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Antimicrobial activity of *Acanthus ilicifolius*: Skin infection pathogens

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PEER REVIEW

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Comments

Skin infection is a worldwide health problem. So this is a good study in which the authors work in the pathogens which are antibiotic resistance. The final results are interesting and the extracts are having the potential compounds for its activity.
(Details on Page 182)

ABSTRACT

Objective: To investigate the antimicrobial activity of *Acanthus ilicifolius* against the skin infecting bacterial and fungal pathogens. Through the literature survey, the mangrove plant *Acanthus ilicifolius* was used in skin infection diseases and have potential anti-inflammatory activity. **Methods:** Antimicrobial activity of the leaf extracts was tested using agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were carried out. **Results:** Among the different extracts, chloroform extract showed maximum activity against the bacterial pathogens methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Candida albicans* and *Trichophyton rubrum*. Methanol and acetone extracts showed maximum activity against *Staphylococcus epidermis* and *Lactobacillus plantarum* respectively. Chloroform extracts showed the lowest MIC (0.5 mg/mL) and MBC (2 mg/mL) values against the skin pathogens compared with other extracts. Phytochemical screening revealed the presence of resins, steroids, tannins, glycosides, sugars, carbohydrates, saponins, sterols, terpenoids, phenol, alkaloids, cardiac glycosides and catechol. **Conclusions:** Further, the separation of potential compounds from the crude extracts will be useful for control the skin infection pathogens.

KEYWORDS

Mangrove, *Acanthus ilicifolius*, Skin infection, Methicillin-resistant *Staphylococcus aureus*, Antimicrobial activity, Phytochemical

1. Introduction

The human skin is a vast protective system enclosing the entire body system. Skin plays an important role in avoiding skin infections. But there are several pathogens that can impact on the human skin. Bacterial and fungal species can cause severe infections in skin, especially the skin and soft tissue infections caused by antibiotic resistant *Staphylococcus* and *Streptococcus* species[1]. Approximately 5% of the general population develops a skin infection each year, leading to a significant number of outpatient visits to the primary care physician[2]. Generally to treat the skin diseases antibiotics are used, but these antibiotics give adverse effects on patients. Bacterial and fungal pathogens are highly resistance to the antibiotics. Therefore, an alternative source is needed to treat the skin diseases. Mangroves have long been a source of astonishment for the layman and of interest for scientist. Mangroves are biochemically unique, producing a wide array of novel natural products. Mangrove ecosystem was a large ecosystem in the tropical region, especially in Indo-Pacific region. The chemical constituents (salts, organic acids, carbohydrates, hydrocarbons, benzoquinone, naphthofurans, sesquiterpenes, triterpenes, alkaloids, flavonoids, polymers, sulfur derivatives and tannins) isolated from mangrove plants have potential application in medicine[3-6]. Substances in mangroves have long been used in folk medicine to treat diseases. *Acanthus illicifolius* (*A. illicifolius*) is a shrub present along the Indian coastal regions. Traditionally, this plant was used for paralysis, asthma, skin disorders, boils and wounds[7,8]. Therefore, the present study was carried out to evaluate the antimicrobial activity of *A. ilicifolius* against skin infection pathogens.

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2. Materials and methods

2.1. Collection of plant material and extraction

The leaves of *A. ilicifolius* were collected from Muthupet mangrove forest, Tamilnadu. Collected leaves were cut in to small pieces and shade dried at room temperature for 10 d. The extraction of leaves was carried out using different solvents (aqueous, ethanol, methanol, acetone and chloroform) in Soxhlet apparatus. For each extraction, 100 g of leaves material was used. The samples were subjected to below the boiling point of the each solvent for 6-8 h in order to extract the compounds into the solvent. Finally, the extraction was concentrated using vacuum distillation.

2.2. Phytochemical analysis

The leaves extracts were undergone for the phytochemical screening for the secondary metabolites using the method of Trease and Evans[9].

2.3. Antimicrobial assay

Methicillin resistance *Staphylococcus aureus* (MRSA), *Staphylococcus epidermis* (*S. epidermis*), *Streptococcus pyogenes* (*S. pyogenes*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Lactobacillus plantarum* (*L. plantarum*), *Candida albicans* (*C. albicans*) and *Trichophyton rubrum* (*T. rubrum*) were used in the present study. Antimicrobial activity of the extracts was tested by agar well diffusion method using Mueller Hinton agar and Sabouraud dextrose agar (SDA) medium. Each experiment was performed in triplicate and the average value of inhibition and standard deviation were calculated. Gentamicin (1 mg/100 μL) and clotrimazole (1 mg/ 100 μL) were used as a positive control.

2.4. Minimum inhibitory concentration (MIC)

The MIC of extracts was carried out by using the method of Akinpelu and Kolawole[10]. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism. Two-fold dilutions of the crude extract was prepared and 2 mL aliquots of different concentrations of the solution were added to 18 mL of pre-sterilized molten nutrient agar and SDA for bacteria and fungi respectively at 40 °C to give final concentration regimes of 0.5 and 10 mg/mL. The medium was then poured into sterile Petri dishes and allowed to set. After that, 18-hour old bacterial and fungal cultures were streaked in the plates and the plates were later incubated at 37 °C for 24 h for bacteria and at 25 °C for up to 72 h for fungal respectively. After the incubation period, plates were examined for the presence or absence of growth.

2.5. Minimum bactericidal concentration (MBC)

The MBC of the extracts was determined by the method of Spencer and Spencer[11]. Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar plates and SDA plates, and later incubated at 37 °C for 48 h and 25 °C for 72 h for bacteria and fungi respectively. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

3. Results

3.1. Phytochemical analysis

Phytochemical screening of *A. ilicifolius* leaf extracts revealed the presence of proteins, resins, steroids, tannins, glycosides, sugars, carbohydrates, saponins, sterols, terpenoids, phenol, alkaloids, cardiac glycosides and catechol.

3.2. Antimicrobial assay

All the five extracts showed different degrees of activities against the bacterial and fungal pathogens. The antimicrobial activity of chloroform extract was compared favorably to that of antibiotics. In this study, chloroform extract showed maximum inhibition against the MRSA with mean zone of inhibition of 17.3±1.69, followed by acetone, methanol, ethanol and aqueous extracts. Aqueous extract showed minimum activity against the MRSA (9.800±1.027) (Figure 1). Chloroform extract of *A. ilicifolius* showed maximum activity against the bacterial as well as fungal pathogens (*S. pyogenes, P. aeruginosa, C. albicans,* and *T. rubrum*). Methanol extract showed maximum activity against *S. epidermis* (18.066±0.410) followed by chloroform, acetone, ethanol and aqueous extracts. *L. plantaram* (19.330±1.027) was highly sensitive to acetone extract compared with other extracts. Compared with all the extracts, aqueous extract showed minimum activity against the tested bacterial and fungal species.

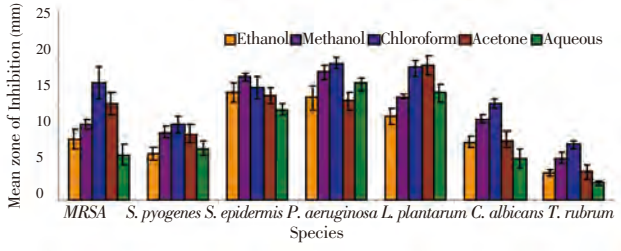

Figure 1. Mean zone of inhibition produced by the extracts of *A. ilicifolius*.

3.3. The MIC and MBC

The antimicrobial activity of the extracts potency was quantitatively assessed by the MIC and MBC values of the extracts. The MBC and MIC values were between 0.5 to 3 mg/mL and 2 mg to 4 mg/mL respectively for chloroform extract of *A. ilicifolius* which were found better than the MIC and MBC values of other extracts (Figures 2 and 3). The highest MIC and MBC value were recorded between 6 mg/mL and 9 mg/mL for the aqueous extract. The lowest MIC and MBC was 1 mg/mL and 3 mg/mL and the value ranged up to 4 mg/mL

and 6 mg/mL respectively for methanol extract. For acetone extract, the MIC and MBC values ranged between 1 to 6 mg/mL.

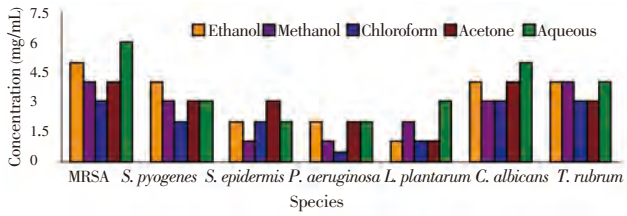

Figure 2. MIC values of *A. ilicifolius* leaf extracts.

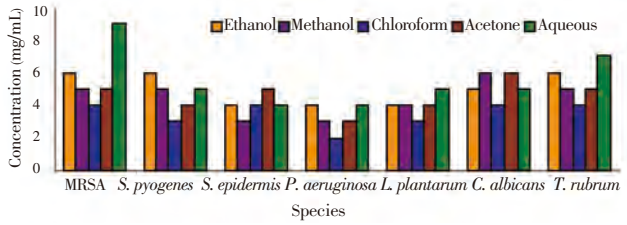

Figure 3. MBC values of *A. ilicifolius* leaf extract.

4. Discussion

A. ilicifolius was a good remedy for skin diseases because there was a report already documented that this plant have analgesic and anti-inflammatory property. *A. ilicifolius* reported in literature as rich sources of long chain alcohols, terpenes, steroids and triterpenoidal saponins[12]. GC-MS analysis of methanolic extract of *A. ilicifolius* evidenced that this plant *A. ilicifolius* contains saponin, tannins, cardiac glycosides, terpenoids, flavanoids, anthraquionones and alkaloids[13]. Compared with all the extracts, chloroform extract shows maximum activity and aqueous extract shows minimum activity against the skin infection pathogens. The previous study revealed that the alcoholic and chloroform extract of leaves exhibited strong inhibitory action against *B. subtilis*, *S. aureus*, *C. albicans*, *A. fumigatus* and *A. niger* and moderate inhibitory action against *P. aeruginosa* and *P. vulgaris*[14,15]. Khajure and Rathod reported that *A. ilicifolius* chloroform extract of leaves showed maximum activity against *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *C. albicans*, *B. subtilis*, *A. fumigatus* and *A. niger* than methanol and hexane extracts of leaves and roots[16]. Chloroform extract containing protein from *A. ilicifolius* had great potential activity against *Vibrio cholerae* and also showed great effect of hemolytic activity in chicken blood[17]. Chandrasekaran *et al*. reported that the mangroves plants have potential activity against the MRSA, methanol extract of *A. ilicifolius* showed maximum activity (13 mm) than the aqueous extract[18]. In previous study, Naidu *et al*. reported that the methanol extract of *A. ilicifolius* showed maximum antimicrobial activity against both the bacterial and fungal phytopathogens than the *Rhizophora mucronata* plant extracts[19].

The antimicrobial activity of the crude extracts due to the secondary metabolites or compounds present in this mangrove plant. Lignan glucosides were isolated from the plant *A. ilicifolius*; generally these lignan glucosides have potential antimicrobial activity[3]. Tannin is a polymeric phenolic substances and these tannins from the mangrove plant sonneratia alba are showed higher antimicrobial activity. The saponins which are called as steroid saponins and triterpenoidal saponins exhibit divergent antimicrobial activity against the human pathogens[20]. This type of triterpenoidal saponins was isolated from the roots of *A. ilicifolius*[20]. Flavonoids and phenolic compounds were isolated from the mangrove plant *A. ilicifolius* showed high antimicrobial activity and antioxidant activity in rats[21]. 2-Benzoxazoline is a synthetic compound form the *A. ilicifolius* extensively used as central nervous system depressant, also exhibiting antipyretic, hypnotic and muscle relaxant activity has been isolated from this plant. It is also reported to be resistant to fungi and also used as anti-viral agent[5,12,22]. The ribose derivative of this compound is an active anticancer and antiviral agent[20,23]. Benzoxazinium derivatives, *i.e.*, compounds with the 1, 4-benzoxazin-3-one skeleton, exhibit phytotoxic, antimicrobial, antifeedant, antifungal and insecticidal properties activities[24]. The aqueous leaf extract of *A. ilicifolius* prevents DNA alterations and provide chemopreventive efficacy on transplantable Ehrlich ascites carcinoma (EAC)-bearing murine model[25].

Based on the result of the present investigation, the mangrove plant *A. ilicifolius* played a major role in the defense and potential source of metabolites against the skin infection diseases. Further work is progressing towards the phytochemical characterization of the extracts and the identification of responsible bioactive compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Human skin is immunological organ system encloses the human body. Skin plays an important role in avoiding and protection from out side pathogens infection. Some of the bacterial and fungal species can cause the skin infections, which are persistent and prefer damp places of human body. Nowdays, the bacterial and fungal pathogens are resistance to the antibiotics. The treatment for the pathogens is complicated one. Due to this reason, identifying noval compounds for the treatment of skin infection is more important.

Research frontiers

The studies were carried out to investigate the antimicrobial activity of the mangrove plant *A. ilicifolius* against the skin infection pathogens. Among different extracts, chloroform extract showed maximum antimicrobial activity.

Related reports

Compared with all the extracts, chloroform extract from *A. ilicifolius* showed maximum activity and aqueous extract showed minimum antimicrobial activity against skin infection pathogens. Bose and Bose (2008), Kumar *et al*. (2011) reported that alcoholic and chloroform extract showed strong inhibitory action against *B. subtilis*, *S. aureus, C. albicans, A. fumigatus* and *A. niger* and moderate inhibitory action against *P. aeruginosa* and *P. vulgaris*.

Innovations & breakthroughs

The study showed that the mangrove plant have some phytochemicals, which are responsible for the activity against the skin infection pathogen particularly MRSA and fungal pathogens.

Applications

The present investigation revealed the plant *A. ilicifolius* plays a major role in the defense mechanism against the skin infection diseases. This work is the baseline for carry out the further studies, like the isolation and characterization of compounds which is responsible for the activity against the pathogens.

Peer review

Skin infection is a worldwide health problem. So this is a good study in which the authors work in the pathogens which are antibiotic resistance. The final results are interesting and the extracts are having the potential compounds for its activity.

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