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Assessment of Antibacterial Activity of *Neem* and *Coriander* Leaves Extract against *Staphylococcus epidermidis* and *Propionibacterium acnes*: Development and Evaluation of Herbal Anti-acne Gel

Tayyaba Sadiq^{1*} and Muhammad Azeem²

¹Akhtar Saeed College of Pharmaceutical Sciences, Lahore, Pakistan

²Hamdrad institute of Pharmaceutical sciences, Islamabad, Pakistan

ABSTRACT

Due to the increase in number of resistant bacterial strains, there is an urgent need for the development of alternative therapies for bacteria induced diseases. Herbal formulations presented good alternative due to their enhanced effect and less side effects. This study was aimed to develop a herbal formulation (gel) with a new combination containing extract of *Neem* (*Azadirachta indica*) and *Coriander* leaves (*Coriandrum sativum*) in the ratio of 2: 1.75. *Neem* leaves extract contain active compounds such as Azadirachtin, Nimbidin and Nimbinin, Gallic acid, Epicatechin, Margolone and Catechin which are responsible for antibacterial and anti-inflammatory activity that can help in the treatment of *acne vulgaris*. *Coriander* leaves contain many important chemical constituents which are having pronounced antibacterial effects such as Plantaricin, an antimicrobial peptide containing 26 amino acids. Herbal gel formulations were developed by using the extract of *Neem* and *Coriander* leaves. Phytochemical analysis of extracts was performed to determine major chemical constituents in each of extract. Antibacterial activity was determined by disc diffusion method against strains of *Staphylococcus epidermidis* and *Propionibacterium acnes*. *Propionibacterium acnes* contributes to development of *acne* and *Staphylococcus epidermidis* considered to trigger inflammation along with *Propionibacterium acnes* and it is well documented. Activity of extracts was compared against standard antibiotic Clindamycin. Combination of Extracts of *Neem* and *Coriander* (2:1.75) showed maximum zone of inhibitions due to synergism but less as compared to Clindamycin. Two gel formulations G1 and G2 were developed and evaluated for physical parameters such as color, consistency,



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greasiness, odor, pH, viscosity and extrudability. These gels differs by the relative ratio of extracts of Neem and Coriander leaves, 1.75:2 for G1 and 2:1.75 for G2. G2 proved best formulation in term of antibacterial activity.

KEYWORDS

Acne vulgaris, antibacterial activity, herbal acne gel, Neem and Coriander extract



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INTRODUCTION

Acne vulgaris is one of the most common skin problem occurring in age group of 18-25 years¹. It is characterized by the formation of comedones, inflammatory lesions and seborrhea. It is disorder of sebum producing gland. Condition often starts as non-inflammatory follicular papules, pustules and nodules, become colonized by the opportunistic bacteria such as *Propionibacterium acnes* and *Staphylococcus epidermidis* leading to inflammation². Many other factors may contribute in triggering acne for example change in climate, dietary habits, allergy and mental stress. Acne vulgaris can cause depression, embarrassment and ultimately social withdrawal³.

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 dryness, darkening of skin and recurrence after withdrawal of therapy⁴. Due to development of resistance in bacteria towards the antibiotics, there is need for an alternate system of medicine for the treatment of acne. Among the alternate systems of medicine, herbal products serve as good option as they have less side effects⁵. Neem (*Azadirachta indica*,

Meliaceae) is evergreen, fast growing tree and commonly found in Pakistan, India, Bangladesh and Srilanka⁶. Neem has been used widely in Unani, Ayurveda and Homeopathic medicines and considered as “wonder tree” in the modern medicine⁷. It is reported to possess chemical constituents such as Azadirachtin, Nimbidin, Nimbinin, Gallic acid, Epicatechin, Margolone and Catechin that are responsible for antioxidant, anti-inflammatory and antimicrobial effects and could provide an alternative for treatment of bacterial induced acne⁸.

Coriander (*Coriandrum sativum*, *Apiaceae*) is an annual herb that is cultivated in different regions like Russia, Turkey, Pakistan, India and Europe. Leaves and dried fruit are used as spice in culinary preparations⁹. Coriander is reported to have therapeutic activities like anti-bacterial, anti-inflammatory, antioxidant and anti-scarring property due to the presence of different chemical constituents primarily due to Plantaricin an antimicrobial peptide containing 26 amino acids¹⁰⁻¹³.

Local effects can be achieved by the topical delivery. For local dermatological diseases, topical delivery gives better absorption by penetrating deeper in to skin. Topical application also provides benefit of less side



effects by minimizing systemic effects. Topical applications also avoids GIT irritation and by passes liver metabolism¹⁴. Topical gels are better candidate for many drugs than the ointment and creams due to their increased stability, controlled release of medicament from the formulation and better bioavailability¹⁵. Objective of this study was to develop a gel formulation containing Neem and Coriander Leaves extract due to antibacterial and anti-inflammatory activity of both extracts. Gel formulation provided good formulation in terms of application, absorption and bioavailability. Herbal formulation provide minimum side effects as compared to antibiotic therapy. Neem and Coriander leaves extract combination (as determined below) was formulated into a gel. Antibacterial activity for combined extract of Neem and Coriander was determined and best ratio was selected. In this way a herbal gel formulation could provide combined effects of topical gel preparation (more absorption, increased bioavailability) and herbal preparation (less side effects, combating antibiotic resistance).

MATERIALS AND METHODS

Neem leaves and Coriander leaves were procured from herbal market of Lahore.

Strains of *Staphylococcus epidermidis* (ATCC14990) and *Propionibacterium acnes* (ATCC11827) were obtained from Akhter Saeed College of Pharmaceutical Sciences, Lahore. Standard Clindamycin was obtained from chemical laboratory, Lahore.

PREPARATION OF EXTRACTS

Preparation of Coriander extract

Leaves of Coriander (200 grams) were dried in the shade and were grinded to 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. 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 extracts. Distilled water and ethanol (60:40) was used for hydroethanolic extract whereas ether was used for ethereal extract¹⁶

Preparation of Neem Extract

Fresh leaves (200 grams) of neem were taken, shade dried for 24 hours and cut into small pieces. This material was then refluxed in reflux condenser for 3 hours by using water and ethanol (80:20). Extract was



cooled at room temperature and filtered for further use^{17, 17}.

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. *Propionibacterium acnes* was incubated in ASLA agar medium for 48 hours under anaerobic conditions. The agar plates were swabbed with inoculums. Polysorbate-80 (0.05%) was added to the agar base used for Coriander extract. The sterile filter paper disc of diameter 6 mm were aseptically placed on the inoculated plates and were impregnated with the test material (20 μ l of Coriander extract). For pre-diffusion before the incubation, the plates were kept at room temperature of 25⁰C for 30 minutes and then incubated at the temperature of 37⁰C for 72 hours in anaerobic bag. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain

anaerobic conditions. The indicator tablet of methylene blue changed color from dark pink to blue and then to light pink, which indicated achievement of anaerobic condition. Incubation condition for *Staphylococcus epidermidis* were kept aerobic, nutrient agar medium used. Temperature was kept at 37⁰C and plates incubated for 24 hours¹⁸. The anti-bacterial activity was estimated by measuring the diameter of the zone of inhibition against cultures of *Staphylococcus epidermidis* and *Propionibacterium acnes*. Ratios of Combined extracts G1 and G2 as shown table number 1 and antibacterial activities are shown in Table number 2¹⁸.

Table 1: Concentration of Extracts used in Gel Formulation

Serial Number	Plant Extract	Concentration of Extract	
		G ₁	G ₂
1	Neem Extract	3.5grams	4 grams
2	Coriander Extract	4 grams	3.5grams

GEL FORMULATION

Distilled water (50 ml) was taken and 1 gram of Carbopol-934 was dispersed with

Table 2: Antibacterial activity of formulation

Serial number	Bacteria	Zone of Inhibition (mm)				
		Neem Extract (200mg/ml)	Coriander Extract (200mg/ml)	G1	G2	Clindamycin (microgram/ml)
1	<i>Staphylococcus epidermidis</i>	14	13	18	20	33
2	<i>Propionibacterium acnes</i>	17	15	22	23	36

continuous stirring. Methyl paraben (0.15 gram) and Propyl paraben (0.03 gram) were

separately dissolved in 5 ml of distilled water by heating on water bath. After

**Table 3** Formulation of anti

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

cooling this solution to room temperature, Polyethylene glycol-200 (15ml) and Propylene glycol-400 (5ml) were added.

To this solution, extracts were added volume was made up to 100 ml. Carbopol-934 was added to the solution with continuous stirring. Triethanolamine was added drop by drop to adjust the pH near physiological range of pH of the skin (6.8-7)¹⁹⁻²⁴

Table 4 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	white	7.1	32	532

Odor: The odor of the gel was checked by mixing the gel in water and then testing the odor by inhalation.

pH: About 20mg of the formulation was taken in a beaker and pH was measured using a digital pH meter within 24 hours of preparation²⁵.

Viscosity

Viscosities of formulated gels were determined using Brookfield viscometer spindle number 7 at 50 revolutions per minute and 25°C. The corresponding dial reading on the viscometer was noted²⁵.

Extrudability

Formulation of Gel shown in Table number 3¹⁹⁻²⁴.

EVALUATION OF GEL FORMULATION

Physical Parameters

Physical appearance: The physical appearance of the formulation was checked for the following parameters-

Color: The formulations were checked for its color.

Consistency: The consistency was checked by application to skin. *Greasiness:* The greasiness was assessed by application to the skin.

Gel formulations were filled in aluminum tubes (collapsible) and sealed. Tubes were clamped between two slides and 500g weight was placed and caps of tubes were removed. Formulation came out in form of ribbon and length of ribbon was recorded²⁶. All these parameters shown in Table number 4.

PHYTOCHEMICAL SCREENING

Neem and Coriander leaves were subjected to phytochemical screening for the analysis of phytochemical groups.

Test for Tannins



Dry powders (0.1 gram) of Neem and Coriander leaves was taken separately and mixed with 10 ml of distilled water and filtered. From each filtrate, 2ml of filtrate was taken in separate test tubes and few drops of 1 % ferric chloride solution was added. A blue green precipitate indicated presence of tannins²⁷.

Test for alkaloids

Dry powder (0.1 gram) of Neem and Coriander leaves was dissolved in 5ml of methanol and filtered. To the 2 ml of filtrate, 5ml of 1 % HCl was added and heated on water bath and then filtered. The filtrate was divided into 1 ml portion in two separate test tubes. To the first test tube few drops of Dragendorff's reagent was added. Appearance of an orange red precipitate indicated presence for alkaloids. To the second test tube, few drops of Mayer's reagent was added, a buff colored precipitate indicated presence of alkaloids²⁷.

Shinoda's test for flavonoids

Crude powder 0.5 gram of each was dissolved in 5ml of ethanol, heated and solution was filtered. To the filtrate, three pieces of Magnesium chips were added followed by addition of few drops of concentrated HCl. Change of color of solution into to red indicated presence of flavonoids²⁷.

Test for Saponins

Crude powder (1 gram) was added to 5 mL of distilled water, boiled and filtered. Each filtrate was then diluted by 3 ml of distilled water and further shaken for 5 minutes. Solutions were heated. Formation of froth indicated presence of saponins²⁸.

Liebermann-Burchard test for steroids

Crude powder (0.2 gram) of each sample dissolved in 2ml of acetic acid. Few drops of concentrated H₂SO₄ was added. Blue or violet color indicated presence of steroids²⁸.

Test for Terpenoids

Each of plant extract (0.5 ml) was mixed with 2 ml of chloroform. To this solution, 3 ml of concentrated H₂SO₄ was added carefully along the wall of the test tube. Reddish brown color at the interface indicated presence of Terpenoids²⁸. Phytochemical analysis for Neem and Coriander shown in Table number 5

Table 5 Phytochemical screening of Neem and Coriander leaves

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



RESULTS AND DISCUSSION

This present work was aimed to develop herbal formulation (anti-acne gel) by the use of herbal ingredients like Neem leaves extract and Coriander leaves extract. 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 were prepared G₁ and G₂. These formulations differed by the relative amount of extracts of Coriander leaves and Neem leaves. G₁ was developed by using 3.5 grams of Neem extract and 4 grams of Coriander extract (1.75:2) where as G₂ was developed by using 4 grams of Neem extract and 3.5 Grams of Coriander extract (2:1.75) as shown in Table Number 1.

Extracts were evaluated for its antibacterial activity against *Staphylococcus epidermidis* and *Propionibacterium acnes* which are opportunistic bacteria reside in papules and pustules and trigger inflammation. 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 of Neem

and Coriander were tested for antibacterial activity in combination by two ratios (2:1.75) and (1.75:2), combination showed synergistic effect than antibacterial activities of extract. Combination of extract G₁ showed 18 mm zone of Inhibition against *Staphylococcus epidermidis* and 22 mm against *Propionibacterium acnes*. G₂ showed 20mm of zone of inhibition against *Staphylococcus epidermidis* and 23mm 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. Antibacterial activity of combination of extracts were less than clindamycin which showed 33mm zone of inhibition against *Staphylococcus epidermidis* and 36 mm against *Propionibacterium acnes*. The best formulation selected in terms of physical parameters and antibacterial activity was G₂. G₂ was having more activity against *Propionibacterium acnes* and *Staphylococcus epidermidis* than the extracts of Coriander and Neem leaves as shown in Table number 2.

Formulations G₁ and G₂ were evaluated in terms of color, pH, viscosity, extrudability and phytochemical analysis G₁



formulation was colorless and G2 was white in color. The formulations were glossy and produced soothing effect on application. The pH for G1 was recorded to be 7.3 while that of G2 was 7.1. Viscosity of formulation G1 was 30 cP and while for G2 was 32 cP. Viscosity was controlled by changing gelling agent concentration and selecting best viscous formulation with reference to skin. Value of extrudability was 536 grams for G1 and 532 gram for G1. These parameters are shown in Table number 4.

Neem leaves extract and Coriander extract were analyzed for their phytochemical constituents. Test for Tannins, Alkaloids, Flavonoids, Steroids and Terpenoids were performed on Neem and Coriander by using standard procedures from Trease and Evans. Neem extract showed positive result towards the presence of Tannins, Alkaloids, Flavonoids and Steroids and Terpenoids were absent. In Coriander leaves extract, test was negative for the presence of Tannins, Alkaloids and Flavonoids and positive for Steroids and Terpenoids as shown in Table number 5

Results of antibacterial activity of Neem leaves are similar to many other documented studies confirming its potential of containing bioactive compounds which could be used as antibacterial agents. One of these studies

was conducted by Okemo and coworkers (2001) on the crude extract. He concluded that extract of Neem leaves had good activity against *Staphylococcus aureus* and *Escherichia coli*²⁹. In a similar study by Aslam and his coworkers (2009), determined antibacterial activity of Neem extract against *Corynebacterium bovi*, *Staphylococcus aureus* and *Escherichia coli*³⁰. Subapriya and Nagin (2005) concluded that antibacterial activity of Neem leaves extract might be due to high concentrations of azadirachtins, quercetin and β -sitosterol³¹. Maragathavalli (2001) determined antibacterial activity of extract of Neem leaves against many pathogenic bacteria such as *Pseudomonas aeruginosa*, *Bacillus pumillus* and *Staphylococcus aureus*³².

Antibacterial activity of Coriander leaves extract as determined by this study strengthened by a previous work of Shyamapads (2015), he discussed antibacterial activity of fruits and leaves of *Coriandrum sativum*. He reported that many components especially essential oils and some peptides possess promising antibacterial, antioxidant and antifungal activity³³.

This studies focused on synergistic effects of Neem and Coriander leaves in order to enhance antibacterial activity and improving



acne vulgaris. Gel formulation was developed as dosage form to enhance absorption and bioavailability for this synergistic extract combination of (2:1.75) of Neem and Coriander Leaves respectively.

RECOMMENDATIONS

Bioactive compounds responsible for antibacterial activity can be isolated by advanced research and scientific techniques. This formulation can be commercialized for future use after animal and human testing and could prove an effective addition in anti-acne therapy regimen instead of antibacterial agents available in market, along with the benefit of cost effectiveness.



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