	Production of blue colour.	-ve

- **Solubility test of extract:**

Table no.6. Stability test of *Ipomoea carnea* flower extract.

Sr.no.	Solvents	Result
1.	Water	Soluble
2.	Ether	Sparingly soluble
3.	Methanol	Soluble
4.	Ethanol	Sparingly soluble
5.	Sulphuric acid	Soluble

- **Thin layer chromatography:**

Table no.7. Observation TLC of *Ipomoea carnea*.

Solvent system	UV Light	No. of component	<i>Ipomoea carnea</i> Rf value
Methanol: Water (8:1)	366 nm	1	0.346
Ethyl alcohol:Acetic acid(5:5)	366nm	1	0.258

Antibacterial evaluation:
Table no.8. Table for Antibacterial Evaluation.

Sr.no	Test culture	Positive (1mg/ml)	Plant extract (0.1g/ml)	Zone of inhibition in (mm)						
				DMS O	Positive control	Plant Extract	Formulation			
							F1	F2	F3	F4
1.	E.coli	Streptomycin	<i>Ipomoea carnea</i> flower extract	-	06	04	2	3	5	7
2.	S.aureas	Streptomycin	<i>Ipomoea carnea</i> flower extract	-	07	04	2	4	5	5

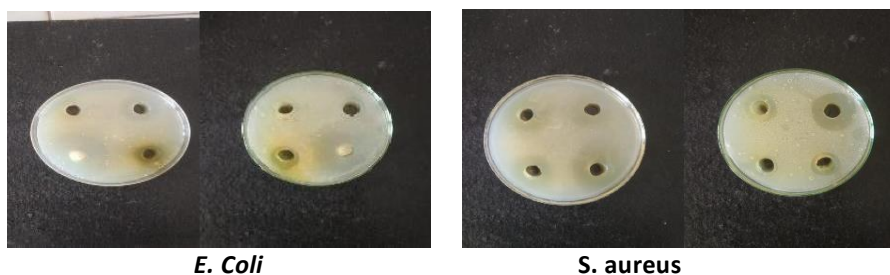
- Minimum Inhibitory Concentration of *Ipomoea carnea*.**

Table.no.9. Minimum Inhibitory Concentration of *Ipomoea carnea*.

Sr. no.	Microorganism	Concentration(µg/ml)										MIC (µg/ml)
		50	100	150	200	250	300	350	400	450	500	
1.	<i>Ipomoea carnea</i> S.aureus	-	-	-	-	+	-	-	-	-	-	250
	<i>Ipomoea carnea</i> E.coli	-	-	-	+	-	-	-	-	-	-	200

Turbidity: Present = (+) ; Absent = (-)

MIC value of *I. carnea* for S.aureus and E.coli shows to 250µg/ml and 200 µg/ml.


Fig.6. Antibacterial Activity of crude extract and formulation against s. aureus and E. coli.
Characterization of Herbal gel:

- Colour and Appearance:**

Table no.10. Colour and appearance of Formulated herbal gel.

Test	Result
Color	Light brown
Appearance	Gel like consistency

- pH of the formulation:**

Table no.11. pH of Formulated herbal gel.

Sr. no.	Batch	pH
1	F1	4.97
2	F2	4.99
3	F3	5.13
4	F4	4.55

- Viscosity:**

Table no.12. Viscosity of formulated herbal gel.

Sr.no.	Batch	RPM	CP
1	F1	20 rpm	1088
2	F2	20rpm	1402
3	F3	20rpm	1544
4	F4	20rpm	1229

- Spread ability of Formulation:**

Table no.13. Spread ability of Formulated herbal gel.

Sr. no.	Batch	Spread ability
1	F1	17.3
2	F2	18.2
3	F3	22
4	F4	24.3

CONCLUSION

The present investigation was designed with the objective of formulating and evaluating herbal antibacterial gel of *Ipomoea carnea*. We get the extract of *I. carnea* by using Soxhlet apparatus and concentrated. The obtained quality of concentrated extract is good. The *I. carnea* extract shows effective against *E. coli* and S. aureus. Then the final concentration of extract is obtained, and the concentration varies in F1(0.05%), F2(0.1%), F3(0.15%), F4(0.2%). The formulation was prepared using the above concentration of extract. Accordingly, the formulation F3 (0.15% *Ipomoea carnea* extract, 3gm of carbopol, 1 ml of

rose water) was formulated successfully which gave better zones of inhibition and it shows significant effect against bacteria. Herbal formulation not only enhances the antibacterial activity but also provides alternatives to some of the bacteria which have shown resistance. The formulated gel did not exhibit any clinical science of Erythema and Edema help the gel can be considered safe and non-irritant proven from preclinical studies does the commercial adoption of the antibacterial herbal topical gel seen to be profitable and can be employed in the treatment of various bacterial infection and other skin infection.

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