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FORMULATION AND EVALUATION OF ANTI-ACNE GEL OF *AZADIRACHTA INDICA* EXTRACT HERBAL PRODUCT

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ABSTRACT

The objective of this study was to produce a Carbopol 940 based gel formula containing an *Azadirachta indica* leaf extract and evaluate its anti-acne potential. The ethanolic extract was derived from the dried leaves of *Azadirachta indica* and was subjected to a phytochemical evaluation. Three gel formulations of Carbopol 940 containing an *Azadirachta indica* extract in three different concentrations, i.e., 1, 2, and 3% w/w were prepared. These gels were evaluated for their physical appearance, antimicrobial activity, skin irritability, pH, spreadability, and viscosity. The prepared formulas were stable, greenish and homogeneous. None of them showed irritation to the skin. The spreadability (g.cm/sec), viscosity (cps), and pH of all three formulations was 34.68, 53 270–65 400, and 7–8, respectively. Gel-III exhibited the highest antimicrobial potential against *Propionibacterium acne*, the main causative organism of acne with a zone of inhibition of 16.2 ± 0.6 mm. It was revealed from the acne healing studies that the elimination time for the acne treated with Gel-III was 15 days. A formulation gel containing 3% w/w extract showed better antimicrobial activity, physicochemical characteristics, and pharmacological parameters than the other formulations. It can be concluded that the acne healing process was faster with the gel formulation containing 3% w/w of the *Azadirachta indica* extract, proposing that this formulation is a promising candidate for acne healing.

KEYWORDS: *Azadirachta indica*, Gel, Anti-acne.

INTRODUCTION

The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutics role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants.[1,2]

Azadirachta indica (family Meliaceae) is a medicinal plant, commonly known as neem, found in India, Pakistan, Bangladesh, and Nepal, and is widely used to treat various diseases.[3-5] Neem has been commonly used in Ayurveda, Unani, Homoeopathic, and Siddha medicine and has become prominent in modern medicine.[6] The limonoids and azadirachtin found in neem seeds have insecticidal effects, but are safe for human beings.

The leaf extract, seed oil, and bark of the neem are medicinally used in folk medicine for constipation, respiratory disorders, leprosy, and intestinal helminthiasis and they promote good health. Neem also possesses antipyretic, anti-inflammatory, antimycotic, antimicrobial, immunomodulatory, cardiovascular, anti-hyperglycaemic, and neuropsychological activities. All parts of the neem plants are used to treat itching, burning sensations, blood morbidity, and skin ulcers.[7] Steroids, alkaloids, flavonoids, fatty acids, carbohydrates, and terpenoids[8] are some of the various phytochemicals found in *Azadirachta indica*. The acetone and water extract of *Azadirachta indica* possess antimicrobial activities against Pseudomonas aeruginosa and Staphylococcus aureus.[9]

Neem has been used safely and effectively for more than 5000 years in India to support the treatment of many common skin disorders such as acne, melasma and heal

skin lesions.[10,11] Neem leaf extract has been reported to inhibit the growth of Propionibacterium acnes[12], prevent wrinkles formation[13], and inhibit the growth of fungal species such as Trichophyton rubrum, Trichophyton mentagrophytes and Microsporum nanum.[14]

Biologically active principles isolated from different parts of the plant include: Azadirachtin, meliacin, gedunin, nimbidin, nimbolides, salanin, nimbin, valassin, meliacin forms the bitter principles of Neem oil, the seed also contain tignic acid responsible for the distinctive odour of the oil.[15] Neem kernels contain30-50% of oil mainly used by the soap, pesticide and pharmaceutical industries and contain many active ingredients which are together called triterpene or limnoids.[16] The four best limnoids compounds are: Azadirachtin, Salannin, Meliantriol, and Nimbin. Limonoids contain insecticidal and pesticidal activity.[17]

Acne vulgaris is an extremely common disorder of the skin (pilocebaceous unit) that affects virtually all individuals at least once during life. The incidence of acne peaks of teenage, but substantial numbers of men and women between 20-30 years of age are also affected by the disorder.[18]

Acne may be classified as comedonal, papular, pustular, cystic, and nodular. Comedonal acne is non-inflammatory and divided into two types: whiteheads and blackheads. White heads (closed comedo) present as fresh or white colored, raised bumps whereas blackhead (open comedo) present as open pores containing dark colored skin roughage consisting of melanin, sebum, and follicular cells. Papules appear as red, solid, elevated lesions often less than 5 mm in diameter. Pustules are circumscribed skin elevations containing purulent material. Cysts and nodules are solid, elevated lesions involving deeper dermal and subcutaneous tissue. Cysts are less than 5mm in diameter whereas nodules exceed 5mm. The pathogenesis of acne involves multiple physiological factors. These include follicular hyper proliferation; increased sebum production due to higher androgen levels and colonization of organism, Propionibacterium acnes.[19,20] Novel concepts have emerged to help better understand its pathogenesis; these include variations in target cell sensitivity, biological markers, neuro-endocrine, genetic, and environmental factors. Plenty of herbal as well as synthetic ingredients are reported to have remarkable beneficial effect on acne vulgaris.[21] They may have different mechanisms like, (a) Control sebum secretion, (b) Antibiotics which inhibit Propionibacterium acne, the main causative organism of acne, (c) Keratolytic which removes the keratin layer and prevents the trapping of sebum under the skin, (d) Anti-inflammatory which prevents the worsening of the condition due to inflammation or redness etc. Numbers of formulations are available in the market with a variety of active pharmaceutical ingredients for the treatment of acne.

Topical preparations such as gels, ointments, lotions and creams are the important drug delivery systems due to its convenience in delivering drug to a localized area of the skin. Gel is one of the semi-solid topical preparations providing quick onset of activity, long-term efficacy and high patient satisfaction.

Gels are popular because of the ease in their application and improved percutaneous absorption compared to other preparations.

In this study, gel formulations containing Carbopol 940 and an extract of *Azadirachta indica* were prepared to treatment the anti- acne activity of neem leaves extract.

MATERIALS AND METHODS

Plant Materials and Preparation of Extract

Azadirachta indica leaves were collected from Al-Selw, Taiz, cleaned from foreign material, washed with distilled water, dried in the shade for 72 hours, coarsely grinded, weighed, and stored in airtight jars. One liter of ethanol (95% v/v) was added to 250 g of powdered *Azadirachta indica* for 3 to 4 days. The mixture was stirred with a sterile glass rod after 12 h and was filtered with Whatman filter paper No. 1. In a rotary evaporator, the solvent was removed under reduced pressure at a temperature of less than 50 °C, leaving a dark green residue stored in the airtight glass jars. The extract's weight was recorded and the percentage yield was 10.6%. Carbopol 940, Propylene glycol, Sodium hydroxide and Methyl paraben as a gift from (Shaphaco Pharmaceutical Industry Company-Yemen).

Phytochemical Analysis of The *Azadirachta Indica* Leaves

The phytochemical screening for major constituents was undertaken using standard qualitative methods as described by *Trease and Evans (1989)*[22] , *Harbone (1985)*[23], *Odebiyi and Sofowora (1990)*[24] *and Sofowora (1993).*[25] The phytochemical screening of the alcoholic extracts of the leaf of *A. indica* was carried out for the presence alkaloids, cardiac glucosides, flavonoids phenols, resins, saponins, tannins, terpenes and steroids using standard phytochemical methods. The phytochemical screening of the ethanolic extracts of the plant was carried out in order to elucidate the chemical constituents (bioactive agents) responsible for their antimicrobial and therapeutic activities.

Preparation of Gel Formulations

Distilled water was added to the Carbopol 940 and mixed mechanically by high-speed mixer. To this mixture, sodium hydroxide 10% was added vigorously. In water bath with a temperature not exceeding 50 °C, the *Azadirachta indica* extracts in a concentration of 1, 2, and 3 g were added to prepare three formulations, Gel-I (1% w/w), Gel-II (2% w/w) and Gel-III (3% w/w), respectively. Separately dissolved methyl paraben in propylene glycol were also added to this gel. The remaining quantity of purified water was added, and the

pH was drop wise adjusted with sodium hydroxide 10%. The final weight was adjusted with water *quantum statis* (*q.s.*) to 100 g as shown in Table 1.

Table 1: Composition of Gel Formulations.

Ingredients percentage	Gel I	Gel II	Gel III
***Azadirachta Indica* Extract (w/w)**	1	2	3
Carbopol 940	3	3	3
Propylene Glycol	10	10	10
Methyl Paraben	0.3	0.3	0.3
Sodium Hydroxide 10%	q.s.n.	q.s.n.	q.s.n.
Distilled Water	q.s.p.	q.s.p.	q.s.p.

q.s.n. = quantity sufficient to neutralize gel base, q.s.p = quantity sufficient to prepare 100 grams of gel.

Evaluation of Gel Formulations[25-48]

Physical appearance

The gel formulations were evaluated for their physical parameters like color, odor, consistency, transparency, and homogeneity.

Spreadability of Gel Formulations

A glass slide with standard dimensions was used, where 0.5 g of the gel was placed in a circle 1 cm in diameter on the glass slide, over which another glass slide was placed. A weight of 125 g was set for 5 min so that the gel was sandwiched between the two slides to form a thin layer. Then, the weight was removed and the extra gel was removed. Then the slides were adjusted so that the upper slide was fixed with a weight of about 20 g. The time was noted for the slides to separate from each other.[26] The spreadability was recorded using the following formula.

$S = M / T$

Where:

S – Spreadability in grams/seconds;

M – Mass in grams;

T – Time in seconds.

Viscosity. A Brookfield DV-E viscometer (RVDVE) was used to determine the viscosity of the gels. Spindle No. 07 was inserted in each formulation and was sheared at 3.3, 9.9, and 16.5 *g* at 24 ± 1 °C. The gel formulations were prepared in distilled water.[27]

pH Determination of Gel Formulations

The pH of the gels was detected with a digital pH meter. An amount of 0.5 g of gel was dissolved in 50 ml of distilled water and stored for two hours. Each formulation's pH was measured in triplicate and the average values were taken.[28]

Antibacterial Activity of Gel Formulations

Each formulation was assessed for its antimicrobial effects against the microorganisms on a nutrient agar using a suitable diffusion method. About 0.2 ml of the bacterial test strain was inoculated over a nutrient agar plate with a sterile cotton swab and was allowed to dry. With the help of a cork borer, 6 mm diameter wells were created. Half a milliliter of the *Azadirachta indica* extract was introduced into the wells.

The plates were placed at room temperature for about one hour. Then the plates were placed in an incubator at 37 °C for 24 hours. Then, the zone of inhibition was checked and recorded. Clindamycin was used as standard.

Acne Healing Activity of Gel Formulations

Adults aged from 17 to 22-year-old were divided into three groups, having 4 adults each. Group I, II, and III received Gel-I containing 1% w/w of the *Azadirachta indica* extract, Gel-II containing 2% w/w of the *Azadirachta indica* extract, and Gel-III containing 3% w/w of the *Azadirachta indica* extract. No other medicine was given to the adults during the entire study. The study was evaluated for 15 days.

RESULTS AND DISCUSSION

This study evaluated the anti-acne potential of herbal gels. Three different concentrations of an *Azadirachta indica* extract were used to prepare gel formulations with Carbopol 940. The formulations were evaluated for the physical parameters like the pH, viscosity, and spreadability.

A pharmacological evaluation, like a skin irritation test, revealed that the herbal gels were safe to apply on the skin. The antibacterial activity of these gels against *Propionibacterium acne* bacteria was also tested and confirmed. An anti-acne study was carried out to show that the herbal gels can heal the acne without severe adverse effects.

Phytochemical Analysis of *Azadirachta Indica*

Many phytochemicals were found in the ethanolic extract. Different tests were performed according to the standard methods to check for the presence of phytoconstituents such as alkaloids, flavonoids, tannins, reducing sugars, saponins, triterpenes and glycosides in the ethanolic extract of the neem. The observations were recorded in Table 2.

Table 2: Phytochemical Constituents of Ethanolic Extract of *Azadirachta Indica Leaves.*

Serial No.	Constituents	Test Name	Outcome
1	Glycoside	Legal's test	+
2	Alkaloids	Mayer's reagent test	+
3	Triterpenoids and steroids	Libermann test	–
4	Flavonoids	Alkaline reagent test	+
5	Reducing sugars	Fehling's test	+
6	Carbohydrates	Molish's test	+
7	Tannins	Ferric chloride test	+
8	Saponins	Froth test	+
9	Proteins and amino acids	Ninhydrin test	–

+ = means detected; = means not detected

Evaluation of Gel Formulations

All the formulations were green. The spreadability indicates the extent to which the gel readily spreads on application to the skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value. All the formulations had very slightly alkaline pH which was compatible with normal skin physiology. The results of the viscosity are also shown in Table 3.

Skin Irritation Test

All the gel formulations were found to be safe while being applied on the skin and there was no irritation or sensitivity to the skin.

Antibacterial Activity of Gel Formulations

The antibacterial activity showed Table 4 that the zone of inhibition increased with an increase in the concentration of the herbal extract. It indicates that the *Azadirachta indica* leaf extract possesses an antibacterial activity, helps maintain a sterile acne area, and promotes the acne healing process. Gel- III was found to be more effective in the acne healing when compared to other herbal gels. These gels showed better activity against *Propionibacterium acne* bacteria.

Koona and Budida (2011)[29] reported the antibacterial activity of a methanolic leaves extract of *Azadirachta indica* against *E. coli*. Additionally, an *Aloe vera* extract was used to study its antibacterial effect against *P. aeruginosa*, *S. aureus*, and *E. coli*.[30]

The antimicrobial activity against various microorganisms like *S. aureus E. coli and Bacillus subtilis* bacteria was evaluated.

It was reported that the *Azadirachta indica* extract was effective against all microorganisms when compared to other plant extracts and the standard ofloxacin.[31]

Priadarshini et al. (2013)[48] studied the antibacterial activity of an extract (200, 150, 100, 50, and 25 mg/ml concentrations) obtained from leaves of herbs like *Azadirachta indica* and *Moringa oleifera* against microorganisms. The results were compared with the standard drug, gentamycin. Both plants' extracts showed activity against microorganisms like *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* in ascending order.[48]

Table 3: Physical Parameters Evaluation of Gel Formulations.

Formulation	Color	Appearance	pH	Spreadability (g.cm/sec)	Viscosity (cps)	Homogeneity
Gel-I	Green	Greasy Transparent	7.78	36	55 400	Homogenous
Gel-II	Dark Green	Greasy Translucent	7.69	33	60 200	Homogenous
Gel-III	Dark Green	Greasy Translucent	7.81	31	64 300	Homogenous

Table 4: Antibacterial Activity of Gel Formulations (Zone of Inhibition).

Formulation	*P. acne* (mm)
Standard	18.2 ± 0.7
Gel-I	13.5 ± 0.4
Gel-II	15.4 ± 0.2
Gel-III	16.2 ± 0.6

Acne Healing Activity of Gel Formulations

Gel-III containing 3% w/w showed a better healing activity when compared to Gel-I and Gel-II. The adults' skin treated with the 3% w/w *Azadirachta indica* extract healed in 15 days compared to those who treated with 2% w/w and 1% w/w *Azadirachta indica* extract where the healing occurred in 21 and 26 days, respectively as shown in Figures 1- 3.

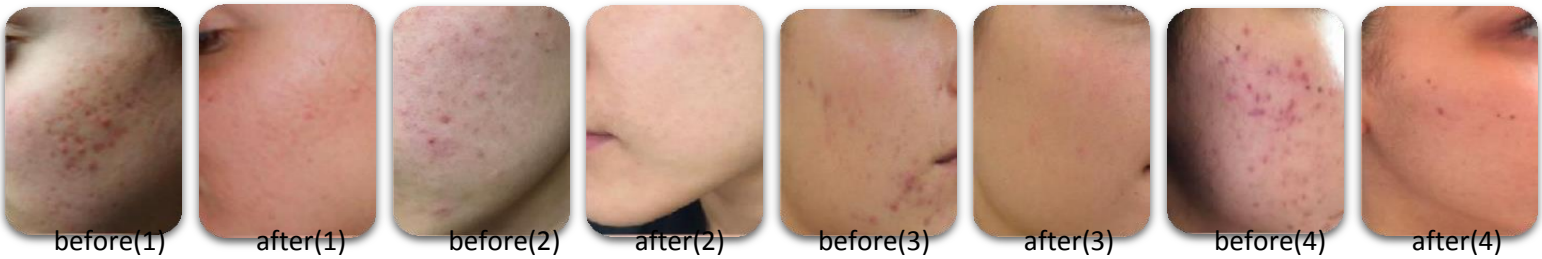

Fig. 1: Adults Treated with Gel-I Containing 1% w/w *Azadirachta Indica* Extract.

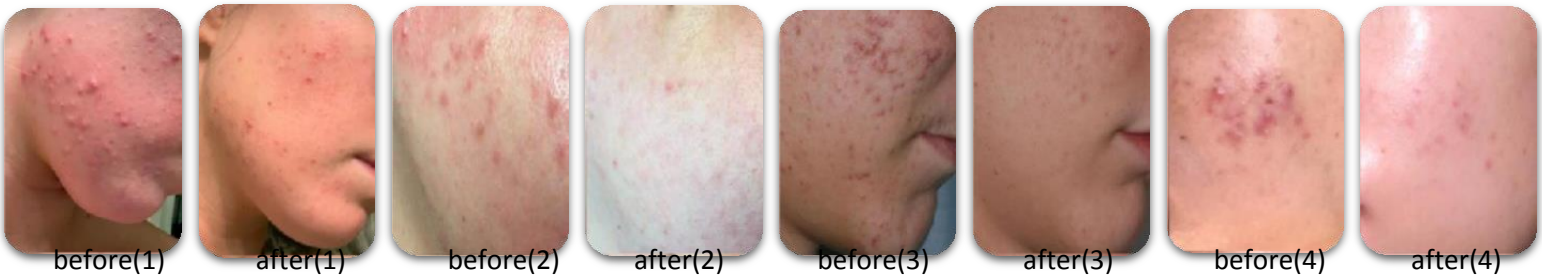

Fig. 2: Adults Treated with Gel-II Containing 2% w/w *Azadirachta Indica* Extract.

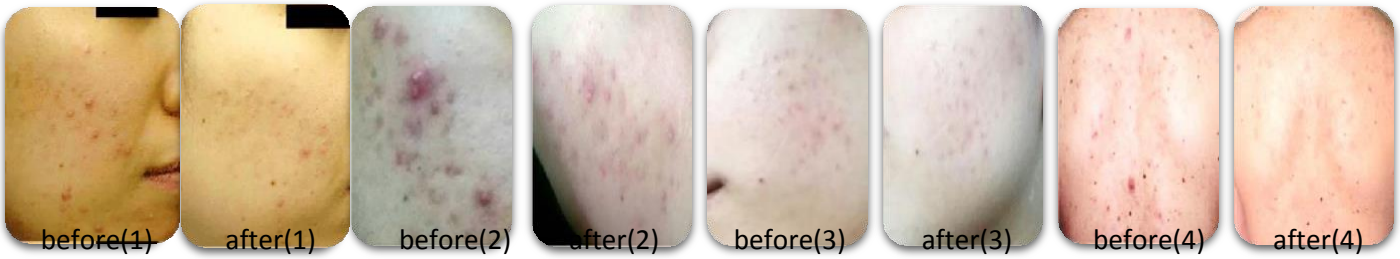

Fig. 3: Adults Treated with Gel-III Containing 3% w/w *Azadirachta Indica* Extrat.

CONCLUSION

According to the present study, the acne elimination improves with the increasing concentration of the herbal extract. Among these formulations, gels containing an *Azadirachta indica* extract in the concentration of 1, 2, and 3% w/w, a formulation gel containing 3% w/w extract of *Azadirachta indica* showed better wound healing and antimicrobial effects. It can be concluded that the extract of *Azadirachta indica* (3% w/w) was a better candidate for acne spots healing.

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