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Development and Biological Evaluation of Herbal Anti-Acne Gel

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Abstract: Topical formulations (Gel) have been developed containing hydro-alcoholic extract of *Ocimum sanctum*, ethanolic extract of *Tabernaemontana divaricata*, *Aloe vera* concentrate gel powder and tea tree oil. In vitro antibacterial activity was performed against *Propionibacterium acnes* (P. acnes), a causative organism for Acne vulgar for the developed formulations using agar well diffusion method. The measured zones of inhibitions of the formulations were compared with standard antibiotic (tetracycline), standard marketed topical herbal preparation for acne and active ingredients of the formulations. Results of the investigation showed that formulation 5 has greater antibacterial activity (zones of inhibition >16 mm) than other formulations and which is comparable to that of standard marketed topical herbal preparation. When compared with hydro-alcoholic extract of *Ocimum sanctum* (Zone of inhibition < 9 mm), ethanolic extract of *Tabernaemontana divaricata* (Zone of inhibition < 8 mm) and tea tree oil (Zone of inhibition 9 mm-10 mm); all formulations had shown greater activity while the activity was found less on comparison with the standard antibiotic solution (zone of inhibition > 17 mm).
Keywords : Acne vulgaris, *Propionibacterium acnes*, Antibacterial assay.

Introduction

Acne vulgaris is an extremely common skin disorder that affects areas containing the largest oil glands, including the face, back, and trunk. *Propionibacterium acnes (P. acnes)*, an anaerobic pathogen[5], plays an important role in the pathogenesis of acne. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils[1, 2]. Although not a serious threat to general health, acne is one of the most socially distressing skin conditions, especially for adolescents, who must deal with a disfiguring disease that erupts just when sexual maturity makes them most sensitive about their appearance. Moreover, severe acne can lead to permanent scarring of the skin that carries the social distress throughout adulthood[3]. For many years, antibiotics have been used to treat acne vulgaris. However, antibiotic resistance has been increasing in prevalence within the dermatologic setting. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases[1].

In the present study, 5 topical formulations (Gel) have been developed containing hydro-alcoholic extract of *Ocimum sanctum*, ethanolic extract of *Tabernaemontana divaricata*, *Aloe vera* concentrate gel powder and tea tree oil., which have been reported for their antimicrobial[6, 7, 8] , anti-inflammatory[6, 7, 8, 9] and antioxidant activities[6, 7, 8, 9]. The developed formulations were examined for antimicrobial activities against microorganism frequently involved in acne inflammation, *P. acnes*.

Materials and Methods

Plants

The plants *Ocimum sanctum* and *abernaemontana divaricata* were collected from local area of Amravati district in the month of August, 2008. Dr. Prabha Y. Bhogaonkar, Director, Governmevt Vidarbha Institute of Science and Humanities had done authentication.

Aloe vera concentrate gel powder is obtained as a free gift sample (10G) from Chaitanya Biologicals Private limited, Malkapur, Buldhana (Maharashtra). Tea tree oil (Dr.Urjita Jain) is purchased from retailer, M/s, Sanjeevan medicals at Nagpur.

Preparation of Extracts

The fresh leaves of *Ocimum sanctum* and *Tabernaemontana divariacata* were dried in shade & powdered. The powdered plant material is defatted with petroleum ether and then subjected to soxhlet extraction till discolorisation for 4 hours to obtain hydroalcoholic and ethanolic extracts resepectively. The extracts thus obtained were filtered, concentrated on water bath to a thick paste & dried under vacuum.

Development of Formulation

Ingredients used for the development of formulations are given in table no. 1.

Procedure

Aloe vera concentrate gel powder and Carbapol 934 were dissolved in sufficient quantity of water and kept overnight. To this sodum hydroxide was added to form a gel. *Aloe vera* gel and Carbapol 934 gel were mixed together with vigorous stirring and kept in a beaker. The beaker was kept on a water bath and the temperature was allowed to reach above 50^0c.To this mixture the weighed quantities of extracts of *Ocimum sanctum* and *Tabernaemontana divaricata* were added. At the same time in another beaker weighed quantities of methyl paraben and proyl paraben were added in water and heated to dissolve. In another beaker weighed quantities of propylene glycol and polyethylene glycol were taken. To this weighed quantity of Tea tree oil was added with continuous shaking. Thus the mixtures obtained were finally mixed to obtain a gel. Then remaining quantity of purified water was added and PH was adjusted with 10% sodium hydroxide solution.

Microorganisms

P. acnes (MTCC 1951) was purchased from the M.T.C.C., Institute of Microbial Technology, Chandigarh (India). Blood agar base nutrient media was used as a growth media.

TABLE NO. 1 DEVELOPMENT OF FORMULATION

Ingredients	**Quantity taken per 100 g (in grams)**				
	1	**2**	**3**	**4**	**5**
***Ocimum sanctum* extract**	0.5	0.5	0.50	1.0	2.0
***Aloe vera* concentrate gel powder**	0.25	1.0	2.0	1.0	3.0
Tea tree oil	1.0	2.0	1.0	2.0	2.5
***Tabernaemontana divaricata* extract**	0.25	0.2	0.3	0.5	1.0
Methyl Praben	0.15	0.15	0.15	0.15	0.15
Propyl paraben	0.03	0.03	0.03	0.03	0.03
Carbapol 934	0.4	0.2	0.2	0.2	0.2
Propylene glycol 200	15.0	15.0	15.0	15.0	15.0
Polyethylene Glycol	5.0	5.0	5.0	5.0	5.0
Sodium Hydroxide (10 %)	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient
Pyrified water	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient

Sample Preparations

Solutions of gels were prepared using 100 mg of gel in 10ml of dimethyl sulfoxide (DMSO). Similarly, Solution of marketed formulation was prepared. Tetracycline (10mg/ml) was used as a positive control and DMSO as a negative control. Solution of Tea tree oil(10mg/ml), *Aloe Vera* gel(10mg/ml), hydroalcoholic extract of *Ocimum sanctum*(10mg/ml and) and ethanolic extract of *Tabernaemontana divaricata*(10mg/ml) was prepared in Dimethyl Sufoxide (DMSO).

Antibacterial Assay

The antibacterial activity of different formulations was determined by modified agar well diffusion method[6]. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *P .acnes*. The plates were allowed to dry for 1 h. A sterile 8 mm borer was used to cut four wells of equidistance in each of plates; 0.5 ml of solutions of formulations, extracts, tea tree oil, Aloe vera gel, marketed herbal formulation and tetracycline were intoduced in to the wells at randomly. The plates were incubated at 37^0c for 24 hours. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition (in mm).The experiments repeated four times.

Results

The results of this investigation showed that all developed formulations had inhibitory effect on the *P.acnes*. Formulation 5 has higher activity than that of other developed formulations. The activity of the developed formulation 5 has been comparable to that of marketed preparation. However, the activity of the standard tetracycline was more than that of all developed formulations, marketed herbal anti-acne preparation, extracts, aloe vera gel and tea tree oil. DMSO did not have significant activity. The diameter of zones of inhibitions is given in table no.2.

Discussion

Acne vulgaris is an extremely common skin disorder that affects virtually all individuals at least once during life. The incidence of acne peaks at teenage, but substantial numbers of men and women between 20-40 years of age are also affected by the disorder. Acne can have important negative psychosocial consequences for the affected individual, including diminished self-esteem, social withdrawal due to embarrassment and depression[3].

Herbal medication are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as like contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, xerosis of the skin etc[3].

The present research work deals with formulation and evaluation of herbal Anti- Acne gels. The Plant material used for the formulations were hydro-alcoholic extract of leaves of *O.sanctum,* ethanolic extract of leaves of *T. divaricata*, tea tree oil and aloe vera concentrate gel powder. Although various topical herbal formulations for acne are available in the market, we propose to make use of T. divaricata leaves extract for the first time in the developed formulations. The plant has been reported in the literature as a good antioxidant[9] and anii-inflammatory agent[9]. The developed formulations were evaluated for their in vitro antibacterial activity against P. acnes.

TABLE NO.2: RESULTS OF ANTI-BACTERIAL ACTIVITY

S.No.	Formulations	Zones of inhibitions in mm				Mean ±S.D (n = 4)
		1	2	3	4	
1	Formulation 1	10.4	10.9	11.2	11	10.875 ± 0.3403
2	Formulation 2	12.3	11.7	12.0	12.6	12.15 ± 0.3873
3	Formulation 3	15	14.2	14.1	14.5	14.45 ± 0.4041
4	Formulation 4	15.3	15.4	16.0	15.7	15.6 ± 0.3162
5	Formulation 5	16.6	16.8	16.2	16.2	16.45 ± 0.3
6	Marketed herbal preparation	16.9	16.2	16.1	15.5	16.15 ± 0.33
7	Tetracycline(10mg/ml)	19	17.5	20.4	18.6	18.875 ± 0.69
8	Tea Tree Oil (10mg/ml)	9.2	9.5	9.8	9.5	9.5 ± 0.24
10	*O.sanctum* extract (10mg/ml)	8.2	8.3	8.3	8.2	8.25 ±0.057
11	*T.divaricata* extract (10mg/ml)	< 8.0	< 8.0	< 8.0	< 8.0	< 8.0
12	*A.vera* gel(10mg/ml)	8.4	8.3	8.5	8.4	8.4 ± 0.081
13	DMSO	---	---	---	---	---

The Zones of inhibitions for the antibacterial activity were compared with the standard tetracycline, herbal marketed preparation for acne vulgaris, active ingredients used in the formulation (*A.vera* gel, *O. sanctum* hydro alcoholic extract, *T.divaricata* ethanolic extract and tea tree oil). Formulation 5 has shown comparable zones of inhibitions to that of the marketed preparation. All the formulations have shown greater zones of inhibitions than that of the tea tree oil. Zones of Inhibitions for tetracyclline were found to be greater than that of all the formulations, marketed preparation and tea tree oil. Tea tree oil with active constituent 1, 4-terpeniol [11, 12] may be responsible for the significant antibacterial activity. The zones of inhibition for tea tree oil are less than 9.8 mm, while the zones of inhibitions for all the formulations are greater than 10.4 mm. This suggests that the other active ingredients of the formulations containing secondary metabolites like triterpenoids, flavonoid, tannins and sapononis may have contributory antibacterial activity. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds[13]. *P. acnes*, an anaerobic pathogen, is implicated in the development of inflammatory acne. The formulations having antibacterial agents inhibiting the P.acnes, may also reduce the development of inflammatory acne.

References

1. Chomnawong Mullika Traidej, Surassmo Suvimol, Nakoolkarn Veena , Gritsanapan Wandee, Antimicrobial effects of Thai medicinal plants against acne inducing bacteria, Journal of Ethnopharmacology,2005, 101,330-333.
2. Chomnawong Mullika Traidej, Surassmo Suvimol, Nakoolkarn Veena , Gritsanapan Wandee , Effect of *Garcinia mongostana* on inflammation caused by *Propionibacterium acnes*, Fitoterapia, 2003, 78,401-8.
3. Gopal M.G, Farahana B, Dr. Kala Suhas Kulkarni, Effectiveness of Herbal Medication in treatment of Acne vulgaris- A pilot Study,The Indian Practioner; 2001, 54, 10, 723.
4. Jappe Utta, Pathological Mechanism of Acne with special emphasis on P.Acnes and related Therapy, Acta Derm Venerol, 2003,83,241-8.
5. *Propionibacterium,* Microbe Wiki, the student edited Microbiology Resource; A microbial Biorealm page on the genus *Propionibacterium.* www.google.co.in
6. WHO Monograph on selected Medicinal Plants, Department of essential Drugs and medicines Policy, World Health Organization. www.google.co.in., 172-178
7. Josias A Hamman. Composition and Application of *Aloe vera* leaf gel. Molecules, 2008, 13,1599-1616.
8. WHO Monograph on selected Medicinal Plants. Department of essential Drugs and medicines Policy, World Health Organization. www.google.co.in. ,206-216.
9. Wasana Pratchayasakul, Anchalee Pongchaidecha, Nipon Chattipakorn and Siriporn Chattipakorn, Ethnobotany and Ethnopharmacology of *Tabernaemontana divaricata*, Ind J Med research, April 2008, 127,317-335.
10. Perez C, A. Pauli, P. Bazerque, An antibiotic assay by agar well diffusion method, Acta Biol Med Exp., 1990,15,113-115.
11. Sean D Cox et al., Determining the Antimicrobial actions of tea tree oil. Molecules, 2001, 6, 87-91.
12. Carson CF, Riley TV., Susceptibility of *Propionibacterium acnes* to the essential oil of *Melaleuca alternifolia.* Letters in Applied Microbiology, 1994, 19:24-25.
13. Marjorie Murphy Cowan**,** Plant Products as Antimicrobial Agents, Clinical Microbiology Reviews, 1999, 12(4), 564-582.

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