https://doi.org/10.48047/AFJBS.6.6.2024.8223-8249

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Development of Poly-herbal Antimicrobial Topical Formulation for Topical diseases

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Volume 6, Issue 6, June 2024

Received: 09 May 2024

Accepted: 1 June 2024

Published: 18 June 2024

doi: 10.48047/AFJBS.6.6.2024.8223-8249

ABSTRACT

Objective: The objective of present research work was formulating and evaluating topical gels containing *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) extracts for the treatment of acne. **Methods:** The phytochemical composition of the plant extracts was determined. The extracts and gels' minimum inhibitory concentration (MIC) was assessed using the microbroth dilution method. The marker compound, clindamycin, in herbal anti-acne preparation, was kept for the comparison with the zones of inhibition for antibacterial activity. All the prepared topical gel formulations were evaluated using 1% Carbopol 940 as a gelling agent and were used different ratio of *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) extracts. **Results:** The phytochemical components of the plant extracts are probably responsible for the antimicrobial activity of the gel formulations. The 5% *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) (1: 1:1) combination gel formulation showed excellent antimicrobial activity against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans, with MICs of 12.50, 25.00, 6.25, 25.00, and 12.50 mg/mL, respectively. The gels generally had good physicochemical and antimicrobial properties and could be used as antiacne remedies. Anti-acne property was explored with the help of a standard curve and by comparing diffusion profiles by taking clindamycin as a reference. **Conclusion:** From the present study it can be concluded that addition of permeation enhancer in the test formulation will improve the diffusion profile and thus it was designed to add permeation enhancer.

Keywords: *Acne vulgaris*, *Propionibacterium acne*, *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs).

INTRODUCTION

Acne vulgaris is a common skin disorder which affects containing the most extensive oil glands, including the face, back and trunk [Firdaus *et al.,* 2015]. *Propionibacterium acne*, an anaerobic pathogen, plays an important part in the pathogenesis of acne. It has also participated in the production of inflammatory acne by its ability to activate antioxidants and metabolize sebaceous triglycerides into fatty acids, which draw neutrophils chemotactically [Borkar *et al.,* 2008]. Acne is one of the most socially distressing skin disorders, particularly for teens, who have to cope with a disfiguring disease that erupts just when sexual maturity makes them maximum sensitive about their appearance. Moreover, permanent scarring of the skin due to severe acne leads to social distress throughout the entirely of adulthood [Bourgou *et al.,* 2012]. The modification and progress of acne is due to abnormal keratinocyte proliferation and desquamation leading to ductal obstruction. Androgen driven sebum production enhances proliferation of P. acne. Excessive production of sebum block the pores and resulted in inflammation [Maltaş *et al.,* 2010]. For many years, the use of antibiotics has shown against *vulgaris*. Nevertheless, the prevalence of antibiotic resistance within the dermatological setting has been increased. The progress of antibiotic resistance is multi-factorial, including the specific nature of the relationship of the bacteria to antibiotics, how the antibacterial is used, host characteristics and environmental factors [Choudhary *et al.,* 2009, Jain *et al.,* 2017]. To avoid the problem of antibiotic resistance, researches on medicinal plants have been done as alternative treatment for the disease [vats *et al.,* 2013].

***Permotrema reticulatum*:-**

Permotrema reticulatum is lichen firstly used traditionally as spices and also as a medicine for centuries in various countries. Lichens are not plant ,they are combine organism that contain algae or cynobacterium or both which living between the filaments of fungus *Permotrema reticulatum* comes under the family permeliaceae having large foliose thali with broad erhizinate marginal zone on lower surface and reticulated molecule upper surface, pharmacologically Permotrema has a broad activity like-antimicrobial, antioxidant since they are symbiotic of mycobiont and photobiont, they generate number of metabolite and despised, dibenzofurans, pulvinic acid and usinic acid due to presence of their compounds they are antiviral, antibiotics ,antioxidant, anticancer and allergenic in activity. Among there usinic acid is effective against

various mechanism of action like Tuberculosis, streptococcus, pneumococcal and other gram positive bacteria. Reason behind the use of this lichen in soap is to provide antibacterial and antioxidant properties in product. The chemical which are commonly found in *P. reticulatum* are Atranorin, chloratranorin, usnic acid, beta-alectoronic acid, alpha-collatolic acid, Praesorediosic acid, Protocetraric acid etc [Jain *et al.,* 2016].

***Tagatus petula*:-**

Tagatus petula is commonly known as the marigold,which is anornamental flower used in the decoration of house and also as a flavouring agent .It belong to the asteraceae family with genus tagatus,species petula.Which have about different 50 species, it is a perennial or annual herbaceous plant. In ancient time it is used in Indian medicine system as treatment of various disease evenly it is used in the treatment of cancer & hair fall. Marigold or *Tagetus petula* are found in the all over world. It is used in anticancer, antibacterial, antifungal activity. *Tagetus* species contains carious phytoconstituents such as thiophenes ,flavonoids, carotenoids and triterpenoids, is rich in the aromatic compound and resinous extract. Tagetus species are generally rich in oil & other phytoconstituents like monoterpine &in acyclic monoterpine ketones with pleasant odour. Chemical which are generally found in *Tagetus petula* plant is monoterpine like as dihydrotagetine, limonin, terpine, terpenon, myrisin. The constituents of tagetus petula alkaloid, terpinoids, flavonoids are found in large quantity. In *T.petula* (root and leaves) are found thiophene steroid and terpenoidal type of constituent. Some of flavonols are also found like: - Quircetin, Quiratagetin, Quiractin-3-g lucoside etc [Reddy 2020].

Curcuma cassia-

Curcuma cassia is characterised by the small rhizome structure, greyish to brown in colour inside the rhizome its persists the light black colour a characteristics aroma and taste the leaf of the plant is generally green and size of leaf typically about 30-60cm long and 15cm broad,deep green colour in the middle are a spike is come out with flower of purple colour .The leaves of the curcuma cassia are green upto the rhizome base. Chemical constituents-curcuma cassia contains 5% of volatile oil; resins are also found curcumonid which have a together portion of the curcuma (50-60%) [Campo 2022].

Pimples may happen when the sebaceous organs associated with pores liable for moving dead cells to the surface space of the skin get hindered. This blockade usually results in bacterial colonization and attack on the sebum, resulting in whiteheads, blackheads, and finally, inflammation and scars when the body's mechanism tries to fight back. *Propionibacterium acnes* and *Staphylococcus epidermidis* assume significant parts concerning inflammatory acne and shallow disease by utilizing sebaceous fatty substances into unsaturated fats, to which neutrophils are pulled in [Dreno 2015, Lee 2014].

Medicinal plants have the advantages of patient tolerance and wide acceptability [Rafieian-Kopaei *et al., 2012*]. *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) have antimicrobial, anti-inflammatory, and antioxidant activities that significantly treat skin infections. They are additionally known to have nutrients and minerals to improve the general strength of the skin. Acne accounted for 5.3% of all skin diagnoses reported, and acne vulgaris was the second most common by gender [Faizi *et al.,* 2008, Jain *et al.,* 2016, Bijayananda Sahoo *et al.,* 2021, Dineja *et al.,* 2023]. In our present study, hydroalcohalic extract of *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) was evaluated for its potential against the skin diases causing microorganism like *Escherichia coli, Candida albicans, Pseudomonas aeruginosa and Acne vulgaris* by in vitro methods.

MATERIALS AND METHODS

Plant material

All the herbs *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) will collected from local area of Bhopal (M.P.) in the month of January, 2019. The microorganisms were obtained from Peoples University Bhopal (M.P.).

Drugs and chemicals

Drugs and chemicals were procured and used analytical grade

Preparation of plant extracts

The plant material was shade dried for about 15 days. The dried each powder sample of herbal drug was macerated separately for 15 days by using 80:20 ratios. Then after evaporated, dried

and was weighed and kept in a refrigerator for further experimental procedure. Separately each plant material extraction process performed and extract will collect.

Determination of percentage yield

The percentage yield of each extracts will be calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

The chemical tests were performed using official reported methods for testing different chemical groups present in extracts like alkaloid, glycoside, volatile oils, tannins, saponins, flavonoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites, all the extract were subjected to battery of chemical tests.

Evaluation of bio-active compounds by using GC-MS

The chemical composition of GCMS was studied by using *T. patula* flower, *Permoterma reticulatum*, and *Curcuma caesia* (Root hairs) extract was determined using gas chromatography-mass spectroscopy [Joshi *et al.*, 2016].

Antioxidant activity

The evaluation of antioxidant activity of different extracts of *T. patula* flower, *Permoterma reticulatum, and Curcuma caesia (Root hairs)* is done through various *in vitro* assays.

Preparation of test sample of extract

Sample Preparation 10 mg each extract taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using Whatmann filter paper no.41 and then sonicated for 10 min.

Assessment of Antioxidant

1, 1-Dipheny-2-Picrylhydrazyl (DPPH) radical scavenging activity

The assessment of antioxidant activity of plant extracts such as *T. patula* flower, *Permoterma reticulatum, and Curcuma caesia (Root hairs)* and L-ascorbic acid (Vitamin C) were measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. For assessment of DPPH radical scavenging activity or the free radical scavenging activity of each extracts. Firstly DPPH solution was prepared by dissolving 4 mg DPPH in 100 ml methanol. A dilution series were prepared for ascorbic acid and extracts. After that 5ml of sample solution will be mixed with 0.5 ml DPPH solution and incubated for 30 min at room temperature in dark condition and absorbance was taken at 517nm and calculated the % inhibition of DPPH radical.

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Absorbance Control} - (\text{Sample with DPPH} - \text{sample without DPPH})}{\text{Absorbance of control}} \times 100$$

Preparation of Mueller-Hinton Broth (Single and Double Strength)

Mueller-Hinton broth was prepared at single and double strengths. For the single-strength broth, 2.1 g of Mueller-Hinton broth was dissolved in 100 mL of sterile water. This solution was then transferred to 10 mL test tubes and sterilized in an autoclave at 121°C for 30 minutes. To prepare the double-strength broth, 4.2 g of Mueller-Hinton broth was dissolved in 100 mL of sterile water. This solution was also transferred into 10 mL test tubes and sterilized in an autoclave at 121°C for 30 minutes. After sterilization, the broth was cooled and stored at room temperature (25°C).

Subculture of microorganisms

Microorganisms including Staphylococcus epidermidis, Staphylococcus aureus (gram-positive), Escherichia coli, Pseudomonas aeruginosa (gram-negative), Candida albicans (fungi), and Acne vulgaris were subcultured. The Mueller-Hinton broth test tubes were sterilized by flaming the mouth with a Bunsen burner under a laminar flow cabinet. Then, 1 mL of the pure isolate was introduced into the broth, flamed again, and capped with a cork. The capped test tubes were incubated at 37°C for 24 hours [Hossain *et al.*, 2012].

Streaking of the Subcultured Organisms to Obtain Pure Isolates

Agar was prepared, transferred into test tubes, and sterilized in an autoclave for 30 minutes at 121°C. After sterilization, the agar was poured onto petri dishes and cooled in a safety cabinet. The subcultured organisms were streaked over the surface of the agar using a sterile inoculum loop. The petri dishes were then incubated for 24 hours [Hossain *et al.*, 2012].

Zone of inhibition of Plant extracts

The antimicrobial screening in vitro was conducted using the Method of the Cylinder. Ointments prepared with hydroalcoholic extracts of plants A, B, C, and D were tested for antimicrobial activity against skin pathogenic bacteria. Standard and test samples were placed in holes bored in the center of solidified nutrient agar medium inoculated with microorganisms. The plates were then incubated at 37°C for approximately 18 hours, and the zone of inhibition was identified after incubation.

Estimation of Minimum Inhibitory Concentrations (MIC) of Plant extracts

The microbroth dilution technique was utilized to estimate the MIC. Microtiter plates were filled with appropriate amounts of Mueller-Hinton broth, sterile water, distinct concentrations of plant materials, and microorganisms (adjusted to the McFarland standard). The plates were then incubated at 37°C for 24 hours. The MIC was determined as the lowest dilution concentration of hydroalcoholic extracts of plants A, B, C, and D with no apparent growth [Kobina *et al.*, 2018].

Drug-excipient compatibility

The investigation into drug-excipient compatibility was conducted through rigorous testing methodologies. Test samples were enclosed in vials and subjected to two distinct environmental conditions: 25 °C with 60% relative humidity (RH) and 40 °C with 75% RH over duration of one month. The drug along with diverse excipients underwent thorough analysis and characterization utilizing the Infrared (IR) spectroscopic method, employing Fourier Transform Infrared (FT-IR) spectroscopy. Spectral data were acquired within the wave number range 4000-500cm^{-1}

Preparation of gel

The concentration of the extracts of herbs is decided according to their activity and base of gel are selected by using the literature references, trials and stability (Table 1).

Table 1: Formulation of gel

Formulation Code	**Gelling agent**	***T. patula* flower extract (µg/ml)**	***Permoterma reticulatum* extract (µg/ml)**	***Curcuma caesia (Root hairs)* (µg/ml)**	***T. patula* + *Permoterma* and *Curcuma* extract**
1	Plant extract	5%	5%	5%	5%
3	Carbopol 940	1	1	1	1
4	Methylparaben	0.1	0.1	0.1	0.1
5	Propylparaben	0.1	0.1	0.1	0.1
6	Triethanolamine	2	2	2	2
7	Propylene glycol	2	2	2	2
8	Distilled water	QS	QS	QS	QS

Gels comprising samples of *T. patula* flower extract, *Permoterma reticulatum* extract, and *Curcuma caesia* root hair extract were formulated at a 5% concentration following preformulation assessments (refer to Table 1). Carbopol 940 was dispersed in distilled water under constant agitation in one container, while propylparaben and methylparaben were dissolved in 5 mL of distilled water in a separate vessel. Subsequently, the extracts were incorporated into the solution and homogenized thoroughly. The resultant mixture was then combined with the carbopol dispersion and mixed adequately. Finally, propylene glycol and triethanolamine were added drop wise to the dispersion under continuous stirring, and the pH was adjusted to 6.8-7.4 to achieve neutralization [Patil *et al.,* 2017].

Physical evaluation (vats 2013)

a. Measurement of pH:

pH was measured using a digital pH meter within 24 hrs of preparation.

b. Colour:

The colour of the formulations were checked against white and black background and documented.

c. Odour:

The odour of the gels was checked by mixing the gel in water and smelling it.

d. Consistency:

The consistency was checked by applying on the skin.

e. Greasiness:

The greasiness was assessed by application on to the skin.

f. Homogeneity:

Homogeneity was tested by visual inspection after allowing them to set in a container. They were evaluated for their appearance and presence of aggregates.

g. Grittiness:

The formulations were evaluated microscopically under 40 x magnifications for the presence of any particulate matter or aggregates.

h. Skin irritant test:

This test was performed on 10 healthy human volunteers of either sex after obtaining consent for the same. About 0.5 gms of gel was applied to an area of about 6cm2 on skin of hand covered with a gauze patch. The patch was held in contact with the skin by means of a semiocclusive dressing for an hr. At the conclusion of exposure period of 1 hr, the gauze was removed and residual test substance was scrapped, without altering the existing response or integrity of the epidermis. The skin was observed at 1 hr, 3 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hrs for any visible response on the skin [Vats et al., 2013]

Zone of inhibition of Topical formulation

In vitro antimicrobial screening of topical formulations was conducted utilizing the Method of the Cylinder. Prepared formulations (F1, F2, F3, and F4) were assessed for their antimicrobial activity against pathogenic skin bacteria. Standard and test samples were placed in wells at the center of solidified nutrient agar medium, which was previously inoculated with microorganisms. Incubation was carried out at 37°C for approximately 18 hours, followed by identification of the zone of inhibition.

Estimation of MIC of Formulated Gels

The microbroth dilution technique was employed to determine the MIC. A microtiter plate was filled with appropriate volumes of Mueller-Hinton broth, sterile water, various concentrations of the formulated gels, and microorganisms, as per the McFarland standard. Incubation was performed at 37°C for 24 hours. Each test organism was tested in triplicate wells. Positive and negative controls were included. MIC was determined as the lowest dilution of the test formulations (F1, F2, F3, and F4) with no visible growth, corroborated by previous studies [Kobina *et al.,* 2018, Wiegand *et al.,* 2008].

***In-vitro* permeation study of topical formulation:**

A calibration curve of a herbal extract mixture (Rason, Mulak, and Marich) was prepared using phosphate buffer pH 7.4. Serial dilutions of the stock solution were analyzed via UV-Visible spectrophotometry at 341nm. In vitro drug permeation studies of the formulated gel were conducted using Franz Diffusion Cells (FDC) with cellophane membrane. Prepared gel was applied to the receptor compartment, and the study was conducted for 12 hours in phosphate buffer pH 7.4 medium, in accordance with previous methodologies [Taofiq *et al.,* 2019]. The donor compartment was supplied with 250mg of gel, while the receptor compartment contained receptor fluid. Temperature was maintained at 37°C, and sampling intervals were set at 0.5, 1, 2, 3, 4, 5, 6, and 12 hours. Samples were analyzed via UV-Visible spectrophotometry

Stability:

Stability studies were conducted for all gel formulations. Products were subjected to temperatures of 4°C, 25°C, and 40°C for 4 weeks, with observation of syneresis. Subsequent exposure to ambient room temperature led to the observation of liquid exudate separation

Statistical analysis of data:

Statistical analysis was performed using INSTAT 1 software, employing analysis of variance. Results were considered significant where $p < 0.05$. Coefficients of variation were calculated for each reading to assess result reproducibility.

RESULT AND DISCUSSION

Determination of percentage yield

Extraction of Selected *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) were prepared by using hydroalcohalic solvent. The yield of extract was found to be 9.3%, 3.1% and 5.6 % respectively. The results of yield of extract indicated that highest bioactive components present in water, chloroform and methanol extract. These extract may have potential therapeutic activities. The percentage yield of each extracts will be calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Table 2: Percentage yield of extract

S.N.	Solvent	% yield w/w
1	*Curcuma caesia* (CC)	9.3
2	*Permoterma reticulatum* (PR)	3.1
3	*T. patula* (TP)	5.6

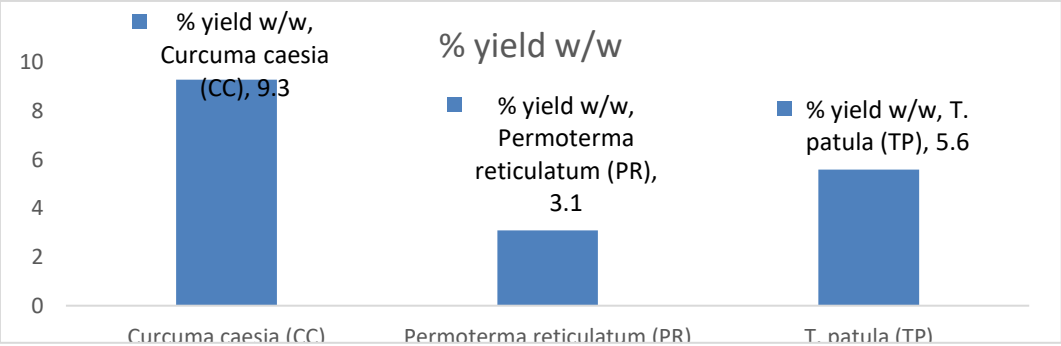

Fig.1: Percentage yield of extract

Phytochemical Screening

The chemical tests will be performed for testing different chemical groups present in extracts like alkaloid, glycoside, volatile oils, tannins, saponins, flavonoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites, all the extract were subjected to battery of chemical tests. According to research, *Curcuma caesia* and *Permoterma reticulatum* has the best potential for bioactivity of any extract when it comes to glycosides, alkaloids, glycosides, phenolic compounds, tannins, saponins, flavonoids, and proteins. Hence, *Curcuma caesia* and *Permoterma reticulatum* chose us for additional research. Results are shown in below Table 1.

Table 3: Results of phytochemical screening test of Hydroalcohalic Extract

S.N.	**Constituents**	***P.reticulatum***	***C. caesia***	***T. patula***
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Phenols	+	+	+
4	Tannins	+	+	-
5	Saponins	+	+	+
6	Steroids & terpenoids	+	+	+
7	Glycosides	-	-	-
8	Carbohydrates	+	+	+
9	Anthraquinones	-	+	-

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

Results showed a significant difference in the extraction yield was indicating that the extraction efficiency favors the nature of solvents (nature of bioactive compounds). It revealed that plant

Curcuma caesia and *Permoterma reticulatum* having highest bioactive compounds. It indicated that these solvent having highest potential for the pharmacological action.

Evaluation of bio-active compounds by using LC-MS-MS

The chemical composition of *T. patula* flower, *Permoterma reticulatum*, and *Curcuma caesia* (Root hairs) extract was determined using gas chromatography-mass spectroscopy. The softcopy findings of LC-MS-MS Turbo Mass utilizing peak area normalization measurements were used to determine the concentration of all the detected chemicals based on peak area/peak height. The chemical composition of *T. patula* flower, Permoterma reticulatum, and Curcuma caesia (Root hairs) extract spectra are shown below [Joshi *et al.*, 2016].

LCMSMS Chemical compositions of poly-herbal extract (Mixture of A, B, C extract)

M/Z ratio	Name of compounds and their activities
118.0905	Zingiberene
123.0448	Dimethylphenol
135.0825	Terpinolene
151.0403	2-Bornanone
155.070	Isoborneol
163.1507	p-Coumaric acid
175.113	Megastigma-3,7(E),9-triene
177.1658	Aesculetin
179.1076	Caffeic acid
181.0506	Methyl β-orsellinate or Demethyliso haematommate
182.9612	Dihydro demethyliso haematommate
191.1798	Quinic acid (Phenolic acid)
201.1658	α-Curcumene, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl
203.1795	ar-curcumene
206.8880	Zingiberene
215.1819	ar-Turmerone, Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene
217.1623	Turmerone, ar-turmerone
219.1762	Curlone, germacrone

222.0208	pentyldivaric acid, Ethyl haematommate
231.1400	Curzerenone
235.1698	Curcumenol isocurcumenol
236.1516	germacrone
248.1375	6-Hydroxy-7-methoxytremetone
249.1848	Dehidro-6-hydroxy-7-methoxytremetone
263.1265	Dehydro-6,7-dimethoxytremetone
271.1560	Naringenin
290.8462	Androstenediol
298.2767	7-Methoxyluteolin
301.2153	Quercetin
313.2757	Isorhamnetin or 7, 3-Dimethoxyluteolin
317.2879	Lecanoric acid,
322.8646	Licheterinic/ Protolichesterinic acid
326.2326	Dihydro lichesterinic acid
342.3003	Patuletin
353.2641	Usnic acid
363.2479	Chlorogenic acid
381.2617	p-Coumaroyoxyltremetone*
387.3240	Praesorediosic acid
391.1372	Divaricatic acid or Salazinic acid, Hypoconstictic acid
407.3708	(+)-2-Bornanone
409.3806	Chloratranorin
419.2732	Dihydrovinapraeso rediosic acid
430.3768	Quercetin-3-O-pentoside, Tetrahydroxytetracosanoic acid
463.3018	Quercetin-3-O-glucoside (isoquercitrin), Quercetina-3-O-galactoside (hyperoside)
495.2011	Patuletin-7-O-glucoside (patulitrin) or 6-O-methyl-quercetin-3-O-glucoside
579.3204	Quercetin-3-O-rhamnosyl-O-xyloside
590.4282	Quercetin-3-O-di-rhamnoside
609.3635	Quercetin-3-O-glycosyl-7-O-rhamnosyl or Kaempferol-3-O-di-hexoside
613.4824	Quercetin-3-O-hexoside-galloyl

617.4316	Orsellinylgyrophor ate
728.4547	Kaempferol-3-O-rhamnoside-galloy

The poly-herbal mixture of extract was studied contained more significant bioactive compounds such as p-Coumaric acid, Quinic acid (Phenolic acid), Quinic acid (Phenolic acid), Naringenin, Quercetin, Patuletin, Usnic acid, Chlorogenic acid and p-Coumaroyoxyltremetone, suggesting that the poly-herbal extract having potential bioactive compounds [Joshi *et al.*, 2016]. These bioactive compounds have been found potent antimicrobial activity against topical infection [Joshi *et al.*, 2016].

Evaluation of bio-active compounds by using following methods

Antioxidant activity

The evaluation of antioxidant activity of different extracts of *T. patula flower*, *Permoterma reticulatum, and Curcuma caesia (Root hairs)* is done through various *in vitro* assays.

T. patula flower (A)

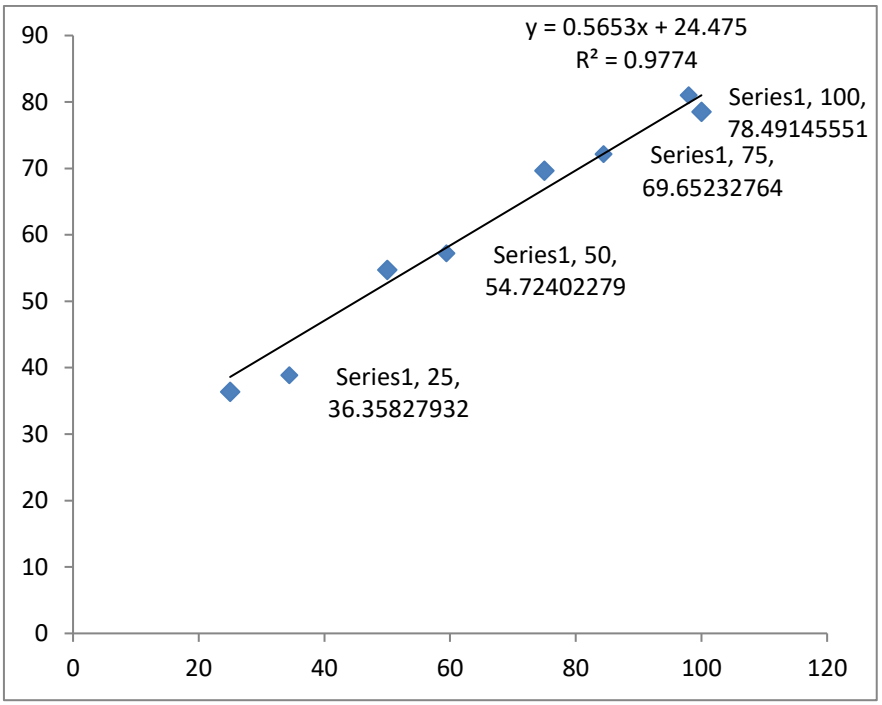

Fig.2: Calibration curve of *T. patula* flower (A)

Table 4: % inhibitions of *T. patula* flower (A)

SAMPLE CODE A	IC 50 (ug/ml)
25	36.35827932
50	54.72402279
75	69.65232764
100	78.49145551

IC 50 = 45.1530161

Permoterma reticulatum (B)

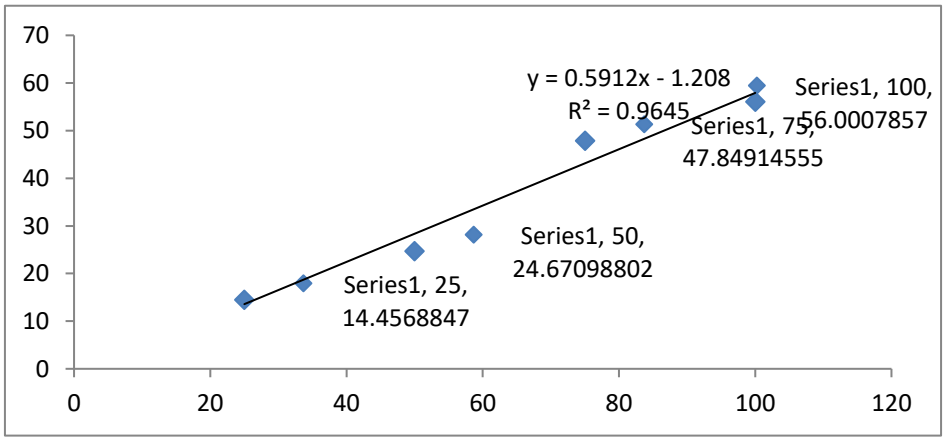

Fig.3: Calibration curve of *Permoterma reticulatum* (B)

Table 5: % inhibitions of *Permoterma reticulatum* (B)

SAMPLE CODE B	IC 50 (ug/ml)
25	14.4568847
50	24.67098802
75	47.84914555
100	56.0007857

IC 50 = 86.61705007

***Curcuma caesia* (C)**

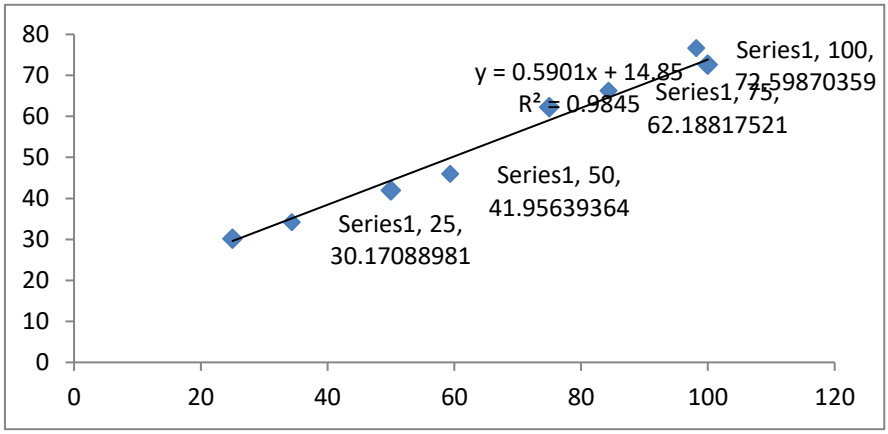

Fig.3: Calibration curve of *Curcuma caesia* (C)

Table 6: % inhibitions of *Curcuma caesia*(C)

SAMPLE CODE C	IC 50 (ug/ml)
25	30.17088981
50	41.95639364
75	62.18817521
100	72.59870359

IC 50 = 59.56617522

Mixture of THE, PRHE and CCHE (D)

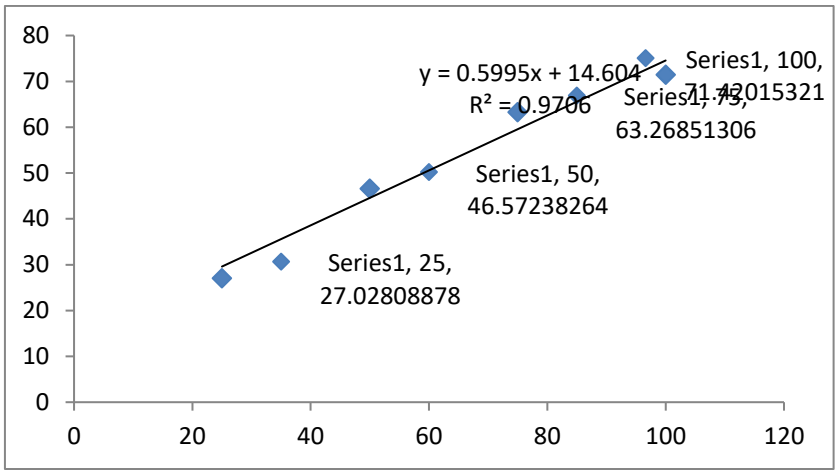

Fig.4: Calibration curve of THE, PRHE and CCHE (D)

Table 7: % inhibitions of THE, PRHE and CCHE (D)

SAMPLE CODE D	IC 50 (ug/ml)
25	27.02808878
50	46.57238264
75	63.26851306
100	71.42015321

IC 50 = 59.04253545

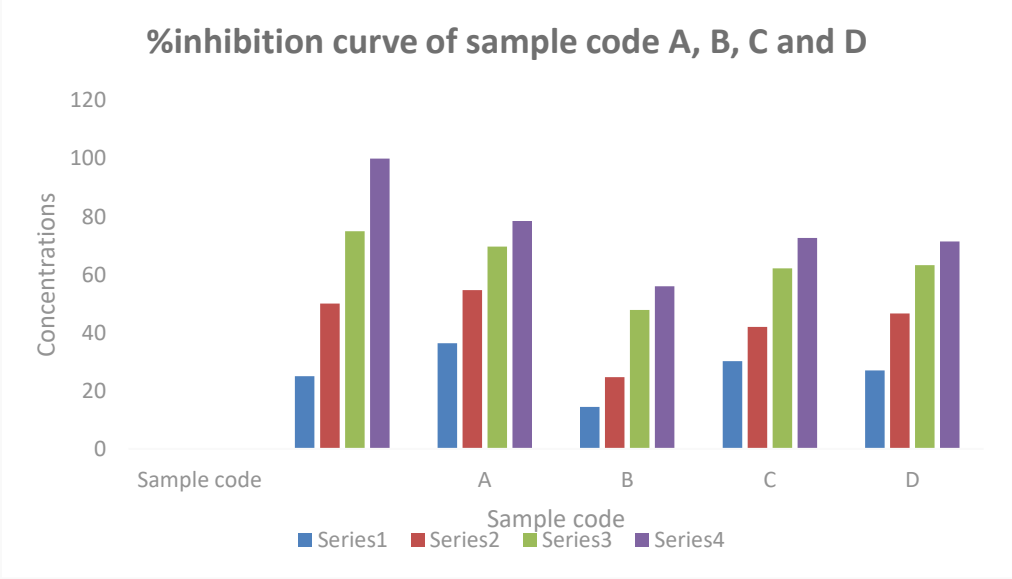

Fig.5: %inhibition curve of sample code A, B, C and D

Free radical scavenging activity of extract

The results of the free radical scavenging activity of *T. patula* flower, *Permoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs) tested by the DPPH method are depicted in Table, Fig. The comparative antioxidant activity among the test samples clearly revealed the effect of A, B, C and D, as compared to the standard Ascorbic acid.

Table 8: Antioxidant effects of PAME and Ascorbic acid

Sample	**% Inhibition at different concentration**	
A	IC 50 = 45.1530161	78.49145551µg/ml
B	IC 50 = 86.61705007	56.0007857 µg/ml
C	IC 50 = 59.56617522	72.59870359µg/ml
D	IC 50 = 59.04253545	71.42015321µg/ml

Table 9: % inhibitions of sample code A, B, C and D

Concentration	**% inhibitions**			
	A	B	C	M
25	36.35827932	14.4568847	30.17088981	27.02808878
50	54.72402279	24.67098802	41.95639364	46.57238264
75	69.65232764	47.84914555	62.18817521	63.26851306
100	78.49145551	56.0007857	72.59870359	71.42015321

Ascorbic acid, *T. patula* flower, *Permoterma reticulatum, and Curcuma caesia (Root hairs)* extract. All values are represented as mean ± SEM, n = 3 for each group. The results of antioxidant activities were studied by using in-vitro methods. The results of in-vitro antioxidant activities revealed that A, B, C and D. The IC50% values of A-78.49, B-56.00, C-72.59 and M-71.42%. The results revealed that mixture of extract having potential antioxidant activity due to presence of polyphenolic and potential bioactive compounds.

Drug-excipient compatibility

Drug excipient compatibility was studied by using FTIR spectroscopy. We were found that no any interaction present between the selected extract and excipients. All the active compounds of selected plant extract peaks were present no any changes were found. The FTIR spectra of mixture of plant extract and excipients both are shown below under.

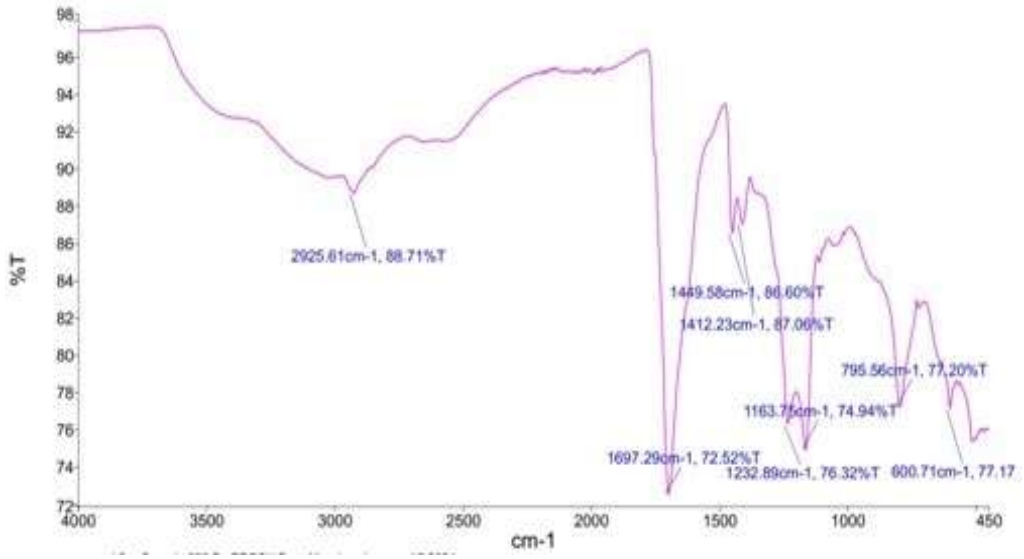

Fig.6: FTIR Spectra of mixture of plant extract

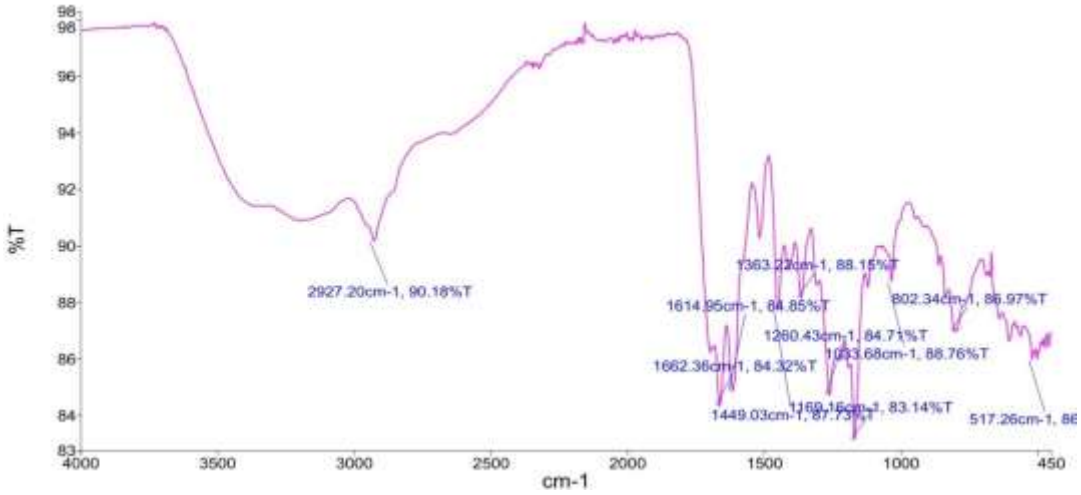

Fig.7: FTIR Spectra of mixture of plant extract and excipients

Table 10: FTIR peaks of mixture of plant extract and excipients

S.N.	FTIR Spectra of mixture of plant extract	FTIR Spectra of mixture of plant extract excipients
1	2925 cm^{-1}	2927 cm^{-1}
2	1697 cm^{-1}	1662 cm^{-1}
3	1449 cm^{-1}	1449 cm^{-1}
4	1232 cm^{-1}	1363 cm^{-1}, 1260 cm^{-1}
5	1163 cm^{-1}	1169 cm^{-1},1033 cm^{-1}

Organic and phenolic chemicals are responsible for these peaks. The main absorption band at about 3300 cm−1, which is connected to O–H stretching and C–H stretching vibrations, was the most noticeable signal for all three dried herbal extracts. In addition to 1379.79 O-H bening, alcoholic group phenol, tertiary alcohol, etc., there is also 1454.88 C=C-C aromatic ring stretching aromatic compound. These peaks also showed that the combination of these particular plant extracts has the potential to be therapeutic and can be utilized to treat skin infections, such as acne etc.

Physical evaluation of prepared gel

Physical and microbiological evaluation criteria were applied to the prepared herbal gels. Each gel had a translucent look and was colored yellowish orange. All gels had pH values between 6.8 and 7. Applying the gel to the skin revealed it to be non-irritating. Results of the microbiological and physical examination demonstrate that formulation F2 was bacteriostatic, whereas formulation.

Table 11: Evaluation of prepared gels

Formulation Code	**F1**	**F2**	**F3**	**F4**
a. Measurement of pH	6.9	6.8	6.9	6.9
b. Colour:	Light orange	Light yellow	Light yellow	Light orange
c. Odour:	Characteristic	Characteristic	Characteristic	Characteristic
d. Consistency:	+++	+++	+++	+++
e. Greasiness:	Nil	Nil	Nil	Nil
f. Homogeneity:	+++	+++	+++	+++
g. Grittiness:	Nil	Nil	Nil	Nil
h. Spreadability	+++	+++	+++	+++
i. Ease of absorbtion	+++	+++	+++	+++
j. Skin irritancy test:	Nil	Nil	Nil	Nil

- (no result), + (moderate), ++ (good), +++ (best)

Antimicrobial evaluation

The antimicrobial activities of extract A, B, C and D were determined by using in-vitro zone of inhibition and MIC activity. The results zone of inhibition and MIC level revealed that *Curcuma caesia (Root hairs)* highest antimicrobial activity. The results of zone of inhibition and MIC level are shown are below under.

Table: Zone of inhibition of plant extracts different plant materials against test organisms

Sample	***Staphylococcus epidermidis***			***Staphylococcus aureus***			***Escherichia coli***			***Pseudomonas aeruginosa***			***B. subtilis***			***Acne vulgaris***		
Concentration	**60µ g/ml**	**40µ g/ml**	**20µ g/ml**	**60µ g/ml**	**40µ g/ml**	**20µ g/ml**	**60µ g/ml**	**40µ g/ml**	**20µ g/ml**	**60µ g/ml**	**40µ g/ml**	**20µ g/ml**	**60µ g/ml**	**40µ g/ml**	**20µ g/ml**	**60µ g/ml**	**40µ g/ml**	**20µ g/ml**
A	12mm	11 mm	10 mm	23mm	22mm	22mm	13mm	11mm	10mm	26mm	16mm	11mm	16mm	14mm	12mm	15mm	10 mm	10 mm
B	19 mm	18mm	16 mm	26mm	22mm	20mm	25mm	24mm	25mm	24mm	22mm	21mm	22mm	14mm	12mm	23 mm	19mm	19 mm
C	20mm	17 mm	17 mm	25mm	23mm	21mm	26mm	24mm	22mm	28mm	26mm	24mm	22mm	24mm	22mm	23mm	21 mm	21 mm
D	20 mm	18 mm	17 mm	27mm	23mm	21mm	27mm	22mm	20mm	27mm	22mm	20mm	26mm	22mm	19mm	28mm	24mm	22 mm

Mean MICs (µg/mL) of the different plant materials against test organisms

Plant extract	***Staphylococcus epidermidis***	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***B. subtilis***	***Acne vulgaris***
A	480µg/ml	480µg/ml	480µg/ml	425µg/ml	350 µg/ml	375µg/ml
B	250 µg/ml	250µg/ml	250µg/ml	250 µg/ml	225 µg/ml	250µg/ml
C	300µg/ml	250µg/ml	250µg/ml	225 µg/ml	225µg/ml	250µg/ml
D	350µg/ml	350µg/ml	350µg/ml	400µg/ml	225µg/ml	300µg/ml

The MIC (µg/ml) levels of different plant materials found to be 200 to 500µg/ml level. Therefore we were selected the 5% concentration of plant extract mixture. The plant mixture having A, B, and C hydroalcohalic extract. The MIC (mg/ml) of the different plant materials (A, B, C, and D) and topical gel formulations F1, F2, F3 and F4 were studied. The results of MIC formulations different plant materials (A, B, C, and D) and topical gel formulations were showed equally potential against antimicrobial strains. The topical formulated gel having potential antimicrobial activities as compare to plant materials. All the topical formulations were found to be 100 to 500µg/ml. The prepared F4 optimized topical gel formulation was found to be more effective therefore we were selected this topical formulation for in-vitro drug release and stability study by using ICH guideline.

Mean MICs (mg/mL) of the different gel formulations against test organisms

Plant extract	***Staphylococcus epidermidis***	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***B. subtilis***	***Acne vulgaris***
F1	475 µg/ml	475µg/ml	450 µg/ml	425 µg/ml	350 µg/ml	400 µg/ml
F2	225 µg/ml	262.5µg/ml	225 µg/ml	250 µg/ml	225 µg/ml	235 µg/ml
F3	225 µg/ml	225 µg/ml	225 µg/ml	225 µg/ml	200µg/ml	200 µg/ml
F4	325 µg/ml	325µg/ml	375µg/ml	425 µg/ml	216µg/ml	300µg/ml

All values are represented as mean ± SEM, n = 3 for each group, Data were analysed by two-way ANOVA, for each bacterial strain, followed by Dunnett's multiple comparisons test Multiple Comparisons Test, ****p< 0.0001, Asterisk (*) denotes significant difference as compared to test drug prepared gel.

In-vitro permeation study of topical formulation

The in-vitro drug release of topical gel was studied and found that prepared topical gel release the drug up to 86.65% at 12h. The absorption of drug was determined by using 341nm. The in-vitro drug release studied by using franz diffusion cell. The F4 formulation was found to be effective and release the drug completely.

Table: Mean % release of the gel

S.N.	Sample taken time (h)	Abs (341nm)	Concentration	% Drug release
1	0.5	0.013	1.103	11.31%
2	1.0	0.027	2.14	21.96%
3	2.0	0.048	3.70	37.95%
4	3.0	0.058	4.15	40.52%
5	4.0	0.067	5.10	52.34%
6	5.0	0.089	6.73	69.05%
7	6.0	0.092	6.95	71.34%
8	12.0	0.099	7.47	86.65%

Stability Studies

The stability studies of prepared topical F4 gel were studied by using ICH guideline. The F4 formulation evaluated with Clarity, pH, Viscosity, Spreadability, Extrudability, Homogeneity, Grittiness etc. The results of F4 formulation were found to be very effective and satisfactory. The evaluated results revealed that F4 formulation was suitable for topical use.

Table 7 **Stability Studies**

Days	Formulation		Clarity	pH	Viscosity	Spreadability	Extrudability	Homogeneity	Grittiness	Syneresis
1	F4	4^0C	+++	7.32	5724	Excellent	Excellent	Good	No	No
15		25^0C	+++	7.32	5734	Excellent	Excellent	Good	No	No
30		40^0C	+++	7.12	5745	Excellent	Excellent	Good	No	No

Turbid: +, clear: ++, very clear (glassy): +++.

CONCLUSION

The results demonstrate that the prepared herbal gels were bacteriostatic at lower concentration and bactericidal at higher concentration. Results show that herbal formulations have potential to

treat skin disease and has efficacy comparable to allopathic drugs and hence can be a better option. However chemical stability of these gels is under progress to establish shelf life and determine chemical parameters of evaluation for these formulations.

ACKNOWLEDGMENT

The facilities provided by the Department of Pharmaceutical Science, Madhyanchal Professional University, Bhopal, Madhya Pradesh, India is gratefully acknowledged.

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SUMMARY AND CONCLUSION

The selection of phytoconstituents for acne treatment was based on a comprehensive literature review. Through phytochemical screening, various compounds such as triterpenoids, tannins, glycosides, flavonoids, polyphenols, carbohydrates, alkaloids, and other antioxidants were identified in extracts from *T. patula* flower, P*ermoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs). Flavonoids and polyphenols, known for their potent antioxidant properties, play a crucial role in activating the body's defense mechanisms against infectious agents. Studies have demonstrated that vicinyl dihydroxyl groups present in compounds like rutin and its metabolites enhance the ability of phenols to inhibit the formation of free radicals catalyzed by iron and copper, possibly by interfering with glucose metabolism. Quercetin and Gallic acid (GA), classified as polyphenols, exhibit both pro- and strong antioxidant activities.

Fourier-transform infrared spectroscopy (FTIR) confirmed the presence of alkaloids, tannins, polyphenols, and flavonoids in the extracts of . *patula* flower, P*ermoterma reticulatum* lichen, and *Curcuma caesia* (Root hairs). Free radicals, continuously generated in living systems, cause substantial damage to tissues and biological molecules, leading to various diseases. Natural antioxidants derived from food supplements and traditional medicine offer an alternative to synthetic medications, which often entail adverse side effects, in combating oxidative damage. This approach provides valuable insights into mitigating oxidative stress-induced pathogenesis using novel chemical compounds alone or in combination with natural sources possessing

antioxidant properties. Extracts from *T. patula* flower, *Permoterma reticulatum lichen*, and *Curcuma caesia* (Root hairs) exhibited strong antioxidant efficacy. The physiologically active compounds present in these herbs contribute to their potent broad-spectrum antibacterial activity, highlighting their potential as effective antimicrobial agents.

Source of Support: Nil.

Conflict of Interest: None declared.

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