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Formulation and Evaluation of Topical Polyherbal Antiacne Gels Containing *Luffa Acutangula, Amaranthus Spinosus* and *Morus Alba*

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ABSTRACT

Anti-acne herbal formulations are used for the treatment of acne vulgaris with the added advantage of not producing adverse effects unlike synthetic drugs. Acne is an inflammatory skin disease that occurs due to blockages in polysebase and inflammation that are caused by bacteria. Topical and systemic antibiotics are always used for treatment of acne, but the gradual resistance to antibiotics can affect the success rate of acne cure. Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defence mechanisms against predator such as microorganism, insects and herbivores which have been proved to be potentially active compounds. There is a tremendous increase in search of antimicrobial plant extracts due to the fact that the resistance offered against antibiotic by the microorganism, in short the effective life span of any antibiotic is limited. *Propionibacterium acnes* are common pus-forming microbes responsible for the development of various forms of acne. In the present study anti-acne gels were prepared using polymer carbopol 940 along with the hydroalcoholic extracts of plants fruits of *Luffa acutangula,* leaves of *Amaranthus spinosus* and *Morus alba* and evaluated for their physicochemical properties, like pH, washability, extrudability, spreadability and viscosity. The formulations (PHG1-PHG6) were tested for the anti-acne activity by well diffusion method against *Propionibacterium acnes.* Results showed that the gels were non-irritant, stable and possess anti-acne activity. The efficacy when tested with a standard was almost same to that of Clintop (Marketed gel). This suggests that fruits of *Luffa acutangula,* leaves of *Amaranthus spinosus* and *Morus alba* have potential against acne causing bacteria and hence they can be used in topical anti-acne preparations and may address the antibiotic resistance of the bacteria.

Keywords: *Luffa acutangula, Amaranthus spinosus*, *Morus alba, Propionibacterium acnes, Acne vulgaris,* Carbopol, Physicochemical properties.

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INTRODUCTION

Acne vulgaris is a cutaneous disorder of multifactorial origin which manifests in the pilosebaceous follicle. It is characterized by open and closed comedones and inflammatory lesions like papules, pustules and nodules[1] [1]. Micro-organisms like *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* proliferate rapidly[2] leading to the development of acne. In clinical management of acne vulgaris, a considerable number of antibiotics and chemotherapeutic agents are available in the global market as topical or systemic treatment modalities[3]. Topical therapy is preferred as first-line treatment in mild acne whereas for moderate and severe type of acne, systemic therapy is required in addition to topical therapy. Topical therapy has associated side effects and the undesirable physicochemical characteristics of certain important agents like tretinoin and benzoyl peroxide affect their utility and patient compliance[4]. The latest treatment regimen followed is the one-step acne solutions[5] , but they too have disadvantages in that, they are 99% oil based creams and contain either (or both) benzoyl peroxide or (and) salicylic acid. Oil based products are counterproductive because they both fight and contribute to acne by clogging pores. Benzoyl peroxide[6] and salicylic acid[7] are generally more irritating than acne itself. So the authors felt a need to develop a formulation that is water based and devoid of harmful chemicals. Herbal therapies on the other hand are gaining attention in comparison to existing formulations which cause enormous side effects like skin dryness, rashes, wrinkling, erythema, pruritis, skin eruption and development of resistance[8]. Several plants with antimicrobial and antioxidant activity such as *Ocimum gratissimum* [9], *Psidium guajava*[10], *Garcinia mangostana*[11] and *Humulus lupulus L.* were found to be effective[12] for prevention of acne vulgaris. Our present study aims to

explore medicinal plants for their Antiacne potential so as to bypass these side effects and to provide natural essence to the skin. Selection of common herbs namely Amaranthus spinosus, Luffa acutangula and Morus alba was based on findings that they possess many pharmacological attributes such as being antibacterial[13,14], antioxidant[15-17]and anti-inflammatory[18-20]. *Amaranthus spinosus* Linn. (Amaranthaceae) is commonly known as Kate Wali Chaulai Kanatabhajii) in Hindi, and is used as a vegetable and cultivated throughout India, Sri Lanka, and many other tropical countries. In Ayurveda (Indian traditional system of medicine) the plant is used as a digestive, laxative, diuretic, stomachic, and antipyretic, to improve appetite, biliousness, blood diseases, burning sensation, leprosy, bronchitis, rat bite, piles, and leucorrhea, while the boiled leaves and root are given to children as a laxative,emollient, and poultice for abscesses, boils, and burns[21,22]. The leaves are used to treat rheumatic pain, stomach-ache, eczema, gastroenteritis, gallbladder inflammation, boils, abscesses, snake bite, colic menorrhagia, and arthritis[23]. The plant has a high concentration of antioxidant components, high nutritive value due to the presence of fiber and proteins, and a high concentration of essential amino acids, especially lysine[24-27]. *Amaranthus spinosus* is also used as an anti-inflammatory, antimalarial, antibacterial, antimicrobial, antidiuretic, and antiviral agent and in hepatic disorders[28-30]. *Amaranthus spinosus* has several active constituents including alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, anthriquinone derivatives, volatile oils, organic acids, betalains, β-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins, polyuronides, and carotenoids. The betalains in the stem bark of *A. spinosus* were identified as amaranthine, isoamaranthine, hydroxycinnamates, quercetin, and kaempferol glycosides[31-33]. It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine, along with stigmasterol glycoside and betaines such as glycine betaine and trigonelline[34,35]. *Luffa acutangula (*L.) Roxb belongs to Cucurbitaceae family, is commonly known as ridge gourd. It is widely found throughout southeastern Asia as a growing vegetative climber. The young fruits usually are taken as vegetables. The plant has been shown to have various medicinal properties such as treatment of jaundice, splenic enlargement and laxative and also proved as CNS depressant used traditionally in insect bites[36,37]. The plant also has potent α-glucosidase inhibitory effect[38]. *Morus alba* L., known as white mulberry, is a short-lived, fast-growing, small to medium sizedtree, native to northern China and is widely cultivated and naturalized elsewhere. Mulberry trees especially *M. Alba* is a widely found plant in Egypt. It is a wild plant available all over the year and found in a large amount in Beni-Suef governorate mainly in Beni-Suef villages[39]. *M. alba* has garnered great attention for its antioxidative and antidiabetic effects and is an important ingredient of herbal tea[40]. Recent studies have shown *M. alba* has antioxidant, antibacterial, antiviral and anti-inflammatory properties[16,20]. The plant is reported to contain the main active principles phytoconstituents like; tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, benzofuran derivatives, morusimic acid, anthocyanins, anthroquinones, glycosides and oleanolic acid as the[41]. Some phenolic compounds (flavonoids, stilbenes and 2- arylbenzofurans) have been reported from *M. Alba* and have been known to show antimicrobial[42].

MATERIALS AND METHODS

Plant materials

Fruits of *Luffa acutangula,* Leaves of *Amaranthus spinosus* and *Morus alba* were collected from local area of Bhopal (M.P.) in the month of March, 2018. Plant material (Fruits and Leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Extraction

Dried powdered of fruits of *Luffa acutangula,* leaves of *Amaranthus spinosus* and *Morus alba* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^{0}C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[43].

Qualitative phytochemical analysis of plant extract

The *Luffa acutangula, Amaranthus spinosus* and *Morus alba* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate and Khandelwal[44,45]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, protein and amino acid.

Quantification of secondary metabolites

Total Phenolic content estimation

The total phenolic content was determined using the method of Olufunmiso *et al*[46]. A volume of 2 ml of extracts or standard was mixed with 1ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids content estimation

The total flavonoid content was determined using the method of Olufunmiso *et al*[46]. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extracts or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Total alkaloids content estimation

The plant extract (20mg) was dissolved in 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and

collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

Formulating anti-acne gel

Measured quantity of methyl paraben, glycerine, polyethylene glycol and hydroalcoholic extract of fruits of *Luffa acutangula,* leaves of *Amaranthus spinosus* and *Morus alba* were dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer (or sonicator). Then carbopol 940 was added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. All the samples were allowed to equilibrate for 24 hours at room temperature prior to performing rheological measurements (Table 1).

Table 1 Formulation of polyherbal Gel

Ingredients (%)	PHG1	PHG2	PHG3	PHG4	PHG5	PHG6
Luffa acutangula extract	1gm	1gm	1gm	1gm	1gm	1gm
Amaranthus spinosus extract	1gm	1gm	1gm	1gm	1gm	1gm
Morus alba extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.25mg	0.5mg	0.75mg	1.0 gm	1.25 gm	1.5 gm
Polyethylene Glycol	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Methyl Paraben	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg
Triethanolamine	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100ml	100ml	100ml	100ml	100ml	100ml

Evaluation of polyherbal gel

Appearance and consistency

The physical appearance was visually checked for the texture of polyherbal gel formulations.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

Extrudability determination of formulations

The polyherbal gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

Determination of spreadability

A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability.

Method:

Two glass slides of standard dimensions (6×2) were selected. The anti-acne gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the anti-acne gel formulation between the two slides was traced uniformly to form a thin layer. The weight was removed and the excess of the anti-acne gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each anti-acne gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams),

l= length of glass slide (6cms), t = time taken is seconds.

Determination of pH

The pH of the anti-acne gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Drug content

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 3 ml of stock solution was mixed with 1 ml of 2 % $AlCl_3$. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 420 nm using a spectrophotometer [47-50].

***In-vitro* anti acne activity**

Preparation of plates

After sterilization, the nutrient agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37°C overnight to check the sterility of plates. The plates were dried at 50°C for 30 minutes before use.

Revival of the bacterial and fungal cultures

The Bacterial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient broth than incubated for 24 hours at 37°C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the agar plates with

loop full of bacteria and further incubated for next 24 hours at 37°C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antibiogram studies

The well diffusion method was used to determine the antibacterial activity of the polyherbal gel prepared from the fruits of *Luffa acutangula,* leaves of *Amaranthus spinosus* and *Morus alba* using standard procedure [51]. There were 3 concentration used which are 25, 50 and 100 mg/ml for antibiogram studies. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells with particular concentration of drug.

RESULTS AND DISCUSSION

The crude extracts so obtained after the maceration extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The percentage yield of extract was given in Table 2. Phytochemical analysis of hydroalcoholic extracts of plants showed the presence of flavonoid, Phenol, alkaloids, carbohydrate and saponins while, protein, glycosides and oils and fats were reported to be absent Table 3. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) total flavonoid content (TFC) and total alkaloid content (TAC) Table 4. From the psychorheological characteristics studies of formulation showed that all of them have clear colour, No clogging, good homogeneity and smooth texture Table 5**.** The results of washability, extrudability, spreadability, pH, viscosity was given in Table 6. In all formulations of gel the spreadibility and viscocity of PHG5 is good was found to be 13.12±0.15 and 3654±25. Extrudability study was performed by gel formulations were filled into aluminium collapsible tubes, the formulation have average extrudability. The skin irritation test performed showed no signs of sensitivity, erythema and edema. So the prepared formulations were considered to be non-irritant. In the all formulation of different gels the percentage of flavonoid content was found maximum in PHG5 Table 7.

Table 2 % Yield of hydroalcoholic extract

S. No.	hydroalcoholic Extracts	% Yield (w/w)
1	*Luffa acutangula* extract	4.6
2	*Amaranthus spinosus* extract	3.9
3	*Morus alba* extract	4.2

Table 3 Result of phytochemical screening of hydroalcoholic extracts

S. No.	Constituents	*Luffa acutangula*	*Amaranthus spinosus*	*Morus alba*
1.	Alkaloids	-ve	+ve	+ve
2.	Glycosides	-ve	-ve	-ve
3.	Flavonoids	+ve	+ve	+ve
4.	Diterpenes	-ve	+ve	-ve
5.	Phenol	+ve	+ve	+ve
6.	Amino Acids	-ve	-ve	-ve
7.	Carbohydrate	+ve	+ve	+ve
8.	Proteins	-ve	-ve	-ve
9.	Saponins	+ve	+ve	+ve
10.	Oils and fats	-ve	-ve	-ve

Table 4 Estimation of total phenolic, flavonoids and alkaloid content

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extracts		
		Luffa acutangula	*Amaranthus spinosus*	*Morus alba*
1.	Total alkaloid (Atropine equivalent (AE) mg/100mg)	-	0.098	0.365
2.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	0.543	0.743	0.645
3.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.897	0.934	0.792

Table 5 Results of psycho rheological characteristics

Formulation	Colour	Clogging	Homogeneity	Texture
PHG1	Brown	Absent	Good	Smooth
PHG2	Brown	Absent	Good	Smooth
PHG3	Brown	Absent	Good	Smooth
PHG4	Brown	Absent	Good	Smooth
PHG5	Brown	Absent	Good	Smooth
PHG6	Brown	Absent	Good	Smooth

Table 6 Results of washability, extrudability, spreadability, pH, Viscosity

Formulation	Washability	Extrudability	Spreadability (gcm/sec)	pH	Viscosity (cps)
PHG1	Good	Average	15.23±0.12	6.82± 0.11	3150±10
PHG2	Good	Average	14.65±0.15	6.95±0.15	3256±15
PHG3	Good	Average	14.15±0.25	7.02±0.11	3365±18
PHG4	Good	Average	13.65±0.35	7.05±0.14	3458±20
PHG5	Good	Average	13.12±0.15	7.00±0.12	3654±25
PHG6	Good	Average	13.25±0.33	7.15±0.13	3562±22

Table 7 Results of Flavonoid Content using $AlCl_3$ method

Formulation	% Flavonoid Content
PHG1	88.25
PHG2	90.25
PHG3	89.98
PHG4	90.25
PHG5	95.56
PHG6	92.25

The efficacy of the anti-acne gels from polyherbal extracts is shown in Table 8. The anti-acne gels could inhibit the growth of the microorganisms that inhabit acnes and the ployherbal gel exhibited comparatively more efficacy to Clintop marketed gel.

Table 8 Anti-acne activity of marketed gel and polyherbal gel formulation against *Propionibacterium acnes*

S. No.	Formulation	Zone of inhibition		
		100mg/ml	**50 mg/ml**	**25mg/ml**
1.	Clintop (Marketed gel)	18±0.5	16±0.94	15±0.57
2.	Polyherbal gel	20±0.74	17±0.86	16±0.5

CONCLUSION

The present study was aimed to developed polyherbal gels for anti acne treatment using hydroalcoholic extracts of fruits of *Luffa acutangula,* leaves of *Amaranthus spinosus* and *Morus alba a*n aqueous based carbopol gel system and evaluated for their physicochemical properties, like pH, spreadability, viscosity and microbial assay. The anti acne activities of the mentioned gel were more than marketed gel, this needs to be fully clarified by further assay methods and using additional concentrations of extracts. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its anti acne activity and to explore the existence of synergism if any, among the compounds.

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