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Antimicrobial potential of plant extracts against acne-causing bacteria

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Abstract
The Indian herbal medication industry is most likely the world's oldest medical care system. Herbs have a long history in ancient India, with one form of herbal medicine being described in the Vedas, India's ancient sacred literature. Ayurveda and Unani are ancient herbal medicine practices that use herbs and natural items to treat various health concerns. Although herbal remedies appear to be novel to Western healers and medical practitioners, the truth is that plant extracts are still used in the majority of prescribed medicines today. Countries around the world now appreciate this old kind of medicine, and Indian herbal pharmaceuticals are in high demand, resulting in rapid growth and an annual growth rate of about 30%. Skin, as the most exposed area of our body to pathogens, requires protection against skin illnesses, particularly acne-causing bacteria. Acne is the most frequent skin condition among teenagers today, accounting for 85% of all cases. They can persist throughout adulthood and primarily affect areas with the greatest oil glands, such as the face and neck. Some bacteria, including *Propionibacterium acnes* and *Staphylococcus epidermidis*, live in the follicles. As a result, these microorganisms can be targeted as a potential acne cure.

Keywords: Acne, anti-microbial activity, soxhelet extraction, well diffusion

1. Introduction
Acne vulgaris is a very common skin ailment (pilocebaceous unit) that almost everyone experiences at least once in their lives. Acne is most common in teenagers, but it also affects a significant number of men and women aged 20 to 30. Acne can be characterised as comedonal, papular, pustular, cystic, or nodular. Comedonal acne is non-inflammatory and consists of two types: whiteheads and blackheads. White heads (closed comedos) seem as fresh or white-colored elevated bumps, but blackheads (open comedos) appear as open pores containing dark-coloured skin roughage made up of melanin, sebum, and follicular cells. Papules appear as red, firm, raised lesions that are typically smaller than 5 mm in diameter. Pustules are confined skin elevations that contain purulent material [1, 2]. Cysts and nodules are solid, raised lesions that affect deeper dermis and subcutaneous tissue. Cysts are smaller than 5 mm in diameter, whereas nodules exceed 5 mm. Acne is caused by a variety of physiological causes. These include follicular hyper growth, increased sebum production due to elevated testosterone levels, and colonisation of the organism, *Propionibacterium acnes* [3].

A wide range of natural and synthetic compounds have been shown to have a significant positive effect on acne. Tropical formulations, available in the market are as follows: Gel, Cream, Lotion, Face mask, Face wash or cleansers. Neem (*Azadirachta indica*, *Meliaceae*) are reported to have very beneficial effect on acne due to anti-microbial, anti-inflammatory and anti-oxidant activities of different chemical constituents [4]. For thousands of years, humans have used plant sources to ease or treat illness. Plants are a source of unique chemical compounds with potential applications in medicine and other fields. Plants contain a variety of active substances, including alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids, which are stored in specialised portions such as leaves, flowers, bark, seeds, fruits, and roots. The positive therapeutic effects of plant materials are often caused by the combination of these secondary metabolites [5].

Researchers have made significant efforts to find efficient extraction methods in order to achieve high efficiency and efficacy. Efficiency refers to the extraction yield, whereas efficacy refers to the extract's potency (magnitude of bioactivity / ability to produce an effect). One of the more sustainable methods for isolating biological components is through plant extraction. To obtain higher quality and more efficient herb extraction, one must optimise the methods [6].

Modern sample preparation techniques offer significant advantages over traditional methods in terms of reduced organic solvent consumption and sample degradation. They also eliminate undesirable and insoluble components from the extract. Modern methods include microwave assisted extraction (MAE), ultrasonication assisted extraction (UAE), supercritical fluid extraction (SFE), solid phase micro extraction (SPME), soxhwave, and so on. The latter is a combination of Soxhlet and microwaves. This combines the rapid heating capability of microwaves with the simplicity of Soxhlet [7]. Standardising extraction procedures for crude drug production aims to obtain the therapeutically desired portion while eliminating inert material through treatment with selective solvents and methods. With an increasing global demand for herbal medicinal products and natural health care products, herbal manufacturers strive to use the most appropriate extraction technologies to produce extracts of defined quality with the least batch-to-batch variation, which can also aid in extraction scale -up. Standardisation of extraction procedures significantly improves the final quality of the herbal drug. To gain a complete understanding of the bioactivity of crude extracts, it is necessary to optimise the extraction methodology in order to obtain the broadest range of phytochemicals. The method used to isolate active components with the highest yield and purity from natural sources is primarily determined by the nature of the compounds and raw materials that will be processed [8].

The Indian herbal drug industry is most likely the world's oldest medical care system. Herbs have a long history in ancient India, with one form of herbal healing even mentioned in the Vedas, India's ancient religious work. Ayurveda and Unani are ancient herbal healing methods that use herbs and natural products to treat various health conditions. Herbal medicine is not a new concept for western healers and medical practitioners, as plant extracts are still used in many prescribed medications today. Countries around the world now appreciate this ancient form of medicine, and Indian herbal drugs are in high demand, resulting in rapid growth and an annual growth rate of nearly 30%. In recent years, there has been a significant increase in global demand for herbal remedies, skin care products, and even herbal cosmetics. [9, 10]. Skin, as the most exposed part of our body to pathogens, requires protection against skin diseases, particularly acne-causing bacteria. Acne is the most common skin problem among teenagers today, accounting for 85% of all cases. They can persist into adulthood and primarily affect areas with the largest oil glands, such as the face and neck. Some bacteria, including *Propionibacterium acnes* and *Staphylococcus epidermidis*, live in the follicles. So, these microorganisms can be targeted for potential acne treatment [11].

This study revolves around the following objectives

- To isolate acne causing bacteria.
- Identify and characterization of acne causing bacteria.
- Preparation of plant extracts.
- Study the anti-acne activity of plant extracts against the acne causing bacteria.

2. Materials and Methods

Experimental situate

The experiment was conduct in Institute of Biotechnology MGM University, Chh. Sambhajinagar. The location provided access to necessary laboratory facilities and equipment essential for conducting microbiological studies, plant extract preparations, and bioactivity assessments.

Collection of samples

The sample of plant materials as leaves and flowers was taken from local surroundings of Chh. Sambhajinagar, Maharashtra. Three types of plant samples were collected:

a) *Rosa rubiginosa* (Rose).
b) *Hibiscus rosa – sinensis* (Hibiscus).
c) *Azadirachta indica* (Neem).
d) *Moringa oleifera* (Drumstick).

Isolation of acne causing bacteria

The bacteria were collected from samples taken from the human skin, targeting individuals with active acne lesions. The isolation was done using purple agar and mannitol salt agar, both of which are designed to select for and support the growth of specific bacteria that are known to cause acne.

- **Purple Agar:** Helps in isolating bacteria by indicating pH changes as bacterial colonies grow.
- **Mannitol Salt Agar:** Selective for bacteria such as *Staphylococcus* spp., this medium contains high salt concentrations and mannitol to differentiate between different species

Identification of acne causing bacteria

The bacteria were identified on morphological as well as biochemical methods.

a. Morphological Methods: Using microscopy, the shape, arrangement, and colour of the bacterial cells were observed. This basic step provides insight into whether the bacteria are cocci (spherical), bacilli (rod-shaped), or another form, as well as their clustering pattern (chains, clusters, etc.).

b. Biochemical Tests

- **Catalase Test:** This test detects the presence of the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen. Bacteria that possess this enzyme (like *Staphylococcus* spp.) produce visible bubbling when exposed to hydrogen peroxide.
- **Coagulase Test:** This is used to differentiate *Staphylococcus aureus* (which produces coagulase and can clot plasma) from other species of *Staphylococcus* that do not produce this enzyme.
- **Nitrate Reduction Test:** This test identifies bacteria capable of reducing nitrate to nitrite, which is a critical step in the nitrogen cycle and can be an important biochemical feature of pathogenic bacteria.

Preparation of plant extracts

The collected plant leaves were first thoroughly washed with distilled water to remove dirt, debris, and surface contaminants. Following that, the leaves were disinfected with diluted ethanol, ensuring that no surface bacteria or fungi remained.

The leaves were cleaned and then dried in a hot air oven at 65 °C for 4-5 days to remove moisture and concentrate the bioactive compounds. After drying, the leaves were ground into a fine powder with a rotatory mixer to maximize surface area for efficient extraction of bioactive compounds in subsequent procedures.

Soxhelet extraction

A fine powder of leaves was transferred in the soxhelet apparatus. A solvent, ethanol, was heated and passed through the plant material multiple times, efficiently extracting the desired compounds. The process was designed to maximize the extraction of bioactive compounds like flavonoids, alkaloids, and other secondary metabolites, which are thought to possess antimicrobial activity. Total of five cycles were to be completed of soxhelet extraction process.

Anti-acne activity

The anti-acne activity of the plant extracts was assessed using the well diffusion method, a commonly used technique for measuring antimicrobial properties. Agar plates were created by spreading the isolated acne-causing bacteria onto a nutrient agar medium. Small wells were then formed in the agar, and the prepared plant extracts were introduced into them. Following incubation, the plates were examined for zones of inhibition, which were visible as clear areas surrounding the wells. These clear zones indicated where the plant extracts effectively inhibited the growth of the acne-causing bacteria.

3. Results

Preparation of agar plates

Various agar media were prepared to facilitate the isolation of acne-causing bacteria. These media included nutrient agar, purple agar, and mannitol salt agar, which provided optimal conditions for the growth and observation of bacterial colonies. Nutrient agar served as a general growth medium, while purple agar and mannitol salt agar were selective, allowing for the specific isolation and identification of certain bacterial strains. These agar plates are illustrated in Fig. 1 (Purple Agar) and Fig 2 (Mannitol Salt Agar).

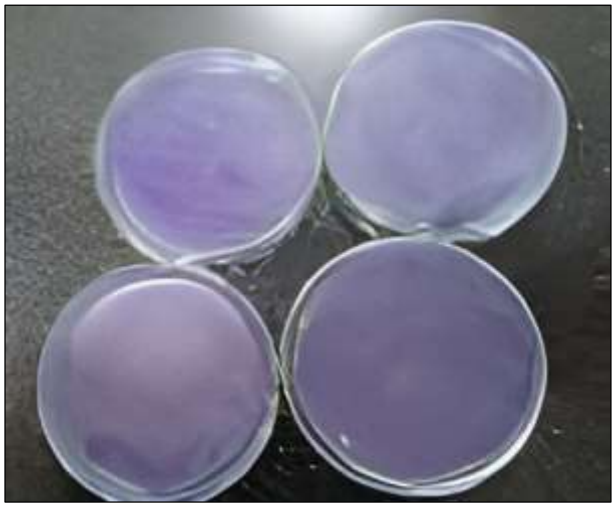

Fig 1: Purple agar

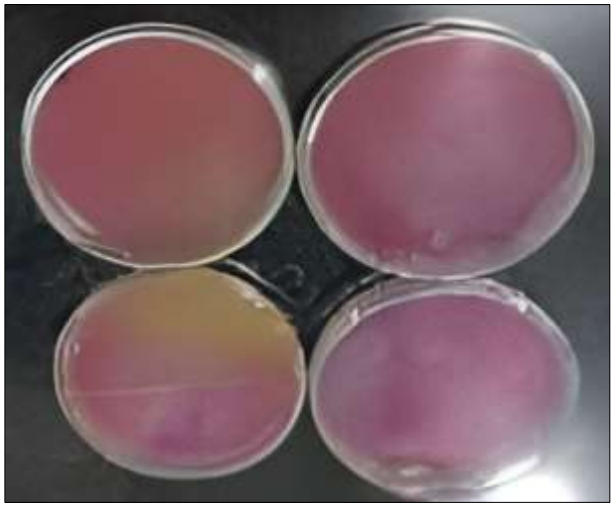

Fig 2: Mannitol salt agar

Isolation of acne causing bacteria

Acne-causing bacteria were successfully isolated from skin samples taken from various human subjects. Bacterial colonies emerged after incubation on purple agar and mannitol salt agar plates, each with its own distinct characteristics. Pink and whitish colonies were observed, with creamy appearances on both types of agar media. This demonstrated the successful isolation of bacterial strains from the skin samples, as shown in Fig 3 (Incubated Purple Agar Plates) and Fig 4 (Incubated Mannitol Salt Agar Plates).

Fig 3: Incubated purple agar plates after inoculation

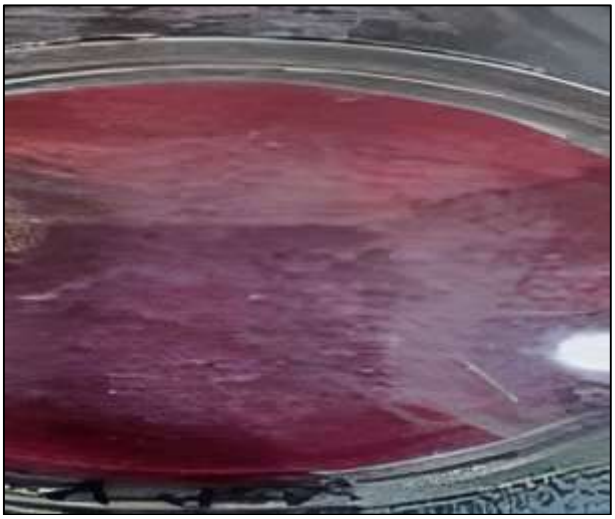

Fig 4: Incubated mannitol salt agar plates after inoculation

Identification of acne causing bacteria by different methods: The acne causing bacteria was identified on the basis of morphological methods and was studied by the biochemical test performed.

a. Morphological Observation

Morphologically, the acne causing bacteria appeared to be cocci in shape and purple in colour. As shown in the following figures.

b. Biochemical tests

The Catalase Test yielded a positive result, indicating that the bacteria produced catalase, an enzyme responsible for breaking down hydrogen peroxide into water and oxygen. The Coagulase Test was negative, suggesting that the bacteria did not produce coagulase, an enzyme typically associated with *Staphylococcus epidermidis*, which aids in clotting plasma. The Nitrate Reduction Test also returned a negative result, meaning the bacteria were not capable of reducing nitrate to nitrite. The results of all the biochemical tests can be summarized into Table 1.

Table 1: Biochemical test for Identification of acne causing bacteria

Test	Results	Comparison
Catalase Test	Positive	Positive
Coagulase Test	Negative	Negative
Nitrate Reduction Test	Negative	Positive

Interestingly, this result differed from previous studies where a positive outcome for nitrate reduction was reported, indicating a possible variation in bacterial strains or experimental conditions.

Fig 5: Cocci shaped bacteria from Sample 1

Fig 6: Cocci shaped bacteria from Sample 2

Preparation of plant extracts

The plant extracts were prepared after drying the leaves for 4-5 days, and crushing them in rotatory mixer to make a fine powder and the powder was then further taken to extraction process by soxhelet method. Soxhelet method was completed after five cycles of the extraction process and the filtrate was obtained from the plant extraction. The filtrate was 120 ml in quantity in Fig 7 and as per research paper the filtrate was obtained 100 ml in quantity.

Fig 7: Soxhelet apparatus containing the plant extract sample

Activity of plant extracts against the isolated acne causing bacteria: The anti-acne activity of the plant extracts was tested using the well diffusion method. In this method, small wells were created in agar plates containing the isolated acne-causing bacteria, and the plant extracts were introduced into these wells. After a period of incubation, zones of inhibition were observed around the wells, indicating where the bacterial growth was suppressed by the plant extracts. These zones of clearance confirmed that the plant extracts had a positive effect against the acne-causing bacteria. The size of the zones of clearance ranged from 1-2 cm, as shown in Fig 8.

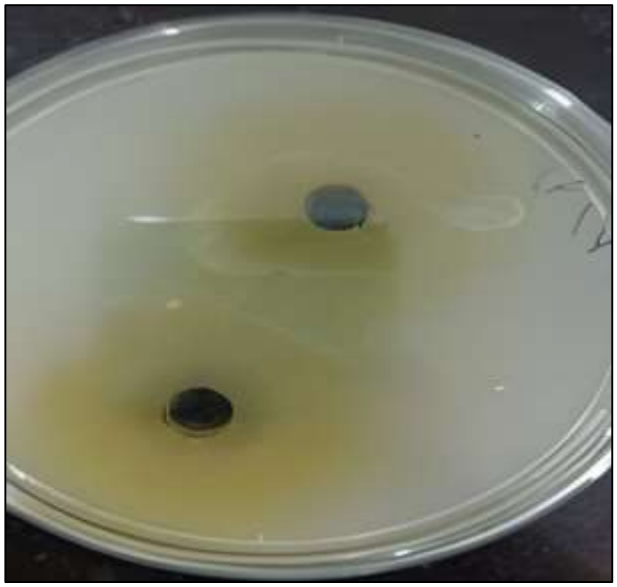

Fig 8: Well diffusion method for anti-acne activity

Fig 9: Well diffusion method for anti-acne activity

In the present study, methanolic extracts of seven plants were analyzed for antimicrobial activity against test bacteria. All extracts showed activity against *P. acnes* and the range of inhibition zone was between 1 and 8 mm. Among these, extracts of *R. officinalis* showed highest inhibition zone against *P. acnes* at 8 mm. For herbal plant extract formation against the acne causing bacteria, we used four types of leaves: Neem, rose, moringa, hibiscus. These leaves have the proper anti-bacterial activity related properties. The anti-acne project contains the isolation, identification of acne causing bacteria and the anti-activity of plant extract against the acne. As in comparison the paper compared the isolation techniques, biochemical test and anti-activity techniques. Plant extracts obtained were different in quantity in comparison. The anti-acne activity differed in zone of clearance as our project showed a less zone of clearance as in compared to the other papers. The zone of clearance appeared to be more as compared to the marketed anti-acne gel.

4. Discussion and Conclusion

The findings showed that plant extracts could help get rid of acne-causing bacteria. Once agar plates were prepared and inoculated with acne causing agents such as *Propionibacterium acnes*, and *Staphylococcus epidermidis*, clear zones of inhibition were viewed around the wells containing the plant extracts. It is evidenced by the sizes of these zones (clear areas) that show effective inhibition in bacterial growth further supporting the use of these plants, although variable depending on plant species used.

Extracts of Plants like *Azadirachta indica* (Neem) and *Moringa oleifera* (Drumstick) shown better antibacterial activity with more Zones of Inhibition. Spearmint and thyme extracts were also effective, although not to the same extent as the neem extract, which can be explained by looking at conventional themes of traditional skincare practices. Moreover, Antibacterial activity was also in *Rosa rubiginosa* (rose) and *Hibiscus rosa-sinensis* (Hibiscus) but smaller zones of inhibition for these plant extracts with compare with neem and moringa.

The activity was measured by the clear zones diameter exerted because of the extracts. Similarly, the bigger zones revealed that the extract has more potential to prevent acne-causing bacteria to proliferate. The extracts attacked the bacteria that causes acne, and thus reduced inflammation and infection of the skin. These results corroborated the hypothesis that topical application of plant extracts is a potential alternative to synthetic acne treatments.

The results also showed some difference in extract amount with some other similar studies. The filtrate from soxhelet extraction of the plant material were 120 ml (volume), while other studies reported a slightly lower quantity of around 100 ml. Additionally, the zone of clearance observed in this study ranged between 1-2 cm in length, with earlier research showing a range of 1-3 cm. To conclude, botanical extracts showed very good potentials in anti-acne activity by inhibiting the growth of acne bacteria (as mentioned above) with neem and moringa as the most active among them all. These results indicate that these plant extracts can (i) a novel natural approach in the development of anti-acne agents and (ii) be retained as alternative chemical-based regimens in acne therapy.

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