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EVALUATION OF ANTIMICROBIAL ACTIVITY AND PRELIMINARY PHYTOCHEMISTRY OF BEGONIA ALBOCOCCINEA HOOK.

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Abstract
The present investigation was aimed to screen the antimicrobial potential of *Begonia albococcinea* Hook. against various human bacterial and fungal pathogens. Antimicrobial efficacy was performed by disc diffusion method against the bacterial pathogens viz., *Escherichia coli, Klebsiella pneumonia*e, *Pseudomonas aeruginosa, Shigella flexneri, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis.*, and *Staphylococcus aureus* incubated for 24 hrs at 37°C as well as the isolates of fungal pathogens *Candida albicans*, *Candida tropicalis, Aspergillus flavus* and *Aspergillus niger*. All the extracts of the plant viz., acetone, methanol, benzene and chloroform investigated exhibited varying degree of inhibitory effect against the selected human pathogens. The investigation reveals that, *B. albococcinea* forms a potentially good source of antimicrobial agent and demonstrates the importance of such plant in medicinal systems for curing various ailments.
Keywords : Antibacterial; Antifungal; *Begonia albococcinea*; Disc diffusion; Plant extracts.

Introduction

Plant kingdom holds many species of plants containing substances of medicinal value which have yet to be discovered. Large number of plants are being constantly screened for their possible antimicrobial activity. Among the estimated 250,000 - 500, 000 plant species, only a small percentage has been investigated phyto-chemically and the fraction submitted to biological or pharmacological screening is even smaller (Ahmad *et* Beg, 2001; Werner *et al*., 1999).

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Antimicrobial drug resistance is a global problem today as the resistant microorganisms have emerged and spread throughout the world because of their genetic plasticity (Kunin, 1993). Over use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistance strain of several group of micro-organisms. For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents (Valarmathy *et al*., 2010). The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed. Literature reports and ethnobotanical records suggest that plants are the sleeping giant of pharmaceutical industry. They may provide natural source of antimicrobial drug that will or provide novel or lead compounds that may be employed in controlling some infection.

The plant of interest *Begonia* of Begoniaceae has about 900 species found in tropical and subtropical regions of the world wherein 45 species are present in India (Santapau and Henry, 1993). *B. albococcinea* is seen distributed in the evergreen forests of Southern Western Ghats, especially towards the southernmost parts of Kerala and Tamil Nadu. It is found at forest margins, usually growing as a lithophyte in areas exposed to direct sunlight. Begoniaceae encompasses more medicinally important species, which help to treat various diseases. Similar to other Begonia species, *B. albococcinea* possess medicinal potential and is effectively utilized for various medicinal properties. In traditional medicinal system of Siddha *B. albococcinea* is used for increasing the vitality and strengthening the body. The dried powder of the plant is given two times with milk to strengthen the body (Mudaliyar, 1988).In this study, we aimed to detect a possible inhibitory effect of different extracts of *B. albococcinea* on the growth of various selected human pathogens tested by using agar disc diffusion method.

Materials and Methods

Collection of plant material:

The whole plants of *Begonia albococcinea* were collected from Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Kerala. The collected specimen was identified with the help of local flora. The plant material were washed thoroughly with normal tap water, followed by sterile distilled water. These samples were then shade dried separately in room condition, powdered and stored for further use.

Sample extraction:

500g of powdered plant samples were weighed and taken separately. These samples were extracted with acetone, methanol, benzene and chloroform individually using Soxhlet's apparatus. The organic extracts obtained were evaporated to dryness by kept open in room temperature. Equal volume of DMSO was added to each extract and was later subjected to microbial bioassays.

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Preliminary phytochemical analysis:

Preliminary phytochemical screening involving chemical tests to determine the presence of Alkaloids, Phenols, Proteins, Tannins, Saponins, Flavanoids, Steroids, Glycosides and Carbohydrates were carried out using the methods described by Odebiyi and Sofowora (1999).

Microbial Bioassays:

Bacterial Isolates and Bioassay:

The extracts of acetone, methanol, benzene and chloroform were screened against eight bacterial strains. The test organisms, *Escherichia coli*, *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis.*, and *Staphylococcus aureus* were obtained from the "Scudder diagnostic Centre", Nagercoil, Kanyakumari.

Preparation of Inoculum:

Stock cultures were maintained at 4ºC on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Mueller-Hinton broth (MHB) for bacteria and were incubated without agitation for 24 hrs at 37 ºC.

Antimicrobial Suceptibility Test:

The disc diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring 15ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The crude extracts (20µl) were loaded on 4mm sterile disc. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37° C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimetre. These studies were performed in duplicates.

Fungal Isolates and Bioassay:

Isolates of fungal pathogens *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus* and *Aspergillus niger* were obtained from "Scudder diagnostic Centre", Nagercoil, Kanyakumari. The cultures were maintained on potato dextrose agar until further study. Aliquotes of potato dextrose agar medium was poured in sterile petridishes. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The crude extract (20µl) was loaded on 4mm sterile disc. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were incubated for 72 hours at 28± 2° C. Observations were made on the growth of fungal mycelium as influenced by the plant extracts. Based on the growth rate of fungi in response to plant extract, the rate of inhibition was measured in millimeter.

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Results

The ethanolic extract prepared from the plant sample of *B.albococcinea* was subjected to preliminary phytochemical analysis. The results exposed the existence of valuable phytochemicals in the species. Quantification of phytochemicals revealed that alkaloids form the major constituent with 148.18 ± 0.08 µg/mL concentration followed by saponins with 124.37 ± 0.04 µg/mL. Phenol shown 103.17 ± 0.08 µg/mL concentration which is followed by Tannins and Flavanoids with 92.66 ± 0.04 and 85.23 ± 0.05 µg/mL concentration respectively. Glycosides, Steroids, Carbohydrate and Protein were quantified with 58.93 ± 0.03, 55.2 ± 0.05, 45.59 ± 0.53, 31.2 ± 0.04 µg/mL concentrations respectively.

The stock crude extracts prepared from the whole plant parts of *B. albococcinea* by using acetone, methanol, benzene and chloroform were subjected to antimicrobial activity against *Escherichia coli*, *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus* and the results were recorded in Table 2 and Fig. 1. The result obtained indicates that all the extracts tested were efficient against the clinical bacterial isolates. The maximum zone of inhibition obtained was with benzene extract (23mm) against *S.flexneri*. This was observed as a best activity when compared with the positive control (Amikacin) tested. This result was followed by the best response with methanol extract (20mm) against *B.cereus*. Comparatively least zone of inhibition was experienced with methanol extract against *S.aureus*.

The results of antifungal assay tested against *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus* and *Aspergillus niger* using the crude extracts obtained from the whole plant parts of *B. floccifera* by using acetone, methanol, benzene and chloroform were recorded in Table 3 and Fig 1. The results obtained indicate that, the chloroform extract was found to be an effective one against *Aspergillus flavus* with a maximum zone formation of 17mm. Similarly, benzene and chloroform extract also exhibited efficient activity against *Aspergillus flavus* and *A.niger* respectively.

Discussion

In the present investigation, *in vitro* antibacterial and antifungal efficacy of the crude extracts of *B. albococcinea* was quantitatively assessed on the basis of zone of inhibition. The results indicates that the plant exhibits a varying degree of inhibitory effect against the selected human pathogens The presence of antimicrobial and antifungal activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids and saponins (Ray et al., 1989). Recently, a number of plants have been reported for antimicrobial properties across the world (Scazzocchio *et al*., 2001; Olowosulu *et* Ibrahim, 2006). The results obtained indicated that solvent derived crude extracts of *B. albococcinea* posses a multipotent efficacy to act against several groups of pathogens. Previous reports in the species specified the presence of varied phytochemicals viz., phenol, flavonoid, saponnin, triterpenoidal saponogenin and carbohydrate, which is ultimately the reason for the presence of such activity in this genus. (Jeeva *et* Marimuthu, 2012). Similar effective response was also reported with

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the hexane and methanolic extracts of *Begonia picta* which was due to the presence of alkaloid, tannin, saponin, terpenoid, glycoside, carbohydrate, flavonoid, phenol, cardiac glycoside and anthraquinone glycosides (Shrestha *et al*., 2016). Tannins are known to possess general antimicrobial and antioxidant activities (Rievere, 2009). Reports show that tannins have potential value as cytotoxic and antineoplastic agents (Aguinaldo, 2005). Other compounds like saponins also have anti-fungal properties (Mohanta, 2007). Phenolic phytochemicals too have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammtory effects (Scalbert, 2005).

From the results it is concluded that, the activity exhibited by *B. albococcinea* against human bacterial and fungal pathogens was significant in benzene and chloroform extracts when compared with the other solvents used. Based on the observations, it is authenticated that most of the pathogens tested was found to be susceptible for the extract tested. Also, the results of the present study supplement the folkloric usage of the studied plant which possess several known and unknown bioactive compounds with antibacterial and antifungal properties. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various infectious diseases. Further sophisticated investigation on this lesser-known plant *B. albococcinea* is absolutely necessary for the successful utility of the species to mankind.

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Table 1. Preliminary phytochemical profile of *B.albococcinea*

Constituents	Concentration µg/mL
Flavanoids	85.23 ± 0.05
Phenol	103.17 ± 0.08
Alkaloids	148.18 ± 0.08
Tannins	92.66 ± 0.04
Saponins	124.37 ± 0.04
Steroids	55.2 ± 0.05
Glycosides	58.93 ± 0.03
Carbohydrate	45.59 ± 0.53
Protein	31.2 ± 0.04

Table 2. Antibacterial assay of *Begonia albococcinea* using different solvents

Sl. No.	Microorganism	Diameter of zone of inhibition					
		Acetone	Methanol	Benzene	Chloroform	Negative Control	Positive Control
1.	*Escherichia coli*	18mm	14mm	17mm	19mm	-	21mm
2.	*Klebsiella pneumoniae*	20mm	15mm	17mm	14mm	-	23mm
3.	*Pseudomonas aeruginosa*	13mm	14mm	14mm	16mm	-	18mm
4.	*Shigella flexneri*	13mm	16mm	23mm	17mm	-	22mm
5.	*Bacillus cereus*	15mm	20mm	12mm	18mm	-	18mm
6.	*Bacillus subtilis*	15mm	18mm	16mm	14mm	-	21mm
7.	*Enterococcus faecalis*	15mm	15mm	14mm	17mm	-	16mm
8.	*Staphylococcus aureus*	13mm	7mm	15mm	15mm	-	16mm

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Table 3. Antifungal assay of *Begonia floccifera* using different solvents

Sl. No.	Microorganism	Diameter of zone of inhibition					
		Acetone	Methanol	Benzene	Chloroform	Negative Control	Positive Control
1.	*Candida albicans*	10mm	11mm	11mm	15mm	-	12mm
2.	*Candida tropicalis*	15mm	13mm	11mm	14mm	-	15mm
3.	*Aspergillus flaves*	13mm	11mm	16mm	17mm	-	21mm
4.	*Aspergillus niger*	15mm	12mm	12mm	16mm	-	17mm

Figure 1 (a) Habit of *B. albococcinea*
Antimicrobial activity of acetone, methanol, benzene and chloroform extracts of *B. albococcinea* on: **(b)** *Candida tropicalis* **(c)** *Shigella flexneri* & **(d)** *Bacillus cereus*