

# Formulation and evaluation of antifungal activity of gel of crude methanolic extract of leaves of *Ipomoea carnea* Jacq.

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**ABSTRACT:** In the last few decades, there has been an exponential growth in the field of herbal medicine. It gets popularized in developed and developing countries owing to its natural origin and lesser side effects. In this experimental study, herbal gel containing *Ipomoea carnea* Jacq. methanolic leaf extract was formulated and evaluated for antifungal activity. The base was prepared by using carbopol 934, propylene glycol 400, methyl paraben, propyl paraben, triethanolamine and required amount of water in a quantity sufficient to prepare 100 g. Different gel formulations were prepared by varying the carbopol 934 concentration (0.8 and 1%), plant extract concentration (2, 4 and 6%) and lavender oil (0.5 and 1%). Eight gel formulations were prepared and evaluated for physicochemical tests that was colour, pH, viscosity, spreadability, extrudability, homogeneity and screened for their antifungal activity by agar well diffusion technique for zone of inhibition. The formulation F8 showed zone of inhibition  $32 \pm 1$ ,  $17.67 \pm 1.15$ ,  $18.67 \pm 0.57$ ,  $26 \pm 1$  mm against *Aspergillus niger*, *Candida albicans*, *Rhizopus* species and *Penicillium notatum* respectively greater than marketed preparations. Skin irritation study (patch test) was carried out on wistar rats with F8 formulation which did not exhibit any clinical signs hence the gel was considered safe and non irritant. The gel can be used in the treatment of vaginal candidiasis, candidiasis of the skin, cutaneous aspergillosis, facial skin manifestations by *Penicillium*, cutaneous mucormycosis and other skin infections.

**KEYWORDS:** *Ipomoea carnea*; antifungal; carbopol 934; extrudability; skin irritation; vaginal candidiasis.

## 1. INTRODUCTION

Now-a-days fungal infection of skin is one of the most common dermatological problems worldwide. It has been investigated that 40 million people suffer from fungal infections. Fungal infections occurring superficially on the hair, skin and nails are common and are usually difficult to treat. Among one of the most common causes of tinea (capitis, manuum, pedis, cruris, corporis, barbae and onychomycosis) are dermatophytes. Some other widespread superficial cutaneous fungal infections are candidal and pityriasis versicolor infections [1-3]. There are numerous synthetic antifungal agents used clinically to treat these fungal infections which can be broadly classified into four major classes, i.e. azoles, allylamines, echinocandins, polyenes [4]. The course to modern treatment although has not been without its problems and complications, particularly the drug resistances. However,azole resistance is well recognized [5-8].

Phytochemistry of various plant species has indicated that the phytochemicals could be a better source of medicine as compared to synthetically produced drugs [9]. There is a need of reduction in the use of chemicals as antimicrobial agents to fight infections caused by aggressive and increasingly endogenous microorganisms that are resistant to the use of synthetic antimicrobials. In this direction, substances derived from plants, such as hydro-alcoholic extracts or essential oils, can certainly play a fundamental role [10].

Bush Morning Glory botanically named as "*Ipomoea carnea* Jacq." from the family of Convolvulaceae is a large, diffuse or straggling perennial shrub grows to a height of 5-6 m [11]. The major anti-fungal fraction of *Ipomoea carnea* leaves was reported to contain two coumarate isomers: (E)-octadecyl p-coumarate and (Z)-octadecyl p-coumarate although no systematic study was conducted to check its antifungal potential in fungi causing infections in human beings. The plant also contains methyl, octyl, dodecyl, hexadecyl, octadecyl, eicosanyl and docosyl coumarates [12]. This plant had immense potential as an anti-inflammatory, antioxidant, antidiabetic, antimicrobial, wound healing, immuno modulatory, embryotoxic, antifungal, cardioactive, hepatoprotective, inhibition and anxiolytic activity [13].

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Herbal treatments applied topically have gained considerable attention due to their widespread use and can be used in the form of ointments, creams, gels, applied directly to an external body surface. Topical application of gel at pathological sites offer great advantages in way of faster release of drug directly to site of action as compared to cream and ointment [14,15].

In present study methanolic extract of leaves of *Ipomoea carnea* and lavender oil were incorporated into the carbopol gel base and investigated for antifungal activity. To ratify the safety of dermal administration of formulated gel, we examined the dorsal skin of rats treated with formulated gel and compared it to that of control group treated with the marketed gel.

## 2. RESULTS AND DISCUSSION

### 2.1. Phytochemical screening of extract

Phytochemical screening of the obtained extract was done to check for alkaloids, coumarins glycosides, saponin glycosides, tannins, phenolic compounds, carbohydrates, flavonoids, cardiac glycosides, amino acids and terpenoids. It was found that alkaloids, coumarins glycosides, saponin glycosides, tannins, phenolic compounds, carbohydrates and flavonoids gave positive results while cardiac glycosides, amino acids and terpenoids showed negative results as shown in Table 1.

Table 1. Phytochemical screening of extract.

Phytochemical tests	Observation	Result
<b>Alkaloids</b>		
Hagers test	Yellow precipitates	Positive
Wagners test	Reddish brown precipitates	Positive
Mayers test	Cream precipitates	Positive
<b>Coumarins glycosides</b>	Yellow fluorescence	Positive
<b>Saponin glycosides</b>		
Foam test	1 cm foam	Positive
<b>Cardiac glycosides</b>		
Killer killani test	No change in colour	Negative
Legal test	No change in colour	Negative
<b>Tannins and phenolic compounds</b>		
Lead acetate test	Yellowish precipitates	Positive
Ferric chloride test	Greenish black colouration	Positive
<b>Carbohydrates</b>		
Molish test	Violet ring was formed at the junction of two layers	Positive
Benedict test	Light green precipitates	Positive
Felhing test	Brick red precipitates	Positive
<b>Amino acids</b>		
Ninhydrin test	No change in colour	Negative
<b>Terpenoids</b>		
Salkowski test	No change in colour	Negative
<b>Flavonoids</b>		
Lead acetate test	Yellowish precipitates	Positive

### 2.2. Physicochemical evaluation of methanolic extract

Results of physicochemical evaluation have been given in Table 2. The extract after partial evaporation of methanol, gave a product semi-solid in nature with a yield of  $4.44 \pm 0.031\%$ , pH  $5.45 \pm 0.012$  and total ash  $2.94 \pm 0.011$  per cent.

The extract appeared to be greenish in colour. Retention factor for the extract's thin layer chromatography was observed  $0.94 \pm 0.018$  which confirmed presence of coumarins in the extract along with other test. Similar results were also observed by Chandrakasan *et al.* [16].

### 2.3. Identification test for coumarins

When extract was treated with ammonium hydroxide solution it produced intense fluorescence of yellow colour which was observed under UV light that confirms coumarins in the extract (Table 1).

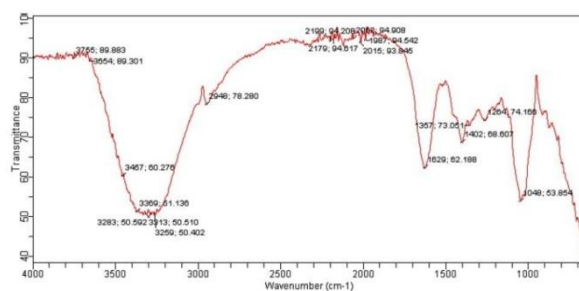
**Table 2.** Results of physicochemical evaluation of methanolic *Ipomoea carnea* extract.

Parameter	Observations (Mean±SD) Units
Percentage yield	4.44 ± 0.031%
pH	5.45 ± 0.012
Total ash	2.94 ± 0.011%
TLC	0.94 ± 0.018

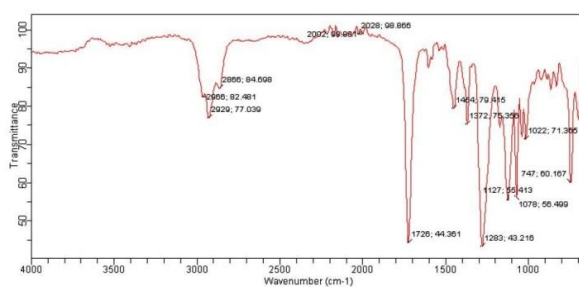
All the values are the mean of 3 observations (n=3).

#### 2.4. Fourier Transform Infra-red (FTIR) study of extract, lavender oil and F8 formulation

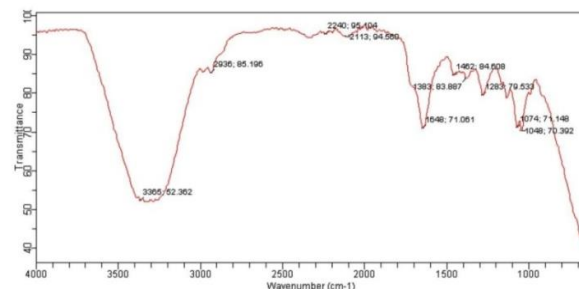
FTIR spectra of extract {Figure 1 (a)} showed broad peak around 3369 cm<sup>-1</sup> due to hydroxyl group, ester (1357, 1048, 1264 cm<sup>-1</sup> due to C-O stretching, around 980 cm<sup>-1</sup> due to C=C conjugated to C=O), and 2948 cm<sup>-1</sup> due to C-H stretching in CH<sub>3</sub>, 1629 cm<sup>-1</sup> due to C=C stretching. The same groups were found to be present in coumarates as reported in literature [17] establishing the presence of coumarates. FTIR spectra of lavender oil given in Figure 1 (b) where the peaks around 3200-3400 cm<sup>-1</sup> due to OH stretching vibrations, 2866, 2966, 2929 cm<sup>-1</sup> due to -CH<sub>2</sub> stretching, 1726 cm<sup>-1</sup> due to C=O stretching vibrations, 1640 cm<sup>-1</sup> due to C=C stretching, 1462, 1372 cm<sup>-1</sup> due to CH bending vibrations, 1127 cm<sup>-1</sup> due to C-O stretching vibrations [18]. FTIR spectrum of final optimized formulation F8 (having methanolic extract and lavender oil), on comparison with spectrum of methanolic extract and lavender oil, showed presence of all major characteristic peaks without any significant shifts indicating absence of any interactions {Figure 1 (c)}. Slight changes in the spectrum with respect to size and shape of the characteristics are unobjectionable on formulation of active ingredients with excipients.



(a)



(b)



(c)

**Figure 1.** FTIR spectra of (a) methanolic extract of *Ipomoea carnea* (b) lavender oil (c) F8 optimized formulation.

## 2.5. Gas Chromatography- Mass Spectroscopy (GC-MS)

The pure methanolic extract was analysed by GC-MS method. The parent ion peak was not identified by the instrument because the ionization chamber broke the compound into smaller fragments hence the fragments were observed at M/Z 274.9, 211.4, 177.3, 164, 148, 225, 118, 43, 57, 85, 71, 141, 119, 169 and 113 in the methanolic extract which confirms the presence of octadecyl-p-coumarate was present in the extract. Fragments of methyl coumarate and hexadecyl-p-coumarate were also observed in the extract at M/Z 41, 43, 119, 186.9, 187.3, 254.1, 177.3, 123.2, 298.6, 55, 69, 109 and M/Z 41, 43, 45, 55, 57, 69, 71, 199.2, 91, 105, 109, 123.2, 225, 85, 154, 82 peaks respectively [19-21]. Figure 2 and Table 3 showed the M/Z peaks and retention time of different coumarates.

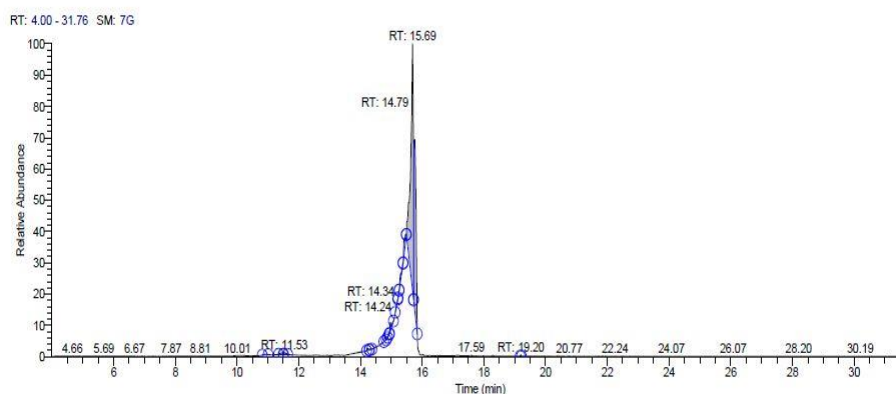


Figure 2. GC spectra of methanolic extract of *Ipomoea carnea*.

Table 3. GC-MS of methanolic extract of *Ipomoea carnea*.

Octadecyl-p-coumarate		Methyl coumarate		Hexadecyl-p-coumarate	
M/Z	Retention time	M/Z	Retention time	M/Z	Retention time
274.9	15.17	41	10.90	41	10.90
211.4	11.48	43	10.90	43	10.90
177.3	15.42	119	15.76	45	11.48
164	19.20	186.9	15.17	55	11.53
148	14.97	187.3	14.92	57	10.90
225	14.24	254.1	15.29	69	14.79
118	14.24	177.3	15.42	71	19.20
43	10.90	123.2	19.20	199.2	19.20
57	10.90	298.6	19.20	91	15.42
85	11.53	55	11.53	105	14.24
71	19.20	69	14.79	109	19.20
141	14.92	109	19.20	123.2	19.20
119	15.76			225	14.24
169	19.20			85	11.53
113	19.20			154	19.20
				82	19.20

## 2.6. Physicochemical evaluation of gel

All gel preparations including control gel were evaluated for their physical characteristics like organoleptic properties, pH, homogeneity, spreadability, viscosity, extrudability. Colour of all the gels was yellowish brown to brown in colour and pH were in range of  $6.82 \pm 0.1$  to  $7.08 \pm 0.2$ , which is to the side of neutral and do not irritate the skin while viscosity of all gel formulations were between  $16038 \pm 2.5$  to  $101498 \pm 1.5$  mpa.s . Data in the Table 4 represent that with increase in the concentration of polymer viscosity, viscosity of gels was increased. Viscosity of gels decreased with increasing concentration of extract in formulations F1 to F6. Addition of lavender oil in formulation F6 also decreases the viscosity in formulation F7 and F8. All tested formulations were homogenous with spreadability values ranging from  $4.0 \pm 0.1$  to  $5.5 \pm 0.2$  g.cm/s [22,23] and extrudability values ranging from  $0.140 \pm 0.007$  to  $1.530 \pm 0.010$  g. The addition of lavender oil did not alter the physicochemical characteristics of the F6 batch to any significant extents and the

resulting gels F7 and F8 were also found to be suitable with respect to results of physicochemical characteristics in Table 4.

**Table 4.** Physicochemical evaluation of gel.

Formulations	Colour	pH	Viscosity (mpas)	Homogeniety	Extrudability (g)	Spreadability (g.cm/s)
Control	Transparent	7.01 ± 0.2	101498 ± 1.5	Good	0.140 ± 0.007	4.0 ± 0.1
F1	Yellowish Brown	6.92 ± 0.2	51265 ± 2.5	Good	0.470 ± 0.018	4.8 ± 0.1
F2	Yellowish Brown	6.89 ± 0.3	98820 ± 2.0	Good	0.160 ± 0.011	4.2 ± 0.2
F3	Brown	7.02 ± 0.3	36374 ± 2.0	Good	0.780 ± 0.015	5.1 ± 0.1
F4	Brown	6.98 ± 0.2	55118 ± 1.0	Good	0.610 ± 0.014	4.8 ± 0.2
F5	Brown	6.82 ± 0.1	16038 ± 2.5	Good	1.530 ± 0.010	5.4 ± 0.2
F6	Brown	7.08 ± 0.2	38776 ± 1.1	Good	1.020 ± 0.008	5.2 ± 0.3
F7	Yellowish Brown	6.94 ± 0.2	32905 ± 1.0	Good	1.290 ± 0.015	5.3 ± 0.1
F8	Yellowish Brown	6.88 ± 0.1	28287 ± 2.0	Good	1.450 ± 0.016	5.5 ± 0.2

All the values as mean ± standard deviation (n=3).

## 2.7. Antifungal evaluation of formulated gels

Antifungal activity of all the formulated gels indicated that gels were not only effective against *Candida albicans* but also produced fungicidal effect against *Aspergillus niger*, *Penicillium notatum* and *Rhizopus* species. Among the methanolic extract gels, F6 showed maximum antifungal activity against all the 4 fungi tested and was selected for enhancement of antifungal activity by addition of (0.5 and 1%) lavender oil. The resulting batches of gel with incorporation of lavender oil at 0.5% (F7) and 1% (F8) showed better antifungal activity as compared to F6 with F8 showing maximum activity as can be seen from zone of inhibition. Thus F8 was chosen as the final optimized batch. Control gel on antifungal activity evaluation showed negligible zone of inhibition indicating antifungal activity due to the presence of extract. (Table 5)

**Table 5.** Antifungal activity in terms of zone of inhibition (mm) of formulated gels, marketed preparations against *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus* species.

Formulations	<i>Aspergillus niger</i> (mm)	<i>Candida albicans</i> (mm)	<i>Rhizopus species</i> (mm)	<i>Penicillium notatum</i> (mm)
Control	Negligible	Negligible	Negligible	Negligible
F1	8.00 ± 1.00	8.00 ± 1.00	11.33 ± 0.57	14.67 ± 0.1
F2	8.00 ± 1.00	9.00 ± 1.00	14.67 ± 0.57	15.33 ± 0.57
F3	12.33 ± 0.57	9.33 ± 0.57	15.00 ± 1.00	15.00 ± 1.00
F4	14.33 ± 0.57	10.67 ± 0.57	16.67 ± 0.57	15.67 ± 0.57
F5	18.67 ± 0.57	12.33 ± 0.57	18.33 ± 0.57	16.67 ± 0.57
F6	23.67 ± 0.57	12.67 ± 0.57	18.67 ± 0.57	18.33 ± 0.57
F7	27.00 ± 1.00	14.33 ± 0.57	18.67 ± 0.57	22.00 ± 1.00
F8	32.00 ± 1.00	17.67 ± 1.15	18.67 ± 0.57	26.00 ± 1.00
Itraconazole gel	21.00 ± 1.00	17.33 ± 0.57	15.33 ± 0.57	18.67 ± 0.33
Himalaya V Gel	29.00 ± 2.00	13.33 ± 0.57	14.33 ± 0.57	19.00 ± 1.00

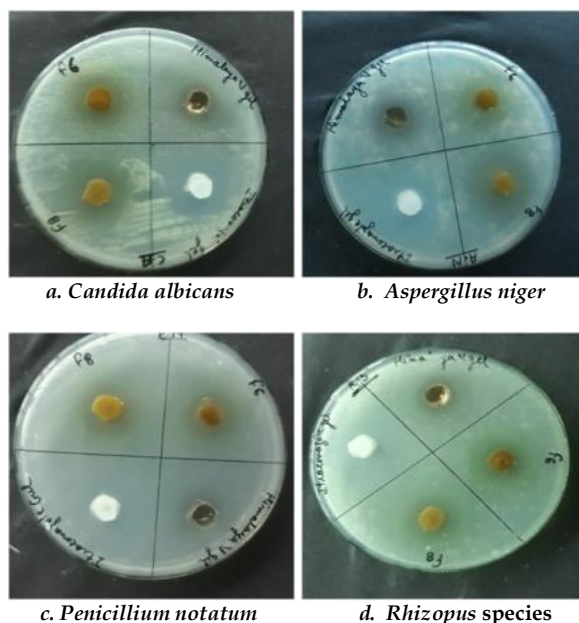
All values as mean ± standard deviation (n=3).

## 2.8. Comparison of F6 and F8 batches with marketed Itraconazole gel and Himalaya V gel

Comparison of antifungal activity of F6 with itraconazole gel (Itromed, Leeford pvt ltd, 1%) and Himalaya V gel showed it has better activity against all the 3 fungi *Aspergillus niger*, *Rhizopus* species and *Penicillium notatum* but lesser activity against *Candida albicans*. The F8 which was prepared by incorporation



of 1% lavender oil in the formulation F6 had better antifungal activity against *Candida albicans*, *Aspergillus niger*, *Penicillium notatum*, *Rhizopus* species with  $17.67 \pm 1.15$ ,  $32.00 \pm 1.00$ ,  $26.00 \pm 1.00$ ,  $18.67 \pm 0.57$  mm zone of inhibition respectively. The formulation F8 was finalized on the basis of its better antifungal activities against the fungal strains used. It was also compared with the herbal antifungal Himalaya V gel and it was established that it had antifungal activity higher than Himalaya V gel against all the strains as can be seen from Table 5. The formulation F8 has also higher activity than Itraconazole gel against all the four fungi. (Table 5, Figure 3)



**Figure 3.** Zone of inhibition of F6, F8, Itraconazole gel and Himalaya V gel against *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus* species.

### 2.9. Skin irritation test/Patch test

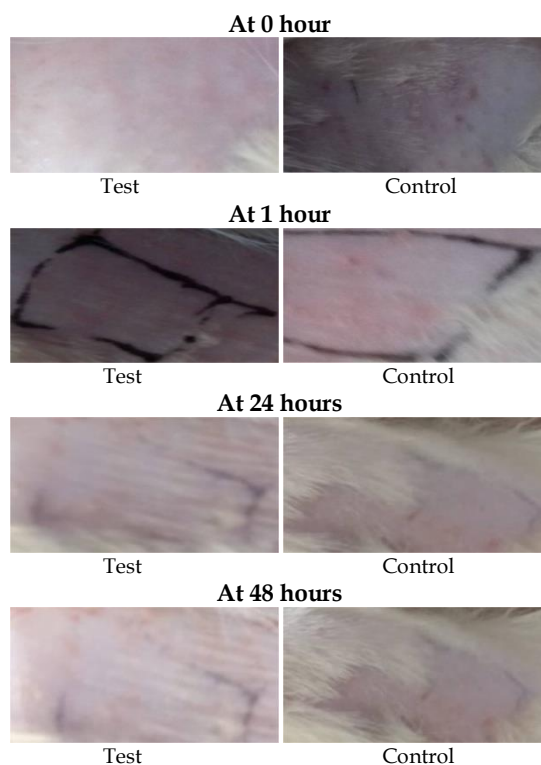
Skin irritation test was conducted on final formulation F8 on wistar rats and found no clinical signs of erythema and edema were found when compared with marketed preparations. Figure 4 ascertains its safety in rats.

### 2.10. Stability studies

After storing the F8 formulation for 3 months at  $40 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  RH the gel was checked for physicochemical screening. The colour of the gel was yellowish brown, homogenous, smooth texture and no crystallization of any kind was observed. The pH of F8 formulation was observed to be  $6.85 \pm 0.1$ , viscosity was  $28270 \pm 3.0$  m.pas and zone of inhibition for antifungal activity with respect to *Aspergillus niger*, *Candida albicans*, *Rhizopus* species, *Penicillium notatum* was  $31.8 \pm 1.5$ ,  $17.5 \pm 0.62$ ,  $18.2 \pm 1.2$ ,  $25.6 \pm 1.1$  mm respectively. Thus all the phytochemical characteristics of the gel and its antifungal activity was similar to the initial time (0 month) giving evidence for its stability.

### 3. CONCLUSION

The present investigations were designed with the objective to formulate herbal antifungal gel of *Ipomoea carnea*. Accordingly, the formulation F8 (6% *Ipomoea carnea* extract, 1% carbopol, 1% lavender oil) was formulated successfully which gave better zone of inhibition even above antifungal allopathic gels (Itraconazole; Itromed 1% and herbal marketed Himalaya V gel) prevailing in the market. Herbal formulation not only enhanced the antifungal activity but also provide alternative to some of the fungi which have shown resistance. The formulated gels did not exhibit any clinical signs of erythema and edema hence the gel can be considered safe and non irritant proven from preclinical studies. Thus, the commercial adoption of the antifungal herbal topical gel seems to be profitable and can be employed in the treatment of vaginal candidiasis, candidiasis of the skin, cutaneous aspergillosis, facial skin manifestations by *Penicillium*, cutaneous mucormycosis and other skin infections.



**Figure 4.** Skin irritation test for F8 (Test) and marketed itraconazole gel (control) at 0, 1, 24 and 48 h.

## 4. MATERIALS AND METHODS

### 4.1. Materials

Carbopol 934 was obtained from Burgoyne Burbidges and co., propylene glycol, methyl paraben, propyl paraben were procured from SD fine chem ltd, methanol was procured from Loba Chemie Pvt Ltd. and lavender oil was obtained from Yarrow chemicals, Mumbai.

### 4.2. Microbial strains

*Aspergillus niger*, *Candida albicans*, *Penicillium notatum*, *Rhizopus* species were obtained from Institute of Microbial Technology, Chandigarh.

### 4.3. Collection and authentication of plant

The whole plant of *Ipomoea carnea* was collected from Pinjore area and sent to the department of forest products, University of Horticulture and Forestry Nauni Solan H.P for its authentication. The herbarium sheet of the said sample was linked to UHF-Herbarium No. 13596 (Voucher specimen number) on dated 29 september 2018.

### 4.4. Preparation of methanolic extract of leaves

Fresh leaves of *Ipomoea carnea* was collected, washed and sorted out. The leaves were shade dried for approximate 15 days, further dried in an oven at 40°C to get a constant weight and finally powdered in a pulverizer. The dry powder (25 g) was exhaustively extracted with 300 ml of methanol using a soxhlet apparatus at 50°C up to 10 times/cycle extractions. The solvent obtained after extraction was filtered using whatman filter paper. Then, the methanol was partially evaporated out at 80 ± 2°C to get the semi-dried extract.

### 4.5. Phytochemical Screening

Preliminary qualitative phytochemical screening of the methanolic extract was carried out to determine the presence of secondary metabolites by using standard phytochemical screening methods. Change in color was noted for the result [24-29]. Phytochemical screening of the obtained extract was done to check for alkaloids, coumarins glycosides, saponin glycosides, tannins, phenolic compounds,

carbohydrates, flavonoids, cardiac glycosides, amino acids and terpenoids by performing their identification tests on extracts.

#### 4.6. Physicochemical characterization of extract

Extract was characterized for pH, total ash and TLC. pH meter was calibrated by using pH 4, 7, 9.2 buffers and then pH was determined by directly dipping the electrode into the extract solution till a constant pH was obtained. Total Ash was measured by weighing 1 g of the semi-solid crude drug in a tared silica dish and incinerated at a temperature not exceeding 450°C until free from carbon, cooled in a desiccator and weight was taken. The process was repeated till constant weight was obtained. The percentage of ash was calculated with reference to semi-solid drug. Thin layer chromatography was done by using the precoated TLC plates made up of silica gel G were cut into strips and the position of the origin marked by a straight line. The methanol extract of *Ipomoea carnea* was spotted on the origin line of TLC plate and put in a development chamber containing a solvent system (ethyl acetate: n-hexane; 9:1). The solvent level in the development chamber was about 1 cm below the origin. Capillary action caused the solvent to travel up the plate till it reached the solvent front (also marked by a straight line). The strip was then taken out and dried before it was viewed by iodine vapour. The retention factor (Rf) values of all the spots were determined by the following formula: Retention factor = Distance travelled by plant extract to the distance travelled by solvent front.

#### 4.7. Identification test for coumarins

The extract residue was dissolved in 2 ml of hot distilled water and divided into two parts. Half of the total volume was taken as control and 0.5 ml 10% ammonium hydroxide was added to another volume. Two spots were put on filter paper and examined under UV light.

#### 4.8. Fourier Transform Infra Red spectroscopy (FTIR)

The FTIR spectra were obtained by using ATR FTIR Spectrophotometer (Make: Agilent technologies; Model: CARY 630). FTIR spectra were recorded for methanolic extract of *Ipomoea carnea* leaves, lavender oil and carbopol gel formulation having methanolic extract and lavender oil. The spectra were recorded in the range of 4000-650 cm<sup>-1</sup> and were used to study active ingredient-excipient interactions by determining major shifts in peaks.

#### 4.9. Gas Chromatography - Mass Spectroscopy (GC-MS)

GC-MS analysis of the methanolic extract of *Ipomoea carnea* was performed using a Thermo Trace 1300 GC coupled with Thermo TSQ 800 Triple Quadrupole MS and equipped with a TG 5MS (5% diphenyl; 95% dimethyl poly siloxane) fused a capillary column (30 m X 0.25 mm, 0.25 µm). Helium gas (99.999%) was used as a carrier gas at a split flow rate of 45 ml/min, and an injection volume of 4 µl was employed (splitless). The injector temperature was maintained at 280°C, the ion-source temperature was 230°C, the oven temperature was programmed from 60°C (Hold time 7 min) with an increase of 15°C/min to 280°C (Hold time 10 min). The total GC/MS running time was 31.76 min. The mass-detector used in this analysis was MS TSQ 8000 detected in the range of 25-1000 and the software adopted to handle mass spectra and chromatograms was XCalibur 2.2SP1 with Foundation 2.0SP1. The mass spectrum obtained was interpreted with the help of NIST library and other reported literature for determination of fragments of coumarates present in methanolic extract of leaves of *Ipomoea carnea*.

#### 4.10. Preparation of gel

The required quantity of carbopol 934 was dispersed in small amount of distilled water and kept overnight for hydration. Weighed amount of extract was added to propylene glycol and filtered. Methyl paraben, propyl paraben were added to the filtered solution. Then the above mixture was added to hydrated dispersion with constant stirring. Finally, pH was adjusted up to pH around 6.8 using triethanolamine until a clear consistent gel was obtained. Various batches of gel were prepared for optimization of plant extract and carbopol. A control gel was also prepared in the same way but without incorporating methanolic extract and lavender oil in it. Table no. 6 shows the composition of different batches of gel formulated. Carbopol was chosen as the gelling agent as it shows good gelling capacity at low concentration, is easily available and process able. Propylene glycol was used for solubilization of extract and as a penetration enhancer. Methyl paraben and propyl paraben were used as preservatives. The batch of gel (F6) showing maximum antifungal activity and all the parameters at appropriate levels was selected. It was further used for enhancing



antifungal activity by addition of lavender oil in two concentrations i.e 0.5 and 1% w/w as lavender oil has been reported to possess antifungal activity [30].

**Table 6.** Composition of methanolic *Ipomoea carnea* extract, lavender oil, gelling agent and other excipients for the preparation of gels.

Ingredients	Control	F1	F2	F3	F4	F5	F6	F7	F8
Plant extract	-	2%	2%	4%	4%	6%	6%	6%	6%
Lavender oil	-	-	-	-	-	-	-	0.5%	1%
Carbopol 934	1%	0.8%	1%	0.8%	1%	0.8%	1%	1%	1%
Methyl paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Propyl paraben	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Propylene glycol	10.35%	10.35%	10.35%	10.35%	10.35%	10.35%	10.35%	10.35%	10.35%
Triethanola mine	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8
Distilled water(q.s)	100 g	100 g	100 g	100 g	100 g	100 g	100 g	100 g	100 g

#### 4.11. Physico-chemical characterization of gel

The gel was characterized for physical appearance, viscosity by brookfield viscometer, pH by digital pH meter.

##### 4.11.1. Physical appearance

Physical parameter such as color and appearance were checked visually.

##### 4.11.2. Viscosity

Viscosity of gel was measured by brookfield viscometer (Acutek A220B) using spindle no. 3 and 4.

##### 4.11.3. pH

pH of the gel was measured by using digital pH meter after calibrating with buffer pH 4, 7, 9.2.

##### 4.11.4. Spreadability

The spreadability was measured by placing 0.5 g of gel within a premarked circle of diameter 1 cm on a glass plate over which a second glass plate was placed and 50 g weight was allowed to rest on the upper glass plate for a period of 5 min. Spreading of the gel caused an increase in diameter of the circle, which was measured in cm and noted down. These results were taken as comparative values for spreadability [31,23].

##### 4.11.5. Extrudability

The formulation was filled taken into collapsible aluminium tubes. The gel was allowed to set in the container. The extrudability of formulation was then determined by applying the pressure of 1 Kg/cm<sup>2</sup> from Monsanto hardness tester [32].

##### 4.11.6. Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels had set in the container. They were checked for their appearance and presence of any aggregates.

#### 4.12. Antifungal study

Antifungal activity of gel against fungi was determined by well diffusion method [33]. A 24 hours old culture of fungus was grown in nutrient agar (NA) broth and used as fungal suspension. Nutrient agar solution was sterilized in an autoclave at 121°C (1.05 kg/cm<sup>2</sup> pressure) for 20 minutes and poured in sterilized petri plates. Agar plates were inoculated with 500 µl of each fungal suspension by streaking the surface of the plates with sterile spreader until the entire surface was covered. Wells of 5 mm were cut in

the solidified media with a sterile cork borer and gel was added into each well [34]. After incubation at 30°C for 24 h, the diameter (mm) of the inhibition zone around the well was measured [35]. Itraconazole gel (Itromed 1%, Leeford Healthcare ltd) and Himalaya V gel was used as a reference antifungal formulation. All antifungal studies were performed in triplicate.

#### 4.13. Enhancement of antifungal activity of extract gel

It has been reported in literature that certain volatile oil like *Curcuma longa*, *Eucalyptus oil*, *Coraindrum stauvum*, lavender oil, neem oil etc. possess antifungal activity [10]. The antifungal activity of the selected extract gel (having maximum antifungal activity based on results of assessment of antifungal activity) was enhanced by incorporation of lavender oil at a level of 0.5 and 1% w/w. The antifungal activity of the resulting gel was also assessed by the well diffusion method stated above.

#### 4.14. *In vivo* skin irritation test (patch test)

CPCSEA guidelines were followed for carrying out animal studies. The protocol was approved by IAEC of LR Institute of Pharmacy, Solan vide their number LRIP/IAEC/2019/PH-04. Animals having an average weight of 250 - 300 g were anaesthetized by using ketamine hydrochloride injection (0.2 ml), to prevent any movement. Organization for Economic Co-operation and Development (OECD) test guidelines 404 were followed for acute dermal irritation and corrosion. 24 h before the application of sample, the back of the animals was shaved with an electric clipper and methylated spirit was applied as an antiseptic to prevent infections. An area of 6 cm<sup>2</sup> was marked on the shaved skin surface with the help of ruler. 0.5 g of gel was topically applied on the surface of the skin, for each group of rats. After 24 hours the test materials was removed and the skin surface was cleaned with distilled water. The sites were then examined for skin irritation at 0, 1, 24, 48 h [36,37]. The observations were recorded as numerical scores as for each animal as follows [38].

- 0 = no visible reaction
- 1 = mild erythema
- 2 = intense erythema
- 3 = intense erythema with edema
- 4 = intense erythema with edema and vesicular erosion

The scores of formulation F8 were then compared with marketed formulation (Itraconazole gel).

#### 4.15. Stability studies

Accelerated stability studies for the optimized, selected F8 formulation were carried out at a temperature of 40 ± 2°C and relative humidity 75 ± 5% RH for a period of 3 months. The gel was stored in aluminium tubes for the stability studies about 3 months and was checked for its physicochemical properties, physical appearance and antifungal activity by the methods used for the evaluation of formulated gels stated above.

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**Ethics committee approval:** *In vivo* studies were approved by IAEC of LR Institute of Pharmacy, Solan vide their number LRIP/IAEC/2019/PH-04.

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