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Assessment of antibacterial activity of Neem and Coriander Leaves extract against *Staphylococcus epidermidis* and *Propionibacterium acnes*: Development and formulation of herbal anti-acne gel

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ABSTRACT

Due to the increasing resistance to bacteria, there is urgency for development of alternative therapies on bacterial induced diseases and disorders. Herbal formulations can serve as good alternative due to their enhanced effect and lesser side effects. This study was aimed to develop herbal formulation (gel) with a new combination of Neem and Coriander leaves extract, owing to the presence of chemical constituents that can lead to the treatment of acne vulgaris in terms of their antibacterial, antioxidant and anti-inflammatory properties which provide an enhanced and targeted effect in this formulation. Herbal gel formulations were developed by using extract of neem and coriander leaf extracts. All formulations were evaluated for physical parameters like color, consistency, viscosity and extrudability. Phytochemical analysis of extract was done to determine major chemical constituents in each extract. Antibacterial activity was determined by disc diffusion method against strains of Staphylococcus epidermidis and Propionibacterium acnes both of which contributes to the development of acne and compared against standard antibiotic clindamycin. Combination of Extracts of Neem and coriander shows maximum zone of inhibitions in synergism but less as compared to Clindamycin.

Keywords: Acne vulgaris, herbal formulation, antibacterial activity.

1. INTRODUCTION

One of the most commonly prevailing disorder is *Acne vulgaris* which usually occurs at the age of 18 – 25 years [1]. It is a skin disorder characterized by the formation of comedone, inflammatory lesions and seborrhea etc. It is disorder of sebaceous gland. This condition often expresses itself by non-inflammatory follicular papules pustules and nodules and by inflammatory papules, the opportunistic bacteria *Propionibacterium acne* (*P.acne*) is causative agent which resides within the pilosebaceous follicle and cause inflammation when exposed to the dermis with ruptured follicle [2]. Many other factors may contribute towards this condition for example change in climate, dietary habits, allergy and mental stress and can cause depression and embarrassment and ultimately social withdrawal [3].

Different agents like Benzoyl peroxide, anti-androgens and antibiotics are used to treat this disorder but there is occurrence of several side effects like dermatitis, skin, and dryness, darkening of skin and reoccurrence after withdrawal of therapy [4].

Due to development of resistance mechanism in bacteria towards the antibiotics there is need of an alternate system of medicine for the treatment of acne. Among the alternate systems of medicine herbal agents serve as good option as they have less side effects and topical application provides ease of application.

Neem (*Azadirachta indica, Meliaceae*) is reported to possess chemical constituents that are responsible for anti-oxidant, anti-inflammatory and antimicrobial effects and could provide an alternative option for treating bacterial induced acne [5].

Coriandrum sativum is the member the family *Apiaceae*. Mostly in Mediterranean countries, annually growing herb. Growth of this herb is favored by high temperature climates. Whole plant is beneficial in term of nutrients and leaves are referred as Cilantro [6].

Coriandrum sativum is reported to have therapeutic activities like anti-bacterial, [7] anti-inflammatory [8], antioxidant [9] and anti-scarring property due to the presence of different chemical constituents primarily due to Salicylic acid [10].

2. MATERIALS AND METHODS

Neem leaves and coriander leaves obtained from Lahore, selected due to their possession of antibacterial activity. Strains of *staphylococcus epidermidis* (ATCC14990) and *Propionibacterium acnes* (ATCC11827)

2.1 PREPARTION OF EXTRACTS

2.1.1 Preparation of Coriander extract

200 grams of leaves were dried in the shade and were grinded in powder form. This powder was macerated with distilled water for seven days at room temperature (25°C). Frequent agitation and circulation of solvent was maintained. After seven days, the extracted solution was filtered and marc was pressed. Both the strained liquid and the expressed liquid were combined together. Then the enriched extract was concentrated in a rotary vacuum flash evaporator under reduced pressure. The procedure was adopted to prepare the hydroethanolic and the ethereal extract with a slight modification in the type of solvent used as distilled water ethanol (60:40) was used for hydroethanolic extract whereas ether was used for ethereal extract. [11]

2.1.2 Preparation of Neem Extract

200 grams of fresh Leaves of neem were taken, shade dried for 24 hours cut into small pieces. This material is then reflexed in reflex condenser for 3 hours by using water and ethanol (80:20). Extract was cooled at room temperature and filtered to be further used in the studies. [12]

2.2 DETERMINATION OF ANTIMICROBIAL ACTIVITY

The antibacterial activity was determined by disc diffusion method. This experiment was performed by following the method of Hayes and Markovic (2002) with some modifications. *P. acne* was incubated in ASLA agar medium for 48 hrs. under anaerobic conditions. The agar plates were swabbed by with inoculums. 0.05% polysorbate 80 was added to the agar base used for coriander oil. The sterile filter paper disc of diameter 6mm were aseptically placed on the inoculated plates and were impregnated with the test material (20µl of coriander oil). The plates were left at ambient temperature for 30 min to allow exceed pre diffusion prior to incubation at 37 0C for 72 hrs under anaerobic conditions in an anaerobic bag (Hi-Media) with gas pack and indicator tablets and the bag was kept in an incubator for 72 hrs at 37 ± 1 ^{0}C .Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobic conditions. The indicator tablet of methylene blue changed from dark pink-blue-light pink finally, which indicated the achievement of anaerobic condition. The culture of *S. epidermidis* was prepared in nutrient agar medium at 24 hrs under aerobic conditions. Test samples of this aerobic bacterium were incubated at 370C for 24 hrs under aerobic conditions. The anti-bacterial activity was estimated by measuring the diameter of the zone of inhibition. All disc diffusion tests were performed in three separate experiments and antibacterial activity was noted. [24]

2.3 GEL FORMULATION

50 ml of distilled water taken and 1 gram of Carbopol 934 was dispersed in it with continuous stirring. Methyl paraben and propyl paraben were separately dissolved in 5 ml of distilled water by heating on water bath. After cooling this solution to room temperature, polyethylene glycol 200 and propylene glycol 400 were added in amounts as shown in table 1. To this solution, extracts are added and made up volume up to 100ml. this solution was then added to the solution of Carbopol 934 along with continuous stirring. Triethanloamine was added drop wise to adjust the pH near physiological range of pH of the skin (6.8-7). Same method repeated for combinational product of both extracts. [13-19]

2.4 EVALUATION OF FORMULATION

2.4.1 Physical Parameters

Physical appearance: The physical appearance of the formulation was checked visually which comprised of

Consistency: The consistency was checked by applying on skin.

Color*:* The color of the formulations was checked out against white background.

Greasiness: The greasiness was assessed by the application on to the skin.

Odor: The odor of the gels was checked by mixing the gel in water and smelling.

pH: About 20mg of the formulation was taken in a beaker and was subjected to the pH measurement using a digital pH meter within 24 hrs of manufacture. [20]

Viscosity: Viscosities of formulated gels were determined using Brookfield viscometer spindle # 7 at 50 RMP and 25°C.The corresponding dial reading on the viscometer was noted. [20]

Extrudability: The gel formulation was filled in a standard capped collapsible aluminum tubes and sealed by crimping to the end. The tubes were placed between two slides and were clamped. 500g weight was placed over the slides and then the cap was

removed. The length of the ribbon of the formulation that came out in 10 secs was recorded [21].

2.5 PHYTOCHEMICAL SCREENING

Neem and coriander extracts were subjected to Photochemical screening for the analysis of photochemical groups.

2.5.1 Test for Tannins

0.5 gram of powder in crude form was mixed with 10 mL of distilled water and filtered. To the 2 ml of filtrates, few drops of 1 % ferric chloride solution was added. Blue green precipitate indicated presence of tannins [22].

2.5.2 Test for alkaloids

0.1 gram of each of powered dissolved in 5ml of methanol separate and filtered. To the 2 ml of filtrate, added 5ml of 1 % HCl and heated on water bath, filtered. Divide this filtrate into portion of 1 ml in two separate test tubes. To the first test tube containing filtrate, add few drops of Dragendorff's reagent. Orange red precipitate indicate presence for alkaloids and reverse for absence. To the second test tube, few drops of Mayer's reagent added and buff colored propitiates indicated presence of alkaloid [23].

2.5.3 Shinoda's test for flavonoids

0.5 gram of each of crude powder dissolved in 5 ml of ethanol separately, warmed it and filtered it. To the filtrated, add three pieces of Magnesium chips followed by few drops of concentrated HCl. Red to purple color indicated presence of Flavonoids [22].

2.5.4 Test for Saponins

1 gram of both crude powder was added to 5 mL of distilled water, boiled and filtered. Each filtrate diluted by 3 ml of distilled water and further shaken for 5 minutes. Warmed it, if frothing persist on warming it indicated presence of Saponins [23].

2.5.5 Liebermann-Burchard test for steroids

0.2 gram of crude powder of each sample dissolved in2 mL of acetic acid. Solutions were cooled, added concentrated H_2SO_4. Blue or violet color indicated presence of steroids [23].

2.5.6 Test for Terpenoids

0.5m of each of plant extract was mixed with 2 ml of chloroform. To this solution, 3 mL of concentrated H_2SO_4 added carefully along the wall of test tube. Reddish brown color at the interface indicated presence of Terpenoids. [23].

Table 1: Formulation of anti-acne gel.

Serial number	Ingredient	Quantity 100 grams
1	Carbopol 934	1 gram
2	Propyl paraben	0.03 gram
3	Polyethylene Glycol	15ml
4	Propylene Glycol	5ml
5	Methyl Paraben	0.15 gram
6	Distilled Water	100 ml

Table 2: Concentration of Extracts used in Gel Formulation

Serial Number	Plant Extract	Concentration of Extract	
		G_1	G_2
1	Neem Extract	3.5 gram	4 gram
2	Coriander Extract	4 gram	3.5 gram

Table 3: Physical Evaluation parameters of the formulations

Serial number	Formulation	color	pH	Viscosity (Cp)	Extrudability (g)
1	G_1	colorless	7.3	30	536
2	G_2	cream	7.1	32	532

Table 4: Phytochemical screening of hydro ethanolic extracts of Neem and Coriander leaves

Phytochemical constituents	Plant Extract	
	Neem	Coriander
Tannins	+	-
Alkaloids	+	-
Flavonoids	+	-
Saponins	+	-
steroids	+	+
Terpenoids	-	+

Table 5: Antibacterial activity of Formulation

Serial number	Bacteria	Zone of Inhibition (mm) Plant extracts (200mg/ml)			
		Neem Extract	Coriander Extract	Neem Extract + Coriander Extract	Clindamycin
1	*Staphylococcus epidermidis*	14	13	20	33
2	*Propionibacterium acnes*	17	15	23	36

3. RESULTS AND DISCUSSION

This present work aimed to develop a herbal formulation (anti-acne gel) which is easy to apply and in compliance to skin conditions and use of herbal extracts like neem leaves extract and coriander leaves extract (cilantro) using natural ingredients so that this herbal formulation possess minimum side effects and can combat phenomenon of resistance which is encountered in current antibiotic therapy.

Two herbal anti-acne gel formulations prepared G_1 and G_2. These formulations differed by the relative amount of extracts of coriander leaves and neem leaves. G_1 was developed by using 3.5 grams of neem extract and 4 grams of coriander extract whereas G_2 was developed by using 4 grams of neem extract and 3.5 grams of coriander extract. Gel formulation without active showed in table 1. These formulations were developed by using reported combination of excipients and innovation by the combination of extracts of neem and coriander.

Formulations G_1 and G_2 were evaluated in terms of color, pH, viscosity, Extrudability, Phytochemical analysis and antibacterial activity. G_1 formulation was colorless and G_2 was cream in color. The formulations were glossy and produced soothing effect on application. The pH for G_1 was recorded to be 7.3 while that of G_2 was 7.1 Viscosity of formulation G_1 was 30 Cp and while for G_2 was 32 Cp. Viscosity was controlled by changing gelling agent concentration and selecting best viscous formulation with reference to skin. Value of Extrudabilty was 536 grams for G_1 and 532 gram for G_2. These parameters showed in Table 2.

Neem leaves extract and coriander extract was analyzed for their phytochemical constituents. Test for Tannins, Alkaloids, Flavonoids, Steroids and Terpenoids were performed on neem and coriander crude powder and extract by using standard procedures. Neem extract showed positive result towards the presence of Tannins, Alkaloids, Flavonoids and steroids and Terpenoids were absent. In Coriander, test was negative for the presence of Tannins, Alkaloids and Flavonoids and positive for steroids and Terpenoids as shown in Table 4.

Extracts were evaluated for its antibacterial activity against *Staphylococcus epidermidis* and *Propionibacterium acnes* which are causative agents for acne vulgaris. Neem extract showed zone of inhibition of 14 mm against *Staphylococcus epidermidis* and 17 mm against *Propionibacterium acnes*. Coriander extract showed 13 mm of zone of inhibition against *Staphylococcus epidermidis* and 15 mm against *Propionibacterium acnes*. Extracts were tested in combination of equal ration and their combination showed synergistic effect than the individual extract. Combination of extract showed 20 mm zone of Inhibition against *Staphylococcus epidermidis* and 23 mm against *Propionibacterium acnes*. Results showed verification to the antibacterial activity of neem and coriander extract and this combination could be used as an effective remedy for topical treatment of acne. These combined extract zone of inhibitions were less than clindamycin which showed 33mm zone of inhibition against *Staphylococcus epidermidis* and 36 mm against *Propionibacterium acnes.* These extract can be combined with the proposed gel formulation to provide an effective natural herbal remedy for acne vulgaris.

RECOMMENDATIONS

This Formulation can be commercialized for future use after animal and human testing and could prof an effective treatment as much as antibacterial agents available in market along with the benefit of cost Effectiveness.

5. REFERENCES

1. Gopal M.G, Farahana B, Dr. Kala Suhas Kulkarni (2001) Effectiveness of Herbal Medication in treatment of Acne vulgaris- A pilot Study, The Indian Practioner; 54, 10, 723

2. Christy C, Riddle, MD; Kathani Amin et al. (2009) A Review of Azithromycin for the Treatment ofAcne Vulgaris, Cosmetic Dermatology, VOL 20 NO, 5, 299-302.
3. Mamgain R.K., (2000) Acne Vulgaris and its Treatment by Indigenous Drugs SK-34 (Purim) and SK-235 (Clarina),The Antiseptic, (97), 3, 76-78.
4. Said H.K., (2005). Traditional medicine in the service of health, Hamadard foundation press, 73-75.
5. Ross JI, Snelling AM, Eady EA et al. (2001) Phenotypic and genotypic characterization of antibiotic-resistant *Propionibacterium acnes* isolated from acne patients attending dermatology clinics in Europe, the USA, Japan and Australia. Br J Dermatol; 144:22
6. www.coriandrumsativum.com
7. Matasyoh J.C.,Maiyo Z.C. (1999) Chemical composition and antimicrobial activity of the essential oil of coriandrum sativum. Food Chemistry 113: 526-529.
8. Chithra V., Leelamma S. (1999) Coriandrum sativum changes the levels of lipid peroxides and activity of antioxidant enzymes in experimental animals. Indian J Biochemistry and Biophysics 36: 59-61
9. Melo A De Enayde, Filho M Jorge. (2005) Characterisation of antioxidant compounds in aqueous coriander extract. Lebensm Wiss u Technol 38: 15-19.
10. Kanlayavattanakul. M., Lourith N. (2011) Therapeutic agents and herbs in topical application for acne treatment. Int. J Cosmetic Sci. 33: 289-29
11. Vats A., Sharma P. (2012) Formulation and evaluation of topical anti acne formulation of coriander extract. Int. J. Pharm. Sci. Rev. Res., 16(2): 97-103
12. Johny JM, Kulandhaivel M, Palaniswamy M, Jose R. (2011) International Journal of Pharmaceutical & Biological Archives 2(4): 1218-1223.
13. Banker GS and Rhodes CT (1990) Modern Pharmaceutics. Marcel Dekker, Inc., Madison Avenue, New York , Edition 2, 40: 263-264
14. Vyas SP and Khar K (2002) Controlled Drug Delivery. Vallabh Prakashan, Edition 1: 416-417.
15. Ansel HC, Popovich NG and Allen LV (2005) Pharmaceutical Dosage Forms and Drug Delivery Systems. B. I. Publications Pvt. Ltd. Edition 8: 415-419.
16. Kesavanarayanan KS and Ilavarasan R (2007) Topical dosage form of valdecoxib: Preparation and pharmacological evaluation. Acta Pharm. 57: 199-209.
17. Chaple DR and Chaware PA (2007) Formulation and evaluation of topical antifungal drug delivery system of henna extract. Indian Drugs. 44(1): 61-62.
18. Reddy M, Mutalik S and Veerabhadra G (2006) Preparation and evaluation of minoxidil gel for topical application in alopecia. Indian J. Pharm. Sci. 432-436.
19. Sahin F, Karaman I, Gulluce M et al. (2003) Evaluation of antimicrobial activities of *Satureja hortensis* L. J Ethnopharmacol. 87: 61–65.
20. Panigarhi L, John T, Shariff A et al. (1997) Formulation and evaluation of Lincomycin HCL gels. Indian J Pharma. Sci. 59: 1997; 610-613
21. Dua Kamal, Pabreja Kavita, Ramana V. M (2010) Aceclofenac topical dosage forms: In vitro and in vivo characterization. Acta Pharmaceutica, 60: 467–478.
22. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002.
23. Sofowora A. Medicinal plants and traditional medicinal in Africa. 2nd ed. Ibadan, Nigeria: Spectrum Books Ltd, Sunshine House; 1993. Screening plants for bioactive agents; pp. 134–156.
24. Vijayalakshmi A., Tripura A., Ravichandiran V. (2011) Development and evaluation of anti acne products from *Terminalia arjuna* Bark. Int J Pharmtech Research 3:320-327.

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