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Research Article

FORMULATION AND EVALUATION OF HERBAL ANTI-ACNE GEL CONTAINING EXTRACTS OF TABERNAEMONTANA DIVARICATA AND PSIDIUM GUAJAVA

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ABSTRACT

This study aims to formulate a herbal anti-acne gel containing extracts of *Psidium Guajava* and *Tabernaemontana Divaricata* and to characterize the formulated gel for its physicochemical properties. The extraction method used was maceration and Continuous hot extraction. The presenceof diverse phyto-constituents in the obtained extracts was also determined. Three formulations containing the extracts of *Psidium Guajava* and *Tabernaemontana Divaricata* were prepared using 1% Carbopol 940 as a gelling agent. Parameters like colour, odour, homogeneity, phase separation, consistency, washability, pH, viscosity, spreadability and anti-microbial activity were evaluated. Theformulated gels were odourless, uniform, consistent, without lumps and washable. It was found that pH of the prepared formulations was closer to skin, had good viscosity and spreadability. The anti-microbial activity of the prepared gel formulation against p-acne was also investigated using disc diffusion method. The anti-microbial activity of the prepared gels was studied with a clear inhibitionzone. The formulated gel F2 has greater anti-microbial activity with a larger diameter of zone of inhibition compared to F1 and F3. It was concluded from study that the extract of *Psidium Guajava* and *Tabernaemontana Divaricata* can be formulated in an aqueous gel-based system for topical therapy of acne vulgaris.

INTRODUCTION

Acne vulgaris is ranked eighth among the top ten most common diseases worldwide in 2010. Microorganisms like Propionibacterium acnes, staphylococcus aureus and staphylococcus epidermidis proliferate rapidly leaving to the development of acne. Ideally, topical therapy is the first-line of treatment in mild acne, whereas for moderate and severe acne, systemic therapy is required in addition to topical therapy. Topical therapy has been associated with side effects and the undesirable physicochemical characteristics of certain important agents like tretinoin and benzoyl peroxide affect their utility and patient compliance. The latest treatment regimen followed is the one-step acne solutions, but they too have disadvantages in that, they are 99% oil-based creams and contain either (or both) benzoyl peroxide or (and) salicylic acid.

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The increasing frequency of taking various antibiotics as treatment can lead to the development of antibiotic resistance. Antibiotic resistance has been increasing in prevalence within the dermatological setting, to overcome the problem of antibiotic resistance medicinal plants have been studied as alternative treatments for diseases. Plants with medicinal value are more difficult to develop drug resistance than its synthetic compounds. *Tabernaemontana divaricatas* indole alkaloids exhibit a wide range of pharmacological activities including antibacterial activity against both gram positive and gram- negative bacteria. This plant has been reported in literature as a good antioxidant and anti-inflammatory agent. In the previous studies it was also known that guava leaves have antibacterial activity against Propionibacterium acnes, staphylococcus aureus and staphylococcus epidermis which are the major causes of acne. [1,2] Based on the background evidence and to overcome the side effects of synthetic active ingredients mentioned above the purpose of the present study is to formulate and evaluate anti-acne gel containing leaf extracts of *Tabernaemontana Divaricata* and *Psidium Guajava.*

MATERIALS AND METHODS

Material

Fresh leaves of *Tabernaemontana Divaricata* and *Psidium Guajava* were collected from home garden, Belagavi. The collected leaves were authenticated by Dr.Ajit Lingayat from Shri B.M.K Ayurveda Mahavidyalaya. The obtained leaves were dried and powder using pulverizer at K.L.E Society Ayurveda Pharmacy. All the other solvents and chemical used in the study were of analytical reagent grade.

Preparation of Ethanol Extracts of *Tabernaemontana Divaricata* leaves Soxhlet extraction

This process is also known as continuous hot extraction. The apparatus is called Soxhlet extractor, made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube, and condenser at the top. A dried, grinded, and finely powder of *Tabernaemontana Divaricata* leaves was placed inside a porous bag (thimble) made using strong filter paper and tightly closed. The extraction solvent that is ethanol was poured into the bottom flask. The solvent was then heated, vapours from round bottom flask evaporates, and passes through the condenser where it condenses and flows down to the extraction chamber and extracts the drug by coming in contact with it.

Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The entire process was continued repeatedly until the drug was completely extracted, until a point when the solvent flowing from extraction chamber does not leave any residue behind. The obtained extract was then subjected to rotary vaccum evaporation process to recover the solvent.[3]

Preparation of Ethanolic Extracts of *Psidium Guajava* leaves Maceration

Coarsely powdered leaves of *Psidium Guajava* were placed inside a conical flask, the menstruum i.e., (ethanol 96%) was poured on top of it until completely covers the drug material. The container was then closed and kept for three days. The contents were stirred periodically, to ensure complete extraction. At the end of extraction, the micelle was separated from marc by filtration Subsequently, the obtained extract was then subjected to evaporation on top of water bath.[3]

Qualitative Chemical Investigation of Obtained Extracts Test for Alkaloids

A sample of 0.5% to 0.6 g was mixed in 8 ml of 1% HCl, heated and filtered. 2 ml of filtrate were treated with the following reagents; after which it was observed whether alkaloids were present or absent with turbulence or formation of precipitates.

Mayer's Test: A few drops of Mayer's reagent in 2-3ml of filtrate indicate a precipitate of cream.

Dragendroff's Test: A few drops of the Dragendroff's reagent show a reddish-brown precipitate in2-3 ml.

Test for Carbohydrates

Molisch's Test (General Test): For 2-3 ml of aqueous sample solution, mix, shake and compress a few drops of alpha-naphthol solution H_2S0_4 from the sides of the test tube. The formation of a violetcolor at the junction of the two fluids indicates the presence of carbohydrates.

Benedict's test: The extract on heating with benedict's reagent, brown precipitate indicates the presence of sugar.

Test for Flavonoids

Shinoda test: In a sample of dried powder, add 5% of 95% ethanol, some drops of conc. HCl and 0.5gm of magnesium turnings. The presence of orange, pink and red to purple confirms the presenceof flavonoids.

Ferric chloride test: Few drops of neutral ferric chloride solutions are added to little quantity of alcoholic extract. A blackish green colour produced indicates the phenolic nucleus.

Test for Tannins

Ferric chloride test: Few drops of neutral ferric chloride solutions are added to little quantity of alcoholic extract. A blackish green colour produced indicates the phenolic nucleus.

Test for Steroids

Salkowski test: 0.5 g samples were carefully mixed in 2 ml of chloroform and 3 ml of concentrate H_2SO_4. A layer of greenish yellow or reddish brown has been formed at the interface indicating a positive result for the presence of steroids.

Tests for Proteins

Millon's test: Treat the test sample with a few drops of Millon's reagents, when it is heated, a white precipitated form that turns brick red or disappears indicating the presence of protein. [4]

Formulation of Gel from Prepared Extracts

The gels were made using various amounts of plant extracts. A total of three formulation batches were prepared according to the composition of formulations shown in table no 1. Carbopol 940 was dissolved in distilled water in a separate beaker with continuous stirring to avoid air entrapment. Methyl Paraben was dissolved in distilled water in another beaker using a water bath and then cooled. The plant extract was added to the above-mentioned mixture. To this solution, the Carbopol 940 mixture was added and thoroughly mixed. After that, propylene glycol and triethanolamine were added and the pH was adjusted to 6.8-7 with continuous stirring. The final volume was made by adding distilled water. To eliminate bubbles, the prepared gels were kept at room temperature for a day.[5]

Table 1: Formulation Table of Gel Containing Extracts of *Psidium Guajava* & *Tabernaemontana Divaricata*

Ingredients	Quantity taken for 100gm		
	F1	F2	F3
Psidium guajava	1%		0.5%
Tabernaemontana divaricata		1%	0.5%
Carbopol-940	1%	1%	1%
Methylparaben	0.1%	0.1%	0.1%
Propylene glycol400	2ml	2ml	2ml
Triethanolamine	q.s	q.s	q.s
Water	q.s	q.s	q.s

Q. S= Quantum Satis

Physiochemical Evaluation of the Prepared Gels

Colour

The colour of the prepared gels was examined by the naked eye against white and black background.

Odour

By dissolving the prepared gels in water, the odour of the gels was tested by smelling.

Homogeneity

By applying the prepared gels to a transparent glass plate, the presence or absence of particles that have not been mixed homogeneously was examined using visual inspection.

Phase separation

By applying the prepared gels to a transparent glass plate, the presence or absence of aggregates was examined using visual inspection.

Consistency

By applying the prepared gels to a transparent glass plate, the presence or absence of coarse particles was examined using visual inspection.

pH determination

The pH meter had previously been calibrated with a standard buffer solution (pH 6.86). Within 24 hrs of production, the pH of formulated gels was measured with a calibrated digital pH meter at a constant temperature. The pH of formulated gels was measured three times and mean values were computed.

Viscosity measurements

The formulated gels viscosity was determined by using (Digital Rotational Viscometer). The measurement of viscosity was repeated three times and mean values were computed.

Spreadability

The spreadability of formulated gels was determined by measuring the spreading diameter of 0.5 g of gel across two 125 g glass plates measuring 2.54 cm x 7.62 cm each. The gel was placed on a circle of 2 cm diameter marked on the first glass plate and then the second glass plate was placed over the gel. For 5 mins, a 500 g weight was applied to the upper second glass plate. The weight was removed and the diameter of the circle after gel spreading was measured by using a vernier caliper from the three sides of the circle. The mean value of the diameters measured was computed. This procedure was repeated three times for all the formulated gels.[6]

Antibacterial Activity Studies

Zone of inhibition also known as zone of clearing refers to the clear zone surrounding the antimicrobial agent. The zone of inhibition results from a complete absence of bacteria on a confluent bacterial lawn, the antimicrobial activity of the agent was screened against a test organism which was used to create a confluent lawn of bacterial growth on an agar plate. The method used to determine the zone of inhibition in the present study was disk diffusion method. The nutrient medium used was brain heart infusion agar, using a sterile loop the colonies of p. acne were transferred to theagar plate. Stock solutions were prepared where 10mg of the prepared gel formulation (F1, F2 and F3) and clindamycin (control) were dissolved in 1ml of DMSO. Hollow tube of 5-millimeter diameterwas used to make a well in the agar plate. The hollow tube was heated, pressed on the inoculated agar plates and were removed immediately by making well in plate, like wise 6 wells were made oneach plate. With the help of a micropipette 75, 50, 25, 10 and 5microlitre of the above prepared solution were added in five wells, clindamycin (75microlitre) which served as a control was added to the sixth well. The above plates were incubated for 24hr at 37 degrees centigrade in an incubator. The diameter of zone of inhibition was measured by using a vernier caliper. [7-9]

Stability Studies

As per ICH guidelines, the stability studies for prepared gel formulation were carried out at room temperature and under accelerated condition (40°±2°C/75%RH± 5%RH) for one month to assess the impact of temperature and humidity on prepared gel formulations. Parameters such as pH, spredability, viscosity, color, odour, homogeneity were assessed to

verify the stability of the prepared gel formulation during the storage period.[10]

Results and Discussion

Phytochemical screening of obtained extracts

The results of qualitative phytochemical screening of the *psidium guajava* and *tabernaemontana divaricata* extracts revealed the presence of diverse type of phytoconstituents namely alkaloids, tannins, flavonoids, triterpenoids, glycosides. It has been reported that the presence of flavonoids and alkaloids are responsible for antibacterial and antioxidant activity. The results are shown in the table no.02

Table 2: Results of Phytochemical Screening of Obtained Extracts

Secondary Metabolites	Results P.G	Results T.D
Tannins	+	++
Alkaloid	++	++
Flavonoids	++	++
Terpenoids	++	+
Carbohydrates	+	_
Proteins	+	_
Steroids	_	+

[-] =Absent [+] =Present in moderate quantity [++] =Present in large quantity

P. G= *Psidium guajava* T. D= *Tabernaemontana divaricata*

Colour and Appearance

The colour, appearance of the formulated gel was found to be good, for F1 it was Brown, F2 Green and F3 Muddy Green respectively. The results shown in table no.3 The prepared formulation were odourless, free from any objectionable odour. The appearance of prepared gel formulations was satisfactory.

pH

The pH all the formulation range between 6.07±0.05 to 6.84±0.02. The values were well within the acceptable limits, and matched the skin pH. The results shown in table no 4.

Spredability

The spreadability of prepared formulations was found in the range of 9.08±0.45 to 9.72±0.54 mm/s. All the formulation had acceptable spredability and were free form any gritty particles. Result showed in table no 4.

Viscosity

The viscosity of prepared gel was in the range of 7356.00±133.24 to 7590.67±300.11 cps. All the formulation had desired viscosity. Result shown in table no 4.

Washability

All the prepared gel formulations had good washability.

Table 3: Results of Organoleptic Characteristics

Formulation	Colour	Odour	Homogeneity	Consistency
F1	Brown	Odourless	Uniform homogeneity	Good
F2	Green	Odourless	Uniform homogeneity	Good
F3	Muddygreen	Odourless	Uniform homogeneity	Good

F1 to F3 showed no phase separation and good washability

Table 4: Results of Evaluation Parameters

Formulation	pH	Viscosity (cps)	Spreadability (mm/S)
F1	6.07±0.05	7452.33±157.24	9.49±0.33
F2	6.67±0.01	7590.67±300.11	9.08±0.45
F3	6.84±0.02	7356.00±133.24	9.72±0.54

Each Value represents mean ±SD n=3

Table 5: Antibacterial Activity of the Prepared Gel Formulations

Samples	Diameter of Zone of inhibition (mm)
Clindamycin [control]	30
F1	13
F2	25
F3	20

F1= *Psidium guajava*; **F2=** *Tabernaemontana divaricata*; **F3=** *Psidium guajava + Tabernaemontana Divaricata*

Antibacterial Activity

Table no 5 and fig no: 1, 2, 3 shows the antibacterial activity of the prepared formulations (F1, F2 and F3) and clindamycin (control) represented by the inhibition zone. All the prepared formulation displayed antibacterial effect against *p.acne*. The diameter of zone of inhibition for F1, F2, F3 and clindamycin were found to be 13 mm, 25mm, 20mm and 30mm respectively. This could be due to the antibacterial activity of the plant extract as reported in previous studies, which caused changes in cell shape in the form of damaged and hollow bacterial cell wall, inhibiting the growth of bacteria. F2 (*tabernaemontana divaricata*) exhibited stronger antibacterial potential against *p.acne* with an inhibition zone of 25mm as compared to F1 (*psidium guajava*) and F3 (*psidium guajava + tabernamontena divaricata*) respectively.

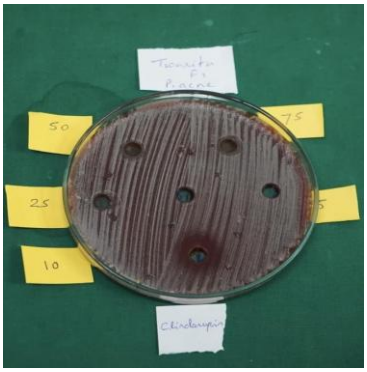

Fig 1: Antimicrobial susceptibility testing showing varying quantities of F1 and clindamycin against Confluent lawn of *P.Acne.*

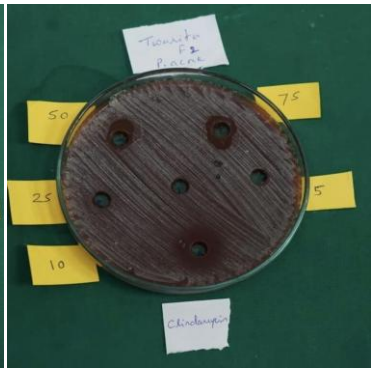

Fig 2: Antimicrobial susceptibility testing showing varying quantities of F2 and clindamycin against Confluent lawn of *P.Acne.*

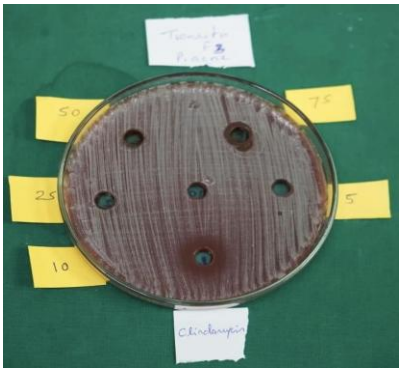

Fig 3: Antimicrobial susceptibility testing showing varying quantities of F3 and clindamycin against Confluent lawn of *P.Acne.*

Stability Studies

Stability studies were carried out to assess the impact of temperature and humidity on the prepared gel formulations. The samples were withdrawn periodically to determine the change in colour, odour, homogeneity, pH, viscosity and spreadability. Data obtained from stability studies shown in table no 6. No significant changes in the evaluated parameters were observed. Hence, we conclude that the prepared gel formulations were stable at room temperature and accelerated conditions.

Table 6: Results of Stability Testing

Condition	**Parameter**	**15 days**			**30 days**		
		F1	F2	F3	F1	F2	F3
At room temperature	Colour	Brown	Green	Muddygreen	Brown	Green	Muddygreen
	Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
	Homogeneity	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform
	pH	6.07±0.05	6.67±0.001	6.84±0.02	6.02±0.03	6.67±0.01	6.94±0.02
	Spreadability (mm/s)	9.49±0.33	9.08±0.45	9.72±0.54	9.39±0.30	9.02±0.42	9.42±0.52
	Viscosity (cps)	7452.33 ±157.2	7590.67 ± 300.11	7356.00 ± 133.2	7346.22 ± 146.2	7380.37 ± 299.31	7256.00 ± 142.2
Accelerated stability condition (40°c±)	Colour	Brown	Green	Muddygreen	Brown	Green	Muddygreen
	Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
	Homogeneity	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform
2°c/75 ± 5% Rh	pH	6.09±0.07	6.69±0.003	6.86±0.01	5.06±0.02	5.52±0.01	5.96±0.1
	Spreadability	9.49±0.34	9.06±0.33	9.73±0.52	8.24±0.12	8.34±0.34	8.23±0.26
	Viscosity	7452.12 ± 123.1	7592.61 ± 300.12	7254.11 ± 131.1	6345 ± 145.2	6178.23± 122.31	6345.00 ± 134.2

CONCLUSION

A satisfactory attempt has been made to formulate and evaluate an herbal anti-acne gel. The following conclusions were drawn from the obtained result, an anti- acne gel extracts of *Psidium guajava* and *Tabernaemontana divaricata* were formulated and evaluated. The extracts were obtainedusing maceration and soxhlet extraction technique. The obtained extract underwent qualitative phytochemical screening. Further the obtained extracts were incorporated into gel using carbopol- 940 as a gelling agent. The prepared gels were evaluated for the following parameters like appearance, colour, odour, homogeneity, consistency, washability, pH, spreadability and viscosity. All the formulation had acceptable organoleptic and physicochemical characteristics. Anti-bacterial studies were performed on all the prepared formulations. The results of the anti- bacterial study revealed that all the prepared formulations had good anti-bacterial potential against p-acne. The stability study revealed that all the formulations were stable at room temperature and accelerated condition. Hence, from the above findings we can conclude that the prepared herbal anti-acne gel could be an effective tool for managing and treating acne. However more investigation is required to strengthen this study.

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