Research Article

FORMULATION AND EVALUATION OF POLYHERBAL GEL CONTAINING NATURAL ANTIMICROBIALS FOR THE MANAGEMENT OF ACNE VULGARIS

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ABSTRACT

Acne vulgaris is collective disorder of the skin affects approximately 80% adolescents during their puberty stage. Medicinal plants are used all over the world to treat various diseases due to its variety of phytochemical constituents. Ideally, topical therapy is the first line treatment for many skin diseases. Among the various topical formulations, gels have been considered as a potential vehicle due to its non-sticky nature, stable and greater aesthetic value. The objective of this proposed study was to develop a polyherbal topical gel formulation containing *Camellia sinensis*, *Cassia auriculata* and *Ocimum gratissimum* to treat acne as a safe, effective and an alternative therapy to the current conventional harmful antibiotics. Medicinal plants used for this study are selected based on antibacterial activity. Extracts of the selected plants were incorporated into a gel base and evaluated for its physicochemical properties such as pH, viscosity, spreadability and antibacterial activity against *S. aureus* and *S. epidermis*. The physicochemical evaluation of the developed formulation showed no lumps, had uniform color dispersion and from any fiber and particle. It was also observed to have easy washability, good spreadability and pH was found to be 6.94, similar to pH of the skin. The antibacterial study of the developed formulation showed dose/concentration proposed inhibitory activity against *Staphylococcus aureus* and *Staphylococcus epidermis*. The study results concluded that the methanolic extract of *Camellia sinensis*, *Cassia auriculata* and *Ocimum gratissimum* in an aqueous gel base system is an appropriate formulation for the for topical therapy of acne vulgaris.

Keywords: *Camellia sinensis*, *Cassia auriculata*, *Ocimum gratissimum*, antiacne, polyherbal gel formulation, antibacterial

INTRODUCTION

In human being, skin is the most susceptible part for entering of various pathogens, microorganisms and spreading of diseases. In general, acne vulgaris originates at puberty stage due to hormonal changes which ultimately results in changes in pathophysiologic factors¹⁻³. Microorganisms such as *Staphylococcus*, *Propionibacterium* and *Escherichia* species are responsible for the formation of acne. *Staphylococcus* is an ubiquitous organism responsible for localizing cutaneous infections and colonizes in the skin^{1,4}.

During the puberty stage, sebum is secreted in higher amount from the sebaceous glands due to increased production of androgens. Sebum is a lipid rich secretion which acts as a media for growing of acne causing bacteria's. The severity of acne formation is depends upon the sebum production. Acne is the 8th most prevalent disease worldwide and the prevalence of acne is about 9.4%. Globally, the epidemiology of the acne were found to be around 85% of adults in the age between 12 and 25 years old, 8% of adults in the age between 25 and 34 years, 3% of adults in the age between 35 and 44 years old and 42.5% of men and 50.9% of women are getting affected in their twenties^{5,6}. Acne is treated by antibiotics either oral or topical application, hormonal therapies, corticosteroids or surgery^{2,7}. Prolong use of antibiotics may leads to develop antibiotic resistance and various side effects such as erythema, photosensitivity, allergic

dermatitis, excessive skin irritation, urinary problem, joint and muscle pain, headache, depression etc⁸.

Due to the increasing frequency of intake of antibiotics, expensiveness and its side effects, there is a need to focus on the scientific exploration of herbal drugs. According to World Health Organization (WHO) estimate, nearly 75- 80% of the world population still uses herbs and other traditional medicines for their primary health care needs⁹. India has about 45,000 plant species among which, medicinal property has been attributed to several thousands.

India is a hub for medicinal plants, about 15% out of the 20,000 medicinal plants of the world is found growing wild in different climatic conditions¹⁰. There is a need for continuous search of indigenous drugs which can provide cheaper and better therapeutic efficacy. Literatures have proven that plants of varying potency when combined theoretically might produce the synergistic therapeutic effect¹¹⁻¹². The reason for the synergistic effect of the polyherbal formulation might be due to the potentiating effects of other plants active constituent's leads to require lower dose to produce the desired therapeutic effect which can improve patient's compliance.

Plants such as *Camellia sinensis*, *Cassia auriculata* and *Ocimum gratissimum* possess many potential therapeutic activities in traditional system of medicinal practice and possess rich

phytoconstituents individually¹³⁻¹⁷. The Plant *Camellia sinensis* is reported to have 4000 bioactive compounds and among these bioactive compounds, one third is contributed by polyphenolic (epigallocatechin gallate, quercetin, kaemferol and luteolin) compounds, which are used in skin care products like burns, wounds, itching, inflammation of insect bites, psoriasis and swelling¹⁸⁻²⁰. The flower part of *Cassia auriculata* is reported to have significant amount of flavonoids (rutin, vicenin, auricouflavonoside) and phenols which are used for various skin diseases²¹⁻²⁴. Plant *Ocimum Gratissimum* reported to have volatile oil mostly phenols, particularly thymol and terpenoids (eugenol, terpineol and β -ocimene) which are used in various skin infections like dermatitis, eczema and scabies²⁵⁻²⁶.

Moreover, these medicinal plants are easily available, cost effective and commonly used by the human beings. Taking these facts into considerations, the present study was designed to systematically explore and analyze the phytoconstituents of these plant extracts and formulate a new polyherbal gel formulation by virtue of their antiacne potential effects.

MATERIALS AND METHODS

Plant Materials

Collection of specimen (flowers of *Cassia auriculata* (L) Roxb. (*Fabaceae*), leaves of *Camellia sinensis* (L) Kuntze (*Theaceae*), and leaves of *Ocimum gratissimum* L (*Lamiaceae*). The specimens for the proposed study were collected and confirmed by Dr. S. Soosairaj, Assistant Professor, Dept. of Botany, St. Joseph's College, Tiruchirappalli. Voucher specimens can be assessed as SJCBOT2159, SJCBOT2152 and SJCBOT2196, Dept. of Botany, St. Joseph's College, Tiruchirappalli.

Chemicals

Chemicals were obtained from Ranchem Laboratory Chemicals Pvt. Ltd., Himedia Laboratories Pvt. Ltd, and Loba Chemie, Mumbai.

Preparation of extract

The leaves of *Camellia sinensis*, flowers of *Cassia auriculata* and leaves of *Ocimum gratissimum* were washed in water to make them free from dust and foreign material, air dried under shade at room temperature. The air dried plant materials were coarse powdered and subjected to methanol extraction separately using soxhlet apparatus by reflux for 6 h at 60 °C. A grey colored semisolid mass were obtained, dried under vacuum and kept in desiccators.

Phytochemical analysis

Methanol extract was analyzed for its phytoconstituents such as saponins, anthraquinone glycosides, phyto steroids, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids²⁷.

Antibacterial Activity

Preparation of inoculum

Uniform suspension of microorganism is obtained by suspending 24 h fresh culture of bacteria (*S. aureus* and *S. epidermis*) in an amount of 15mL of the sterile water.

Determination of zone of inhibition

Agar well diffusion method was used to determine the antibacterial activity of the prepared extract. Transferred 20 mL of liquefied agar medium previously inoculated with 0.1 mL bacteria into the sterile petri dish having an internal diameter of 8.5 cm and allowed to form uniform thickness of the medium in the petri dish. After complete solidification of liquefied inoculated medium, the wells were made aseptically with cork borer having 6mm diameter. Specific quantity of the extract was carefully added into the well and the plates were kept for 30 min for pre-diffusion of the extracts. After pre-diffusion, the petri plates were incubated at 37 °C for 24 h in the incubator and measured the zone of inhibition for its antibacterial activity.

Preparation of Gel

The gel was prepared with defined quantity of carbopol-934 polymer. The specified quantity (1 g) of Carbopol 934 was added in to distilled water with vigorous stirring and left for overnight for dissolving the polymer. Methanol extracts 5% were dissolved in 15 ml of methanol with constant stirring. This methanol extract solution was added into the polymer solution and mixed well. Methyl paraben, was added as a preservative into this mixture and mixed well by magnetic stirrer. After complete dispersion of the extracts and preservatives, the pH of the gel was adjusted to neutral pH 7 by using sodium hydroxide. Glycerin 10 ml was added and mixed well in a magnetic mixer. Distilled water was added and made upto 100 g²⁸⁻²⁹. The gel formulation composition is shown in Table 1.

Physicochemical evaluation of formulations²⁸⁻³¹

Physical evaluation

Physical parameters such as color, appearance and consistency were checked visually.

pH

Aqueous solution (1%) of the formulation was measured by using a calibrated digital pH meter at constant temperature.

Viscosity

Viscosity of the prepared herbal gel formulation was measured by using Brookfield Viscometer (Brookfield Engineering Laboratories, USA) with spindle # C 50-1 having a speed of 50 rpm at room temperature and the determination of viscosity was done in triplicate.

Spreadability

Two sets of glass slide with standard dimension were taken. Polyherbal formulation gel was placed in between the two slides and sandwiched about the length of 60mm. Removed the adhered excess gel on the surface of the glass slides and fixed to a stand without any disturbance. In the upper slide 20 g weight was tied and noted the time taken for movement of upper slide to the distance of 60mm under the influence of weight. Mean time was calculated by repeating the experiment three times and the spreadability was calculated using the following formula.

$$\text{Spreadability} = (\text{Weight} \times \text{Length}) / \text{Time}$$

Antibacterial Activity studies

Transferred 20 mL of liquefied agar medium previously inoculated with 0.1 mL bacteria into the sterile petri dish having an internal diameter of 8.5 cm and allowed to form uniform thickness of the medium in the petri dish. After complete solidification of liquefied inoculated medium, the wells were

made aseptically with cork borer having 6 mm diameter. Four different concentrations 250 mg, 500 mg, 750 mg and 1000 mg of polyherbal gel were weighed and diluted with 2 mL of sterile water in sterile test tubes. The drug solution was carefully transferred into the cup and incubated at 37 °C for 24 h and the zones of inhibition were measured.

Table 1: Composition of the polyherbal gel formulation

Ingredients	Quantity
Carbopol-934	1 g
Extract <i>senna auriculata</i>	5%
Extract <i>Camellia Sinensis</i>	5%
Extract <i>Ocimum Gratissimum</i>	5%
Methanol	15 mL
Glycerin	10 mL
Sodium hydroxide solution (1%)	q.s
Methyl Paraben	0.5 g
Distilled water	q.s upto 100 g

Table 2: Phytochemical analysis

S.no.	Phytoconstituents	<i>Camellia sinensis</i>	<i>Senna auriculata</i>	<i>Ocimum gratissimum</i>
1	Saponins	+	-	+
2	Alkaloids	+	+	+
3	Steroids	+	+	-
4	Tannins	+	+	+
5	Flavonoids	+	+	+
6	Carbohydrates	+	-	-
7	Terpenoids	-	-	-
8	Polyphenol	+	-	-
9	Anthraquinone	+	+	-

+ → Present - → Absent

Table 3: Antimicrobial activity of medicinal plants determined by diffusion technique on solid media

S. No	Name of the medicinal plant	Diameter of zone of inhibition (in mm)	
		<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermis</i>
1	<i>Cassia auriculata</i>	4	3
2	<i>Camellia sinensis</i>	3	4
3	<i>Ocimum gratissimum</i>	6	5

Table 4: Physicochemical evaluation

Parameters	Observation/Values
Clarity and appearance	Good
Color homogeneity	Uniform
presence of fiber and particles	Absence of fibers and particles
pH	6.94±0.2
Viscosity	6505 ± 110 cps
Spreadability	7.1±0.1 (g. cm/sec)

Figure 1a: Zone of inhibition of *Cassia auriculata* against *Staphylococcus aureus*

Figure 1b: Zone of inhibition of *Cassia auriculata* against *Staphylococcus epidermis*

Figure 2a: Zone of inhibition of *Camellia sinensis* against *Staphylococcus aureus*

Figure 2b: Zone of inhibition of *Camellia sinensis* against *Staphylococcus epidermis*

Figure 3a: Zone of inhibition of *Osimum gratissimum* against *Staphylococcus aureus*

Figure 3b: Zone of inhibition of *Osimum gratissimum* against *Staphylococcus epidermis*

Figure 4a: Zone of inhibition of polyherbal formulation against *Staphylococcus aureus*

Figure 4b: Zone of inhibition of polyherbal formulation against *Staphylococcus epidermis*

RESULT AND DISCUSSION

In general, oral or topical antibiotic formulation is used for the treatment of skin diseases. Traditional medicinal and aromatic plants are interesting and explore its various bioactive natural organic compounds for various treatments. In the last two decades, more research has been carried out towards the identification of the bioactive compound from medicinal plants and developing into drug for the various treatments.

Evaluation of extracts

The color of the extracts was found to be dark green. The extracts are tested for its phytochemical analysis like saponins, anthraquinone glycosides, phyto steroids, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids. The phytochemical analysis of the various extracts results are shown in Table 2.

Antibacterial activity

The antibacterial activity study results showed that all the selected herbal plants showed antibacterial activity against acne causing bacteria *staphylococcus aureus* and *staphylococcus epidermis*. The antibacterial activity study results are shown in Table 3 and Figure 1-3.

Physicochemical evaluation of Gel formulation

Physicochemical parameters like color homogeneity, presence of fiber and particles, washability, pH and viscosity are evaluated. The visual inspection of the prepared formulation indicated no lumps and to have uniform color dispersion, free from any fiber and particle, easy washable, pH was found to be 6.94, it is near to the skin pH which indicates that the prepared formulation can be compatible with skin and viscosity was found to be 6506 cps. The physicochemical properties of the observed results are shown in Table 4.

Spreadability

Rheological property of the semisolid formulations gel can be assessed by spreadability. Spreadability test is a qualitative tool to evaluate physical state as well as the bioavailability of the formulation. The spreadability value was found to be 7.1 ± 0.1 (gm. cm/sec) which indicates the better spreadability of the formulation.

Antibacterial activity of the formulation

The antibacterial activity studies were performed by well diffusion method by measuring the zone of inhibition (in mm).

The study results of the polyherbal gel showed antibacterial activity in a dose dependent manner against the bacteria's causing acne. The antibacterial activity study of the formulation is shown in Figure 4.

CONCLUSION

Frequency of intake the allopathic drugs for the treatment of acne vulgaris results to produce adverse side effects. Recently, herbal remedies are considered as safe as the synthetic one and herbal formulations are having growing demand in the global market. Selection of the plants with desired concentration is important to produce the desired therapeutic effect. Herbal plants such as *Camellia sinensis*, *Cassia auriculata* and *Ocimum gratissimum* are selected and incorporated into a 1%w/w carbopol 934 gel base as a non-oily (aqueous) topical polyherbal formulation. The antibacterial study results of the developed polyherbal topical gel showed the antibacterial activity against *staphylococcus aureus* and *staphylococcus epidermis*. The study results conclude that the formulated polyherbal gel with methanolic extract of *Camellia sinensis*, *Cassia auriculata* and *Ocimum gratissimum* can be used for the management of acne vulgaris.

REFERENCES

1. Pratik BM, Ashok GV, Pranita GA. A quest of Anti-acne Potential of Herbal Medicines for extermination of MDR *Staphylococcus aureus*. International Journal of Pharmaceutical Science Invention 2014; 3(6): 12-17.
2. Ray C, Trivedi P, Sharma V. Acne and its treatment lines. International Journal of Research in Pharmaceutical and Biosciences 2013; 3(1): 1-16.
3. Kuver S, Palshikar G. Formulation and evaluation of herbal antiacne face wash. International Journal of Phytotherapy Research 2014; 4(2): 1-9.
4. Sinha P, Srivastava S, Mishra N, Yadav NP. New Perspectives on Antiacne Plant Drugs: Contribution to Modern Therapeutics. BioMed Research International 2014; Article ID 301304. <http://dx.doi.org/10.1155/2014/301304>.
5. Taj JK, Bhate K. A global perspective on the epidemiology of acne. British Journal of Dermatology 2015; 172 (1): 3-12.
6. Nguyen R, Su J. Treatment of acne vulgaris. Paediatrics and Child Health 2011; 21(3): 119-125.
7. Yadav N, Singh A, Chatterjee A, Belemkar S. Evaluation of efficacy and safety of perfect face gel and perfect face tablets in management of acne. Journal of Clinical Experimental Dermatology Research 2011; 2: 118. doi:10.4172/2155-9554.1000118.
8. Aruna MS, Sravani A, Reshma V, Santhi Priya N, Surya Prabha M, Rama Rao N. Formulation and evaluation of

- herbal acne gel. World Journal of Pharmaceutical Research 2015; 4(5): 2324-2330.
9. Verma S, Singh SP. Current and future status of herbal medicines. Veterinary World 2006; 1: 347-350.
10. Maheswari JK. Ethnobotany and Medicinal Plants of Indian Subcontinent. Scientific Publishers, Jodhpur, India, 2000.
11. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. Pharmacognosy Review 2014; 8(16): 73-80.
12. Che CT, Wang ZJ, Chow MSS, Lam CWK. Herb-Herb Combination for Therapeutic Enhancement and Advancement: Theory, Practice and Future Perspectives. Molecules 2013; 18: 5125-5141.
13. Senthilrani S, Renuka Devi P. Biological activities and chemical composition of *cassia auriculata*. Asian Journal of Biochemistry 2014; 9(4): 195-202.
14. Joy V, John Peter MP, Raj JY, Ramesh. Medicinal values of avaram (*cassia auriculata* linn.): a review. International Journal of Current Pharmaceutical Research 2012; 4(2): 1-3.
15. Nille GC, Reddy KRC. A Phytopharmacological Review of Plant – *Cassia auriculata*. International Journal of Pharmaceutical Bio Archives 2015; 6(6): 1-9.
16. Bhatt PR, Pandya KB, Sheth NR. *Camellia sinensis* (L): the medicinal beverage: a review. International Journal of Science Review Research 2010; 3(2): 6-9.
17. Nsofor CA, Chikezie UN, Azuwike CO. Antibacterial activity of *ocimum gratissimum* leaves extract on *escherichia coli* O157. Palgo Journal of Medicine and Medical Science 2014; 1(2): 15-18.
18. Tariq M, Naveed A, Barkat Ali K. The morphology, characteristics and medicinal properties of *camellia sinensis* tea. Journal of Medicinal Plants Research 2010; 4(19): 2028-33.
19. Ur Rashid MM, Sayeed MA, Hassan MM, Jainul MA, Azam S, Rahman MM. Antidepressant and antibacterial activities of *camellia sinensis* (White Tea). e-Journal of Science and Technology 2013; 8(4): 39-46.
20. Sharangi AB. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) – A review. Food Research International 2009; 42: 529-535.
21. Senthil Rani S, Renuka Devi P. Chemical composition and characterization studies of *cassia auriculata* flower extract. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(4): 327-330.
22. Jeeva GM, Jeeva S, Kingston C. Traditional treatment of skin diseases in south travancore, southern peninsular India. Indian Journal of Traditional Knowledge 2007; 6: 498-501.
23. Kingston C, Jeeva S, Jeeva GM, Kiruba S, Mishra BP, Kannan D. Indigenous knowledge of various medicinal plants in treating skin diseases in Kanyakumari district, southern India. Indian Journal of Traditional Knowledge 2009; 8: 196-200.
24. Kainsa S, Kumar P, Rani P. Pharmacological potentials of *cassia auriculata* and *cassia fistula* plants: A review. Pakistan Journal of Biological Sciences 2012; 15(9): 408-417.
25. Nakamura CV, Nakamura TU, Bando E, Melo AFN, Cortez DAC, Filho BPD. Antibacterial activity of *ocimum gratissimum* L. essential oil. Mem orias do Instituto Oswaldo Cruz 1999; 94(5): 675-678.
26. Mann A. Phytochemical Constituents and Antimicrobial and Grain Protectant Activities of Clove Basil (*Ocimum gratissimum* L.) Grown in Nigeria. International Journal of Plant Research 2012; 2(1): 51-58.
27. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 47th ed. Nirali Prakashan publication, India, 2011.
28. Nand P, Drabu S, Gupta RK, Bhatnagar A, Ali R. *In vitro* and *in vivo* assessment of polyherbal topical gel formulation for the treatment of acne vulgaris. International Journal of Drug Delivery 2012; 4 434-442.
29. Bhalani U, Shah K. Preparation and evaluation of topical gel of *Nigella sativa* (kalonji). International Journal of Research Development in Pharmacy and Life Sciences 2015; 4(4): 1669-1672.
30. Yamini K, Onesimus T. Preparation and evaluation of herbal anti-acne gel. International Journal of Pharma and Bio Science 2013; 4(2): 956-960.
31. Bhaskar G, Arshia A, Priyadarshini SRB. Formulation and evaluation of topical polyherbal antiacne gels containing *Garcinia mangostana* and *Aloe vera*. Pharmacognosy Magazine 2009; 5(19): 93-99.

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