Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2019.9.14

Research Article
Antibiogram Profile and Antibacterial Efficacy of Herbal Plants on *Acinetobacter johnsonii* Isolated from Skin Sample

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Abstract

Background and Objective: Acne is a common skin problem affecting individuals of different ages; however post-adolescence acne is a common problem usually seems in females. Present study aimed to identify bacteria from acne-affected individual and to evaluate the efficacy of medicinal herbs and antibiotics. **Materials and Methods:** Samples were collected with sterile cotton swab by gently running it over the acne affected area, followed by culture and isolation of bacteria. The most prevalent bacterial isolates were identified as *Acinetobacter johnsonii*. The antibacterial efficacy of methanol, ethanol and aqueous extract for the plants was determined by agar well diffusion method. The antibiotic susceptibility test was also performed by Kirby Bauer disk diffusion method using the standard antibiotic discs. **Results:** The findings of this study revealed that *Azadirachta indica*, *Curcuma longa* and *Ocimum tenuiflorum* had a maximum to moderate inhibitory effect, while other herbal plants showed minimum to no-zone of inhibition against the *A. johnsonii*. The organic solvent extract showed better efficacy than aqueous extract. The antibiotic susceptibility tests demonstrated that *A. johnsonii* was resistant to cotrimoxazole, cefdinir but showed intermediary resistant and susceptibility to all other antibiotics. **Conclusion:** Therefore, it can be concluded that *A. johnsonii* found in the skin may be one of the common bacteria responsible for causing acne among healthy individuals. Prolonged use of topical antibiotic agents and oral drugs lead to the intermediary resistance to few drugs. Results of the present study highlights the antimicrobial activities of some herbal extracts and thus can be used traditionally to cure ache problems.

Key words: Acne, herbal plants, post-adolescence acne, *Azadirachta indica*, *Curcuma longa*, antibiotics, topical antibiotic agents, *Acinetobacter johnsonii*

Citation: Satabdi Bhattacharjee, Bibhas Deb and Soumitra Nath, 2019. Antibiogram Profile and Antibacterial Efficacy of Herbal Plants on Acinetobacter johnsonii Isolated from Skin Sample. Asian J. Plant Sci., 18: 9-14.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Skin is the most exposed part of human body. The various regions of the skin are noted to have different populations of microbial inhabitants, reflecting their different niches. Many external factors such as temperature, humidity and light exposure can alter the ecosystem of the skin, with resulting changes in microbial populations[1]. Acne is one of the most common cutaneous lesions, among which comedones, papules, cysts, pustules and abscesses which, after regression, may leave scars[2]. Increased sebum production, sloughing of keratinocytes, bacterial growth and inflammation are the primary factors involved in the formation of acne lesions[3]. Acne may occur at any age, but it is most prevalent and severe during adolescence, at the age of 14-17 years in females and 16-19 years in males[4,5].

Common therapies used for the cure of acne are topical agents, systemic, hormonal and herbal[6]. Antibiotics and retinoids, extensively used for the treatment have side effects and the micro-organisms are acquiring resistance against antibiotics which leads to treatment failure[7]. The exponential growth in demand for herbal drugs has increased due to their natural origin, less toxicity and low side-effects, for which the herbal plants are in great focus for a remedy to the skin disease and infection. Some plants are recognised to produce a diverse range of bioactive molecules such as Anthocyanin, Terpenoids, Saponins, Tannins, Flavones, Polyphenols etc. making them a source rich in producing different types of medicines[8]. The need for medications and cosmetic measures to combat skin bacteria continues to be major research in the pharmaceutical industries. Nature acts as an excellent source of salvation for human being by providing different remedies from plants, animals and other sources to cure all ailments of mankind. Plants are playing a leading role in formulating allopathic medicine, herbal medicines, homoeopathy and aromatherapy[9]. The medicinal importance of plants lies in some chemical substances which produce a distinct physiological action on the human body[10]. Present study aimed to identify bacteria from acne-affected individual and to evaluate the efficacy of medicinal plants and antibiotics against the isolate.

MATERIALS AND METHODS

Isolation and identification of bacteria: In the present study, samples were collected from a 26 year old female, reported to get frequent acne even after using Clindamycin. The subject has been using Clindamycin topical gel on the papules since 9 years. A preliminary data was recorded to understand the dietary habit, use of cosmetics and skin type of the individual. During the period of this study the subject has reported of not using any oral and topical antibiotics since three month. Samples were collected with sterile cotton swab by gently running it over the acne affected area. The samples were inoculated on Nutrient agar plates and incubated at 37°C for 24 h. Individual distinct colonies were subcultured and pure cultures were obtained. Bacteria were identified by colony morphology (shape, structure, colour, pattern, size) gram staining, biochemical tests (indole production, Methyl red, Voges Proskauer, citrate utilization and starch hydrolysis)[11]. Genomic DNA was extracted from isolated bacterial cultures[12] and PCR amplification of 16S rDNA gene was achieved by 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3')[13]. Sequencing of 16S rDNA was carried out using ABI 3730xl 96 capillary system using Big Dye Terminator v3.1 kit. The consensus sequence was generated from forward and reverse sequence data using aligner software and BLAST search was performed to find the closest homologous sequence. The 16S rRNA gene sequences were aligned by Clustal-W and a Neighbor-joining tree was constructed using Geneious R8 software package (Biomatters Ltd., Auckland, New Zealand).

Collection of plant material: Fresh leaves of *Azadirachta indica* (neem), *Psidium guajava* (guava), *Curcuma longa* (turmeric), *Ocimum tenuiflorum* (tulsi), *Santalum album* (sandalwood), *Aloe barbadensis Miller* (aloe vera), *Coriander sativum* (coriander), *Carica papaya* (papaya), *Zingiber officinale* (ginger), *Mentha sachalinensis* (mint), *Citrus limon* (lemon) and Eucalyptus were collected from different locations of Cachar district, Assam, India during the month of January-February, 2017. The vernacular name, plant species and parts used for the present study is mentioned in Table 1.

Preparation of methanol, ethanol and aqueous extract of plants: The collected plant parts were washed, shade dried and 2.5 g of plant materials were weighed and crushed in a mortar-pestle[22]. In separate conical flasks 15 mL of methanol and ethanol were added to the crushed plant material and kept on an orbital shaker at 250 rpm for 30 min to evaporate the solvent[23]. For the preparation of aqueous extract 2.5 g of plant materials were weighed and crushed in mortar-pestle using water. The methanol and ethanol extract samples were kept in water bath to concentrate the plant extract. The supernatant of methanol, ethanol and aqueous extracts were filtered and used for further tests.

Table 1: Ethnobotanical extract of herbal plants

Source	Common name	Parts used	Useful forms	References
Eucalyptus	Eucalyptus	Leaf	Oil	Athikomkulchai *et al.*[3]
Coriander sativum	Coriander	Leaf	Powder, oil	Rajeshwari and Andallu[14]
Psidium guajava	Guava	Leaf	Oil	Mailoa *et al.*[15]
Carica papaya	Papaya	Fruit, leaf	Gum, paste	Krishna *et al.*[16]
Azadirachta indica	Neem	Leaf	Oil, powder	Mehrotra and Srivastava[17]
Zingiber officinale	Ginger	Root	Paste	Ali *et al.*[18]
Mentha sachalinensis	Mint	Leaf	Paste	Kunnumakkara *et al.*[19]
Ocimum tenuiflorum	Tulsi	Leaf	Oil	Prakash and Gupta[20]
Santalum album	Sandalwood	Wood	Oil	Kapoor and lata Saraf[6]
Aloebarbadensis Miller	Aloe vera	Leaf	Gel	Mehrotra and Srivastava[17]
Curcuma longa	Turmeric	Rhizome, root	Powder	Kapoor and lata Saraf[6]
Citrus limon	Lemon	Fruit	Juice	Irshad[21]

Antibacterial activity of the plant extracts: The extracts were subjected to screening for antimicrobial activity against the isolated bacteria by agar well diffusion method. Wells were prepared in the agar plates with a syringe puncture. Mueller Hinton Agar (HiMedia) surface of each plate was streaked by a sterile cotton swab with the bacterial strain. About 50 µL of solvent was poured with micropipette onto the wells and incubated[7] at 37°C for 48 h.

Antibiotic susceptibility test: The antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method[24] using the antibiotics Ceftriaxone (30 mcg), Cefdinir (5 mcg), Cotrimoxazole (25 mcg), Ampicillin (10 mcg), Ofloxacin (5 mcg), Ciprofloxacin (5 mcg), Norfloxacin (10 mcg), Kanamycin (30 mcg), Amoxicillin-clavulanate (30 mcg), Meropenem (10 mcg), Streptomycin (10 mcg), Tetracycline (30 mcg), Amikacin (30 mcg), Gentamicin (120 mcg), Polymyxin B (300 mcg) and Clindamycin (2 mcg). The antibiotic disks were placed on the surface of Mueller Hinton Agar plates containing bacterial culture and incubated overnight for determination of zone of inhibition.

RESULTS

Identification of bacteria: The identification was based on morphology, biochemical and 16S rDNA sequencing method. The isolate was Gram-negative rod, aerobic, nonmotile showing positive catalase, nitrate reduction and citrate test. However, the isolate showed negative results in indole, MR, VP, urease, gelatin hydrolysis and oxidase test. The 16S rDNA sequence of the isolated bacteria showed 99.2% pairwise identity with the bacterium recorded in the GenBank. The phylogenetic relationship of the isolates with the database sequence is represented in Fig. 1. Based on these data, the isolate was identified *Acinetobacter johnsonii* strain SB_SK and the GenBank accession number thus assigned was MH482927.

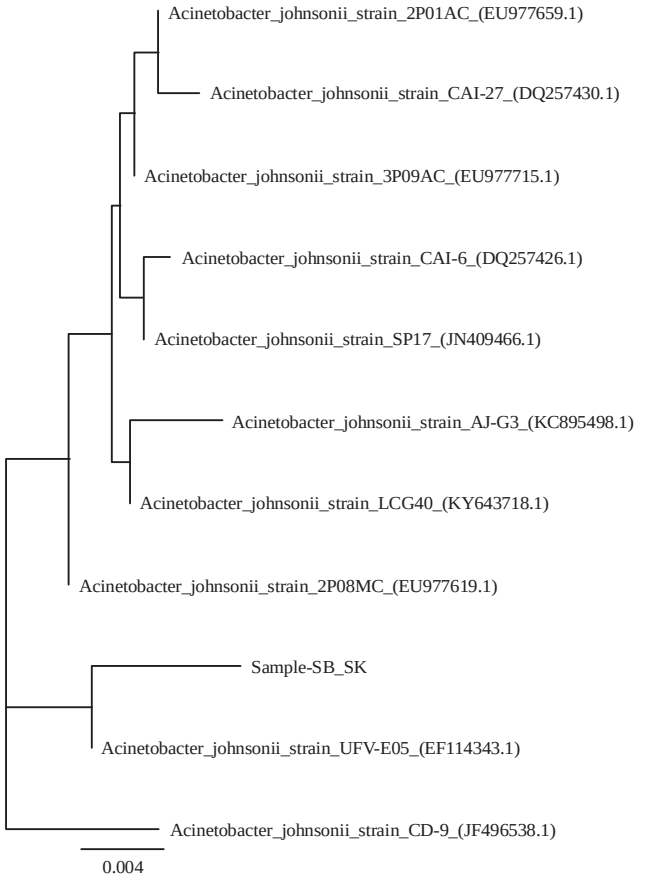

Fig. 1: Neighbor-joining tree, showing the phylogenetic relationship between studied sample SB_SK and other closely related species

Antibacterial activity of the plant extracts: The phytochemical screening against *A. johnsonii* strain SB_SK revealed that all the solvent extracts of neem was most effective in inhibiting the growth showing highest zone of inhibition 14.60±0.81, 10±0.32 and 8.20±0.20 mm, respectively. The methanol and ethanol extract of tulsi (10±0.71 and 8.80±0.37 mm) and turmeric (10±0.32 and

Table 2: Zone of inhibition (mm) of phytochemical extracts against *Acinetobacter johnsonii* strain SB_SK

Samples	Methanol	Ethanol	Aqueous
Eucalyptus	6.60±0.51	6.80±0.66	0
Coriander	6.20±0.37	0	0
Guava	0	6.20±0.20	0
Papaya	0	6.60±0.51	0
Neem	14.60±0.81	10.00±0.32	8.20±0.20
Ginger	0.50±0.28	8.00±0.55	0
Mint	5.80±0.37	0	0
Tulsi	10.00±0.71	8.80±0.37	5.00±0.50
Sandalwood	0	6.00±0.32	0
Aloe vera	0	0	0
Turmeric	10.00±0.32	9.20±0.49	8.60±0.68
Lemon	5.80±0.37	0	0

*Values are the Mean±Standard error of 5 replicates

Table 3: Antibiogram pattern of *Acinetobacter johnsonii* strain SB_SK against standard antibiotics

Antibiotics	Zone of inhibition (mm)
Ceftriaxone	14.60±0.51
Cefdinir	0
Cotrimoxazole	0
Ampicillin	15±0.32
Ofloxacin	20.20±0.37
Ciprofloxacin	20.80±0.66
Norfloxacin	14.40±0.68
Kanamycin	16.20±0.86
Amoxicillin-clavulanate	16.80±0.58
Meropenem	29.20±0.58
Streptomycin	11.60±0.68
Tetracycline	20.40±0.51
Amikacin	18.40±0.68
Gentamicin	22.20±0.37
Polymyxin B	14±0.32
Clindamycin	0

*Values are the Mean±Standard error of 5 replicates

9.20±0.49 mm) possess moderate antibacterial activity, whereas in case of aqueous extract turmeric showed higher zone than tulsi (Table 2). Intermediary resistance was seen towards methanol and ethanol extract of eucalyptus showing zone 6.60±0.51 and 6.80±0.66 mm, respectively. Moreover, the methanol extract of mint (5.80±0.37 mm), coriander (6.20±0.37 mm) and lemon (5.80±0.37 mm) showed minimum zone, but the ethanol and aqueous extract were not effective against the bacteria. The ethanol extract of papaya (6.60±0.51 mm), guava (6.20±0.20 mm), ginger (8±0.55 mm) and sandalwood (6±0.32 mm) possess intermediate effect, whereas methanol and aqueous extract did not show any zone of inhibition. Similarly, all the three extracts of aloe vera were also not effective against the isolate showing no zone of inhibition.

Antibiotic susceptibility test: The overall susceptibility pattern of *A. johnsonii* is displayed in Table 3. Significantly high resistance rates towards Cefdinir, Cotrimoxazole and Clindamycin were documented, on the other hand, high degree of sensitivity was detected towards Ofloxacin, Ciprofloxacin, Meropenem, Tetracycline and Gentamicin having zone of inhibition $\geq$20 mm. Moreover, Ceftriaxone, Ampicillin, Norfloxacin, Kanamycin, Amoxicillin-clavulanate, Streptomycin, Amikacin and Polymyxin B were intermediary resistant showing zone of inhibition $\geq$14 to $\leq$18 mm, respectively.

DISCUSSION

Micro-organisms colonising the skin have been of great importance to dermatologists and microbiologists. Changes in microbiota due to various factors, such as age, climate, hygiene or antibiotic consumption can cause dysbiosis and lead to various skin infections[25]. For acne progression, the microbial inhabitant has to get around the defence barriers of the host skin and be able to defend against them in order to survive[26]. Since long ago, Gram-positive bacteria are recognized as the major component of the skin microbiota causing acne and other skin problems[27]. However, present study reported genus *Acinetobacter* responsible for causing acne lesions, one of the most frequently encountered Gram-negative bacteria in human skin microbiota[1,27,28].

The effectiveness of some herbal plants on *A. johnsonii* strain SB_SK showed that some of the herbs possess moderate antibacterial activity and thus can be used as preventive measures to combat the infection. Neem leaves contain gedunin and nimbidol that has antimicrobial property and the leaves works wonder for various skin problems and effective in curing acne[17,23]. Turmeric and tulsi have been used traditionally since ancient times to cure skin problems and proved to be effective in reducing acne and scars[29]. Basil consists of linolenic acid which has the ability to block cyclo-oxygenase and lipoxygenase of arachidonate metabolism that are responsible for the anti-inflammatory action and hence useful to reduce the inflammation related to acne[6,30]. Kumar *et al.*[31] and Mulay *et al.*[32] reported that sandalwood oil has been used for centuries and is beneficial for reducing acne, healing eczema and dry skin whereas, Aloevera contains flavonoids, fatty acids, cholesterol, tannins, sterols, salicylic acid, organic acids, enzymes, saponins, vitamins, minerals which combat acne-inducing bacteria. In contrary, present study failed to showed the significant efficacy of aloevera towards the tested bacteria. Ethanol extracts of papaya and ginger showed minimum inhibitory effect whereas, aqueous and methanol extracts failed in inhibiting the growth of the isolates. The potent

anti-microbial activity of papaya and ginger in treating bacterial infections have been reported by other researchers[33,34]. Eucalyptus, mint, coriander and lemon showed considerable zone of inhibition against all the tested isolates. Eucalyptus oil from leaves have antiseptic property and work as an astringent to treat acne prone skin[35,36]. Mint contains menthol which provides mild anaesthetic property and the essential oil extracted from coriander leaves has antiseptic property which works as an antibacterial agent[35,36]. The phytochemical composition of the citrus juice includes alkaloids, flavonoids, steroids, terpenoids, saponins and reducing sugars, which act as anti-inflammatory and anti-microbial agents[37]. The results of the present study reveals that the methanol and ethanol extract showed better efficacy than aqueous extract.

It has been reported that overuse of antibiotics have lead to the emergence of antibiotic resistance[38,39]. Antibiotics can alter the skin microbiome and inhibit the growth of skin flora that competes against the pathogenic bacteria that may lead to the colonization of pathogens responsible for developing skin infections. In this study the bacteria showed susceptibility up to 33%, intermediary resistance 53% while conferring 13% resistant. Clindamycin, the most commonly used topical agent in the management of acne[40], failed to prevent the progression of acne in the studied individual. The reason may be due to the overuse of Clindamycin from many years resulted in development of resistance among the pathogenic bacteria. *Acinetobacter johnsonii* have been commonly found in skin but very few studies have been reported so far of this bacteria causing acne. The observed intermediary resistance pattern revealed that, in later years these strain may develop resistance towards antibiotics that are prescribed commonly. Therefore, the use of antibiotics should be limited and to be used only if it is too necessary. Further, knowing the actual cause of acne and the causative organisms may result in reduction of treatment failure and worsening the infection.

CONCLUSION

This study concluded that *Acineobacter johnsonii* found in skin may be responsible for causing acne among individuals. Without knowing proper cause of infection, prolonged use of antibiotics should be restricted to reduce the chances of bacteria becoming resistant. This study demonstrated that some of the herbal extract possess good antibacterial activity and can be used as preventive measures to combat acne. Thus, identification of noble plants, their pharmacological evaluations and toxicological studies may lead to the formulation of new herbal based drug that can be used as an alternative to synthetic medicine for acne.

SIGNIFICANCE STATEMENT

The study demonstrated the efficacy of the medicinal plants, as alternatives in the treatment of bacterial infections so as to overcome or minimize the resistance on commercial antibiotic drugs.

ACKNOWLEDGMENT

The authors sincerely acknowledge Institutional Biotech Hub and Bioinformatics Centre (funded by DBT, New Delhi) of Gurucharan College, Silchar, Assam to carry out the research work.

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