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PREPARATION AND CHARACTERIZATION OF NATURAL ANTIACNE CREAM CONTAINING PLANT EXTRACT

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Abstract:

Acne affects skin having dense sebaceous follicles in areas including face, chest and back. Acne may be of inflammatory or non-inflammatory forms. Due to changes in pilosebaceous units lesions are caused by androgen stimulation. The studied was designed to develop and characterize an anti acne cream containing herbal extract. Many topical agents are available in the market, which interfere with the pigmentation process at different levels. They are often known to cause side effects ranging from irritation to tumor over chronic use. A herbal cream was formulated using neem extract and aloe vera juice and subjected to evaluation of its anti blemish potential against stress augmented. The formulated cream was characterized for solubility, pH, particle size, grittiness, viscosity, stability, phase separation, shelf life and spreadability, and found to be stable. Acne is an infection of the skin that occurs in both men and women during their lifespan. There are various natural or synthetic products available in the market to prevent and cure this disease. The majority of the world population depends on the herbs or natural resources for the relief of acne disease. Further, the study of various patents also revealed that herbs with anti-oxidant properties have been used in most of the herbal anti-acne formulations. Moreover, the various patents also give the idea that herbal formulations also prevent the appearance of pimples on the skin. It has been concluded that the herbal anti-acne formulation is not only used to treat acne but also prevents this disease safely and economically.

Keywords: *Herbal cream, Acne treatment, Neem extract, Aloe vera juice, Natural active materials*

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INTRODUCTION:

Acne vulgaris or simply known as acne is a human skin disease characterized by skin with scaly red skin (seborrhea), blackheads and whiteheads (comedones), pinheads (papules), large papules (nodules), pimples and scarring. Acne occurs commonly during adolescence, affecting about 80–90% of teenagers in the Western world and lower rate are reported in rural societies **[1]**. Acne is usually caused by increase in androgens level like testosterone mainly during puberty in both male and female. The word acne refers to the presence of papules, scars, comedones and pustules. The common form of acne is known as acne vulgaris. Many teenagers suffer from this type of acne. Acne vulgaris shows the presence of comedones. Acne includes papules, nodules (large papules), seborrhea (increased oil-sebum secretion), comedones, pustules and scarring. The appearance of acne varies with skin color and it is also associated with psychological and social problems. Acne scars shows inflammation within the dermis and it is created by the wound healing resulting in collagen deposition at one spot **[2].** Acne develops due to blockage of follicles, hyper keratinization and keratin plug formation and sebum (microcomedo). The naturally occurring commensal bacterium Propionibacterium acnes can cause inflammation and inflammatory lesions like infected pustules or nodules and papules in the dermis around the microcomedo or comedone resulting in redness, scarring or hyperpigmentation **[3].** Acne vulgaris in adult women may be due to underlying condition such as; pregnancy, Cushing's syndrome, hirsutism or polycystic ovary syndrome. Acne climacterica refers to menopause associated acne, occurs as production of the anti-acne ovarian hormones estradiol and progesterone allowing the acnegenic hormone testosterone to continuously exert its effects **[4].** The National Institutes of Health (USA) shows that stress can cause acne flare. In Singapore, study of adolescents observed positive correlation between stress levels and acne severity **[5].** Parasitic Acne is linked with the parasitic mite Demodex but it is not clear whether Demodex or Demodex associated bacteria causes the effects **[6].** Typical features of acne include increased secretion of oily sebum by the skin, microcomedones, comedones, papules, nodules (large papules), pustules, and often results in scarring. The appearance of acne varies with skin color. It may result in psychological and social problems. Post inflammatory hyperpigmentation (PIH) is usually the result of nodular acne lesions. These lesions often leave behind an inflamed darkened mark after the original acne lesion has resolved. This inflammation stimulates specialized pigment-producing skin cells (known as melanocytes) to produce more melanin pigment which leads to the skin's darkened appearance. People with darker skin color are more frequently affected by this condition **[7].** Infection with the parasitic mite Demodex is associated with the development of acne. It is unclear whether eradication of the mite improves acne **[8].** Topical application increases sensitivity to the sun and sunscreen is combined to prevent sunburn. Benzoyl peroxide is often combined with antibiotics. Benzoyl peroxide is found to be equally effective as antibiotics at all concentrations, although it does not produce bacterial resistance. Antibiotics Antibiotics are used in more severe cases due to their antimicrobial activity against P. acnes along with anti-inflammatory properties. They are becoming less effective with increasing resistance of P. acnes worldwide **[8].** Topical Sulfur and Sodium Sulphacetamide Sulfur is used as a drying agent and antibacterial agent. It is present in washes, lotions, creams, foam formulations, prescription and nonprescription masks. It can be useful for treatment of rosacea and seborrhoeic dermatitis. Sodium Sulphacetamide is often combined with sulfur and has anti-inflammatory properties. Sodium Sulphacetamide can treat acne and used for the sensitive skin acne patient **[9].** Salicylic acid has bactericidal and keratolytic properties and hence lessens acne. Salicylic acid open obstructed skin pores and promotes shedding of epithelial skin cells but it causes hyperpigmentation of the skin in individuals with darker skin types. Retinol a form of vitamin A has mild effects and is used in many over the counter moisturizers and other topical products **[10].** Neem is an omnipotent tree and a sacred gift of nature. Neem tree is mainly cultivated in the Indian subcontinent. Neem is a member of the mahogany family, Meliaceae. Today it is known by the botanical name Azadirachta indica (A. indica). As per Ayurveda, Aloe is known as Kumari or "Young Girl". It is because, Aloe is believed to bring back youthful energy and femininity. Aloe is used as a tonic for the female reproductive system. According to Ayurveda, Aloe is said to have alliterative, tonic, rejuvenating, purgative, and vulnerary actions. The antiviral activities of Aloe extracts may be due to indirect or direct effects. Indirectly they show these effects by stimulating the immune system and directly by anthraquinones. The anthraquinone loin inactivates various enveloped viruses such as Herpes simplex, Varicella zoster and Influenza. Aloe is rich in mucopolysaccharides which help in binding moisture to the skin. Aloe stimulates fibroblasts to produce collagen and elastin fibers thereby making the skin more elastic and less wrinkled. Aloe vera gel gloves improved the skin integrity, decreased

appearance of fine wrinkles and erythema in the treatment of dry skin associated with occupational exposure indicating its moisturizing effects **[11].** The greater part of the world's population relies on traditional medicine for their health care. This is also the case in the treatment of wounds. The objective of proposed work is to develop formulation and characterization of natural anti-acne cream containing neem and aloe vera plant extract. The proposed formulation expected to be treating the development of acne on face or other part of body by bacterial infection vulgaris. Thus, the main aim of our work is to develop a herbal cream which can give multipurpose effect, like moisturizer, reduce acne and skin irritation, reduce skin diseases like eczema, psoriasis, dry skin, wrinkles, rashes etc. and also adding glow to the face. Neem is used as an antifungal and anti-inflammatory and it is also used to reduce scar, pigmentation, redness and itching of the skin and aloe vera extract act for moisturizer, to reduce pimples and acne and also used for treatment of burn wounds. The evaluations of all formulations were done on different parameters like pH; viscosity, spreadibilty, and stability were examined. Formulations should not show any adverse effects. The formulation shows no redness, edema, inflammation, and irritation during irritancy studies. These formulations are safe to use for skin. These studies suggest that the composition of extracts and base of cream are more stable and safer, it may produce synergistic action.

MATERIAL AND METHODS:

Collection and extraction of plants: The plants *Azadirachta indica* and *Aloe barbadensis* were collected from market or nursery. Botanical identification is necessary because it ensures the safety and efficacy of the natural plant. The collected material is compared with the published description of the drug and with authentic specimen and identification is verified by an acknowledged expert.

Pharmacognostic study: In view of its diverse medicinal applications and in order to ensure the quality, authenticity and assay, and in view of lack of pharmacognostic study the present investigation was undertaken with an objective to evaluate *Azadirachta indica and Aloe barbadensis* on various pharmacognostic parameters, such as macroscopic, physiochemical, and phytochemical studies of the plant. Fresh galls were taken for morphological and histological studies. Coarse powder was used to study the microscopic characters and physicochemical investigations. The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc.

Physicochemical analysis: Physicochemical values such as % of ash values and extractive values were determined according to the well-established protocols. The following Physicochemical analysis was investigated for the powder drug.

Extraction of plant material: The extracts of *Azadirachta indica and Aloe barbadensis* were prepared and they are extracted with ethanol as coarsely powdered 100g *Azadirachta indica and Aloe barbadensis* was macerated and extracted with 250 ml ethanol at room temperature for 7 days and the extract was concentrated, frozen and lyophilized by lyophilizer **[11]**.

Physical characterization of extract: Different physical parameters of extracts including their colour and percentage yield were obtained and extracts were weighed and percentage yields were calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Preliminary phytochemical screening: Phytochemical screening means to analyze the plant material for its chemical constituents. It involves the isolation of active constituents and their qualitative identification.

Qualitative chemical test: Extracts of the selected plants were subjected to qualitative chemical test to assess the presence of alkaloid, glycosides, proteins, amino acids, steroids, tannins, carbohydrates, phenol compounds by using standard screening procedure **[12]**.

Formulation of polyherbal antiacne cream: The polyherbal antiacne cream was prepared by the oil phase ingredients cetyl alcohol, glyceryl stearate, and almond oil were combined in a beaker and gently heated until they fully melted and mixed. The aqueous phase ingredients glycerin, methylparaben, propylparaben, and xanthan gum were combined in another beaker along with an appropriate quantity of distilled water. This mixture was heated until all the ingredients dissolved. Once the oil and aqueous phases were both prepared and their temperatures were equivalent, the aqueous phase was slowly added to the oil phase under constant stirring. This mixing process continued until a uniform emulsion was formed. The previously prepared plant extracts (*Azadirachta indica* and *Aloe barbadensis*) were added to the emulsion at varying concentrations,

according to the specific formulation (1, 2, or 3). The mixture was stirred until the extracts were evenly distributed throughout the cream. The prepared cream was allowed to cool down to room temperature while being stirred periodically to maintain homogeneity. Once cooled, it was packaged in airtight containers to protect it from contamination and to maintain its integrity. This process was repeated for each of the three formulations, adjusting the concentrations of the plant extracts as per the specifications outlined for Formulation PHC1, Formulation PHC2, and Formulation PHC3. The resulting creams were then stored for further testing and evaluation **[13]**.

Evaluation of Polyherbal Antiacne Cream: The prepared polyherbal antiacne cream was subjected to physical characterization such as color, appearance, pH, viscosity, spreadability. It was also evaluated for its stability property, antimicrobial activity and in vivo skin irritation study **[14]**.

Physical appearance: The formulated Polyherbal cream was inspected visually for their color, odor, homogeneity and consistency. All developed cream was tested for homogeneity by visual inspection after cream has been set in the container. They were tested for their appearance and presence of any aggregates.

Measurement of pH: The pH of various formulations was determined by using digital pH meter. One gram of cream was dissolved in 100ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate.

Determination of Viscosity: The measurements of viscosity of prepared cream were carried out with Brookfield viscometer (Brookfield viscometer RVT) with spindle No.6.

Spreadability: Spreadability denotes the extent of area to which the cream readily spreads on application to skin or the affected part. Two sets of glass slides of standard dimensions were taken. The cream formulation was placed over one of the slides. The other slides was placed on the top of the cream, such that the cream was sandwiched between the two slides in an area occupied by a distance of 6.0 cm along the slide.100gm weight was placed upon the upper slides so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of cream adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide slip off freely by the force of weight tied to it. A 20gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times and the mean time taken for calculation **[15].**

Spreadability was calculated by using the following the formula:

$$S = \frac{(M \times L)}{T}$$

Where, S = Spreadability, M = Weight in the pan (tied to the upper slide) L = Length of the glass slide, T= Time (in sec) taken to separate the slides.

Antimicrobial activity of the optimized polyherbal antiacne cream: The following Standard cultures of American Type Culture Collection (ATCC) strains were used in the study:

1. Staphylococcus aureus (ATCC- 6538P)
2. Escherichia coli (ATCC- 8739)
3. Candida albicans (ATCC- 18804)

The sterile Petri dishes were filled with Muller Hinton Agar medium which was then inoculated with a suitable dilution of a test organism (Staphylococcus aureus (ATCC- 6538P), Escherchia coli (ATCC- 8739) and Candida albicans (ATCC- 18804). Four cylinder or cups were made in the medium with the sterile borer in each plate. The formulated polyherbal cream, standard disc and solvent control were prepared. A uniform amount of 0.2 ml solution was added to the cup and incubated at 370C for 24 hrs. The well diffusion test was performed in triplicates and antimicrobial activity was expressed as the mean of inhibition in diameter (mm) **[16]**.

RESULTS AND DISCUSSION:

Pharmacognostic study: *Azadirachta indica* and *Aloe barbadensis* is an evergreen perennial climber. It is a plant of significant medicinal importance in the indigenous systems of medicine and designated as Rasayana. All the parts of this plant are reported for various ethnobotanical and therapeutic uses.

Physiochemical constants:

The percentage of total ash, acid insoluble ash, sulphated ash and water-soluble ash were shown in **Table 2**. The ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The loss on drying and foreign matter was 9.50 and 0.10 respectively. The extractive values are primarily useful for the determination of exhausted drugs.

Preliminary phytochemical analysis: Investigations on the preliminary phytochemical screening of *Azadirachta indica* and *Aloe barbadensis* extracts revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids and carbohydrates in methanolic and aqueous extracts respectively;

Evaluation of Polyherbal Cream

The formulated cream was checked visually for color, appearance and homogeneity. The pH of all prepared formulation ranged from 5.7-5.9. The pH of the prepared cream formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin. Viscosity is an important property of fluids which describes a liquids resistance to flow and is related to the internal friction within the fluid. This rheological property helps in determining consistency and also the diffusion rate of drug from cream. The measurement of viscosity of the prepared cream was done with Brookfield viscometer with spindle no: 62. The results were shown in **b**y keeping the viscosity below about 15,000 cps, the advantages of more appealing cosmetic characteristics and ease of accurate application through improved flow and pourability are achieved. Spreadability denotes the extent of area to which the cream readily spreads on application to skin or the affected part. The spreading was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the spreadability. Two sets of glass slides of standard dimensions were taken. The cream formulation was placed over one of the slides. Spreadability of different cream formulation were studied. The formulation PHC2 produced good spreadability than the other formulation.

Antimicrobial activity testing by cup plate method: The antimicrobial activity testing was performed by measuring and comparing the diameter of zones of inhibition (in mm). The zone of inhibition can be defined as the clear region around the well that contains an antimicrobial agent. It is known that the larger the zone of inhibition, the more potent the antimicrobial agent. The prepared formulations polyherbal cream (PHC1, PHC2 and PHC3) were evaluated for its antimicrobial property towards the microbial organism such as Staphylococcus aureus, Escherchia coli, Candida albicans. These prepared polyherbal cream was also compared with standard such as Gentamicin (10µg) and Fluconazole (25 µg). From the result it was observed that it showed good zone of inhibition but lesser when compared to standard. Polyherbal cream containing *Azadirachta indica* and *Aloe barbadensis* extracts were formulated and evaluated for various parameters. From the results, obtained from the physical parameters such as spreadability, pH, viscosity and spreadability it was indicated that formulation PHC2 was ideal and it was chosen for further characterization such as antimicrobial activity testing. The formulated polyherbal cream (PHC2) was observed more antimicrobial property towards the organism such as Staphylococcus aureus, Escherchia coli, Candida albicans and it was also compared with standard such as Gentamicin (10µg) and Fluconazole (25 µg). From the result it was observed that it showed good zone of inhibition but lesser when compared to standard.

Table 1: Formulation of polyherbal cream

Ingredients	PHC1	PHC2	PHC3
Azadirachtaindica indica extract	1% w/w	1.50% w/w	2% w/w
Aloe barbadensis extract	1.50% w/w	2% w/w	1% w/w
Cetyl alcohol (emulsifier)	3% w/w	3% w/w	3% w/w
Glyceryl stearate (emulsifier)	2% w/w	2% w/w	2% w/w
Almond oil	10% w/w	10% w/w	10% w/w
Glycerin (humectant)	5% w/w	5% w/w	5% w/w
Methylparaben (preservative)	0.15% w/w	0.15% w/w	0.15% w/w
Propylparaben (preservative)	0.05% w/w	0.05% w/w	0.05% w/w
Xanthan gum (thickener)	0.3% w/w	0.3% w/w	0.3% w/w
Distilled water	q.s. to 100% w/w	q.s. to 100% w/w	q.s. to 100% w/w

Table 2: Physiochemical parameters of *Azadirachta indica* and *Aloe barbadensis*

Parameters	***Azadirachta indica***	***Aloe barbadensis***
Foreign matter (% w/w)	0.1	0.9
Total ash value (% w/w)	5.02	4.53
Acid insoluble ash (% w/w)	1.51	2.31
Water soluble ash (% w/w)	3.22	2.49
Sulphated ash (% w/w)	0.21	0.35
Loss on drying (% w/w)	9.5	8.7
Moisture content (% w/w)	1.1	1.5

Table 3: Physical appearance of formulated cream

Parameters	**PHC1**	**PHC2**	**PHC3**
Physical appearance	Transparent yellow cream	Transparent yellow cream	Transparent yellow cream
Color	Pale yellow	Pale yellow	Pale yellow
Homogeneity	Absence of aggregates	Absence of aggregates	Slight aggregates

Table 4: Measurement of pH of formulated cream

Formulation code	**pH**	**Viscosity [cps]**	**Spreadability (gm.cm/sec)**
PHC1	5.9	1428±0.1	19.37
PHC2	5.7	1425±0.75	21.35
PHC3	5.8	1358±0.25	22.13

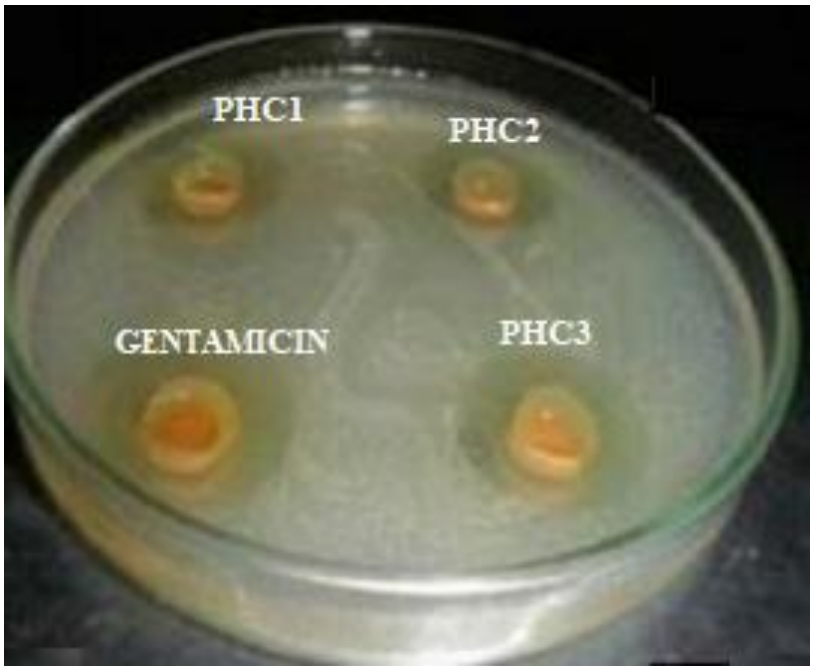

Figure 1: Measurement of Zone of inhibition of formulated cream on *Staphylococcus aureus*

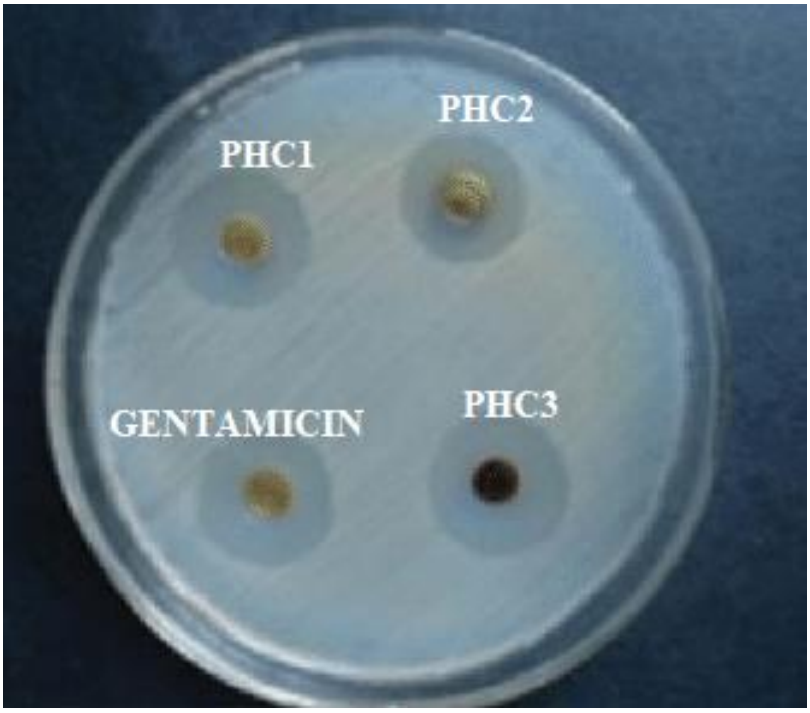

Figure 2: Measurement of Zone of inhibition of formulated cream on *Escherchia coli*

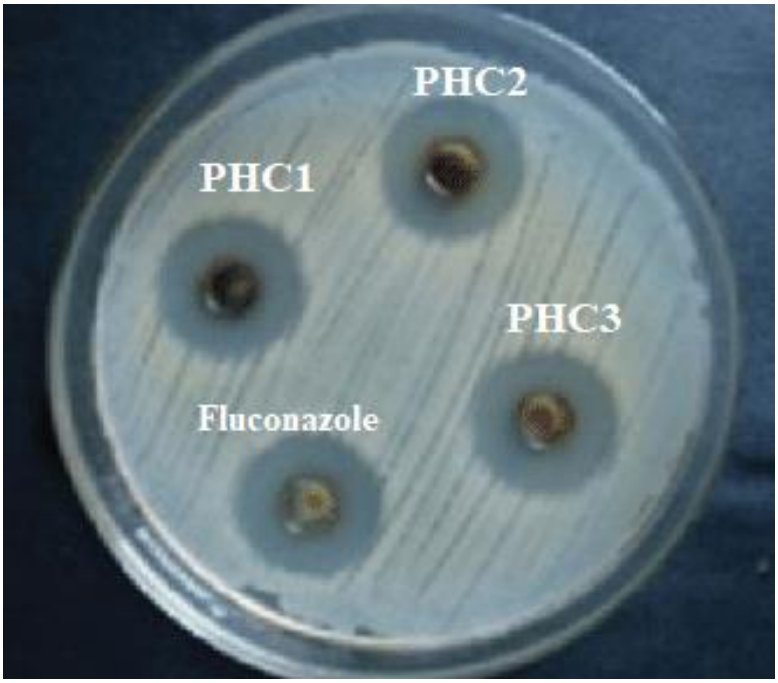

Figure 3: Measurement of Zone of inhibition of formulated cream on *Candida albicans*

SUMMARY AND CONCLUSION:

The conclusion of our comprehensive study has successfully illustrated the potential for a topical cream formulation using extracts from *Azadirachta indica* and *Aloe barbadensis*. The eethanol extraction process resulted in a notably high yield, emphasizing its effectiveness for obtaining bioactive compounds from these plant materials. Subsequently, we incorporated these extracts into a cream formulation, maintaining an appealing texture, color, and skin-

compatible pH, along with an optimal viscosity for easy application and skin absorption. The prepared polyherbal cream was evaluated for study of antimicrobial test against these test organism (Staphylococcus aureus (ATCC- 6538P), Escherchia coli (ATCC- 8739) and Candida albicans (ATCC-18804) by cup plate method. In order to have a good permeation across the skin, cream should have ideal property and stable over the long period of time. From the results, obtained from the physical parameters such as spreadability, pH, viscosity and spreadability it was indicated that formulation PHC2 was ideal and it was also have more antimicrobial activity than other formulations. The results of prepared formulated polyherbal cream indicates that the topical cream formulation harnessing the phytochemical richness of *Azadirachta indica* and *Aloe barbadensis* extracts could be a potential tool for skin health improvement and assess the long-term safety and efficacy of our formulations.

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