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ANTIMICROBIAL ACTIVITY OF *ALTERNANTHERA BETTZICKIANA* (REGEL) *G. NICHOLSON* AND ITS PHYTOCHEMICAL CONTENTS

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ABSTRACT: The present investigation evaluates preliminary phytochemical screening and antimicrobial activity of aerial parts of *Alternanthera bettzickiana* (Regel) *G. nicholson* an edible green medicinal herb tested against various Gram positive, Gram negative bacteria and fungi. The plants were evaluated against two Gram positive bacterial species such as *Staphylococcus aureus* and *Enterococcus faecalis*; two Gram negative bacterial species such as *Escherichia coli* and *Klebsiella pneumoniae*; and fungi such as *Aspergillus niger*, *Epidermophyton floccosum* and *Candida albicans* were tested. Preliminary phytochemical screening was performed with ethanol, petroleum ether, acetone, chloroform and aqueous extracts of *Alternanthera bettzickiana* (Regel) *G. nicholson* that showed the alkaloids, carbohydrates, saponins, phenols, flavonoids, diterpenes, tannin, terpenoids, steroid, oxalate, anthocyanin, leucoanthocyanin, Xanthoprotein, coumarin and glycosides in the ethanol extract. The ethanol extract showed higher phytoconstituents when compared to the other extracts. The extracts were compared with standards like Amoxicillin and Ketoconazole for antibacterial and antifungal activity respectively. The extracts showed greatest antimicrobial activity as measured from the zone of inhibition and results were comparable with that of standard drugs against the organism tested. In conclusion, plant extract of *A. bettzickiana* showed remarkable antimicrobial activity due to the phytochemicals present in them.

INTRODUCTION: Medicinal plants are the nature's gift to human beings to make disease free healthy life. Medicinal plants are the main source of drugs for traditional medicines, food supplements, neutraceuticals and pharmaceuticals etc [1, 2]. Plants have a great potential for producing new drugs of great benefit to mankind.

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There are many approaches to the search for new biologically active principles in higher plants [3]. The use of crude extracts of plants parts and phytochemicals of known antimicrobial properties can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases [4]. In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavonoids [5-7]. Plants used for traditional medicine contain a wide range of

substances that can be used to treat chronic as well as communicable diseases [8]. Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases. Modern technique and pharmacological screening procedure results in new plant drugs usually finding their way into modern medicine.

The most important of these phytoconstituents of plants are alkaloids, flavonoids, tannins and phenolic compounds. In recent years, there has been resurgence of interest in the discovery of new compounds from plants with the aim of finding novel treatment against a variety of illness. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicine [9]. Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [10]. The phytotherapeutic can provide many modern drug developments that provide many invaluable drugs from traditional medicinal plants.

Alternanthera bettzickiana (Regel) *G. nicholson* (Amaranthaceae), native to South America, commonly known in English as Baptist plant, border plant, red calico plant, is an erect and bushy or prostrate perennial herb with food and ornamental values. The leaves are green or reddish and sometimes variegated in red and green. The shoots and tender leaves are commonly consumed like vegetable or spinach and in soups, either cooked alone or mixed with other vegetables such as cowpeas or amaranth, with added coconut milk and served with a staple food like rice or ugali [11].

The whole plant is reported to be useful in purifying and nourishing blood and is claimed to be a soft laxative, a galactagogue and an antipyretic, in addition to its wound healing property [12]. It is commonly used as an ornamental edging plant. It is popular in China where it is cultivated in nearly all the large cities. This genus consists of approximately 80 species and is widespread genus with cosmopolitan distribution [13].

MATERIALS AND METHODS:

Collection and Authentication of Plant Materials: The plants were collected from Pechiparai of Kanyakumari District, Tamil Nadu. The collected plants were identified in the Department of Botany, Queen Mary's College and confirmed by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC) Chennai.

Processing of Medicinal Plants: Fresh plants were washed thoroughly three to four times with running tap water then finally with sterile water followed by shade drying at room temperature for 20-30 days and powdered by using an electric blender and stored in airtight container.

Preparation of Extract: Each sample of 10 g was taken and soaked for 24 hr in 30 ml of ethanol, petroleum ether, acetone, chloroform and aqueous separately. The extracts were filtered using Whatman filter paper No. 1, evaporated to dryness and re-dissolved in DMSO ((Dimethyl Sulphoxide). The extracts were preserved in airtight container and kept at 4-5 °C for further use.

Phytochemical Screening: The plants have primary and secondary metabolites which can be used for medicinal and other uses. There is a need to analyze the plants for such phytochemical screening. Phytochemical screening was carried out by using the standard protocols as described by Harborne [14]. The alkaloids are determined by Wagner's Test [15]; carbohydrates by Benedict's Test; saponin by Foam Test; phenol by Ferric Chloride Test; flavonoids by Lead Acetate Test; diterpenes by Copper Acetate Test, terpenoids by Salkowski's Test [16], aminoacids by Ninhydrin Test; proteins by Biuret Test, Tannins by Ferric Chloride Test); and oxalate by Ethanoic acid glacial [17]. Further detection of steroids was carried out by Harborne; detection of coumarin was done by Mace method [18] and quinone by conc. H_2SO_4. Xanthoproteins by conc. HNO_3 and NH_3 Test [19], cardiac glycosides by Kellerkillani synthesis [20], anthocyanin by HCl and NH_3 [21], leucoanthocyanin by isoamyl alcohol; carboxylic acid by effervescence test and glycosides by Modified Borntrager's Test [22].

Antimicrobial Assay:

Test Organisms: The bacterial cultures used in the study were *Staphylococcus aureus* and *Enterococcus faecalis* (positive), *Klebsiella pneumoniae* and *Escherichia coli* (negative) and fungal cultures were *Candida albicans, Epidermophyton floccosum* and *Aspergillus niger* were procured from Department of Microbiology, Presidency College, Chennai.

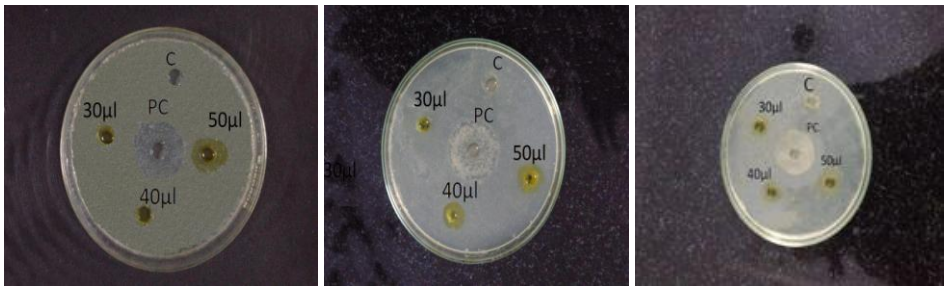
Culture Medium: Mueller Hinton Agar (MHA) medium was used to study the antibacterial activity and Potato Dextrose Agar (PDA) was used to study the antifungal activity**.**

Antibacterial Activity Assay: Antibacterial activity of solvent extracts was determined by well diffusion method on MHA medium. The bacterial culture to be tested was inoculated as lawn culture using sterile swab. Wells were made on to the agar plate using sterile cork borer (6 mm diameter). The extracts were applied to different wells in serially increasing volumes 30µl, 40µL and 50µL. DMSO (Dimethyl Sulphoxide) served as negative control and Amoxicillin (10µg) was used as the reference. The plates were labelled, covered and incubated at 37 °C for 24 hr.

Antifungal Activity Assay: The fungal mycelial suspension was spread on PDA plates and 6 mm diameter wells were made with cork borer. The extracts were applied to different wells in serially increasing volumes of 30µl, 40µL and 50µL. DMSO served as negative control whereas Ketoconazole (10µg) was used as the reference. The plates were labelled, covered and incubated at 28 °C for 48-72 hr. The activity of the extracts was determined by measuring the diameter of zone of inhibition.

A B C D

FIG. 1: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *A. BETTZICKIANA*



E F G

FIG. 2: ANTIFUNGAL ACTIVITY OF ETHANOL EXTRACT OF *A. BETTZICKIANA*

A: *Escherichia coli*; B: *Enterococcus faecalis*; C: *Klebsiella pneumonia*; D. *Staphylococcus aureus*; (C – Control, PC - Amoxicillin); E: *Epidermophyton floccosum*; F: *Candida albicans*; G: *Aspergillus niger* (C – Control, PC- Ketoconazole)

RESULTS AND DISCUSSION: The phytochemical analysis was carried out from the dried plant powder using ethanol, petroleum ether, acetone, chloroform and aqueous, which showed the presence of many bioactive compounds in the plant. Twenty different phytochemical tests were carried out for the five different extracts. Ethanol extracts showed the presence of fifteen major phytoconstituents. Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites. Secondary metabolites are frequently accumulated by plants in smaller quantities [23]. In this present study, the preliminary phytochemical analysis showed

primary and secondary metabolites such as alkaloids, carbohydrates, saponins, phenols, flavonoids, diterpenes, tannin, terpenoids, steroid, oxalate, anthocyanin, leuco-anthocyanin, Xanthoprotein, coumarin and glycosides in the ethanol extract (**Table 1**).

Tannins are phenolic compounds and plant phenolics are a major group of compounds that act as antioxidants or free radical scavengers [24] (Hollman., 2001). Alkaloids isolated from plants are commonly found to have antimicrobial properties [25]. Phytochemicals possess specific physical, chemical and biological activities that make them useful as drugs [26]. The aqueous extract showed the presence of thirteen phytoconstituents; acetone extract showed the presence of eight phytoconstituents; petroleum ether showed the presence of six phytoconstituents; and the chloroform extract showed the presence of five phytoconstituents.

Since these phytoconsti-tuents were found to be present in the extracts, it might be responsible for the potent antimicrobial capacity of *A. bettzickiana*. The presence of phytoconstituents makes the plant parts useful for treating different ailments.

TABLE 1: PHYTOCHEMICAL COMPOUNDS OF *A. BETTZICKIANA*

Name of the test	Ethanol	Petroleum ether	Acetone	Chloroform	Aqueous
Alkaloids	+	-	-	-	+
Carbohydrates	+	-	-	-	-
Saponins	+	+	-	-	+
Phenols	+	+	-	-	+
Flavonoids	+	-	+	+	-
Aminoacids	-	-	-	-	+
Diterpenes	+	-	+	-	+
Tannins	+	+	-	-	+
Terpenoides	+	-	+	-	+
Protein	-	-	-	-	+
Steroid	+	-	+	+	-
Oxalate	+	+	+	+	-
Cardiac glycosides	-	+	-	+	-
Anthocyanin	+	-	+	-	+
Leucoanthocyanin	+	+	-	-	+
Carboxylic acid	-	-	-	-	-
Xanthoprotein	+	-	+	-	-
Coumarin	+	-	-	-	+
Quinones	-	-	+	-	+
Glycosides	+	-	-	+	+

Note: "+" indicates presence and "–" indicates absence of phytoconstituents.

TABLE 2: ANTIMICROBIAL ACTIVITY OF THE ETHANOL EXTRACT OF *A. BETTZICKIANA*

Test organisms	Zone of inhibition (mm)			
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µl	40 µl	50 µl
Staphylococcus aureus	29	11	12	13
Enterococcus faecalis	27	10	11	13
Klebsiella pneumoniae	25	11	13	15
Escherichia coli	26	13	15	17
Aspergillus niger	23	10	11	13
Candida albicans	21	9	10	11
Epidermophyton floccosum	19	-	-	9

TABLE 3: ANTIMICROBIAL ACTIVITY OF THE PETROLEUM ETHER EXTRACT OF *A. BETTZICKIANA*

Test organisms	Zone of inhibition (mm)			
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µl	40 µl	50 µl
Staphylococcus aureus	29	11	13	14
Enterococcus faecalis	27	10	11	12
Klebsiella pneumoniae	25	9	10	11

Escherichia coli	26	11	13	15
Aspergillus niger	23	-	9	11
Candida albicans	21	-	-	9
Epidermophyton floccosum	19	-	-	9

TABLE 4: ANTIMICROBIAL ACTIVITY OF THE ACETONE EXTRACT OF *A. BETTZICKIANA*

Test organisms	Zone of inhibition (mm)			
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µl	40 µl	50 µl
Staphylococcus aureus	29	11	12	13
Enterococcus faecalis	27	10	11	12
Klebsiella pneumoniae	25	9	10	11
Escherichia coli	26	11	13	14
Aspergillus niger	23	-	9	11
Candida albicans	21	-	9	10
Epidermophyton floccosum	19	-	-	9

TABLE 5: ANTIMICROBIAL ACTIVITY OF THE CHLOROFORM EXTRACT OF *A. BETTZICKIANA*

Test organisms	Zone of inhibition in mm			
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µl	40 µl	50 µl
Staphylococcus aureus	29	9	11	12
Enterococcus faecalis	27	9	10	11
Klebsiella pneumoniae	25	-	11	11
Escherichia coli	26	10	11	13
Aspergillus niger	23	-	-	9
Candida albicans	21	-	-	9
Epidermophyton floccosum	19	-	-	-

TABLE 6: ANTIMICROBIAL ACTIVITY OF THE AQUEOUS EXTRACT OF *A. BETTZICKIANA*

Test organisms	Zone of inhibition (mm)			
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µl	40 µl	50 µl
Staphylococcus aureus	29	9	11	13
Enterococcus faecalis	27	-	9	11
Klebsiella pneumoniae	25	9	11	13
Escherichia coli	26	11	13	14
Aspergillus niger	23	9	11	13
Candida albicans	21	-	9	11
Epidermophyton floccosum	19	-	-	9

The ethanol, petroleum ether, acetone, chloroform, and aqueous extracts of *Alternanthera bettzickiana* were tested for growth inhibiting activity against four bacterial strains in three varying concentration. The results (**Table 2, 3, 4, 5** and **6**) show that the plant possess appreciable amount of antibacterial activity against the strains tested. The ethanol extract of the plant was found to be more active than the rest of the extracts. The zone of inhibition was compared to that of the standard. The activity was found to be more against the Gram negative organism (*Escherichia coli*) than the Gram positive organism (*Staphylococcus aureus)*. *A. bettzickiana* exhibited a moderate activity against *Staphylococcus aureus, Klebsiella pneumoniae, and Enterococcus faecalis*. The results of antifungal activity showed highest activity against *Aspergillus niger,* moderate activity against *Candida albicans* and very least activity against *Epidermophyton floccosum.*

The chloroform extract did not show any activity in *Epidermophyton floccosum*. Vidhya *et al*., 2015 reported that the plant leaf showed very poor antibacterial activity. There is no previous report for antifungal activity of this plant. The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment [28].

Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism and many components of plant products have been shown to be specially targeted against resistant pathogenic bacteria [29, 30].

CONCLUSION: The present study suggests that the crude ethanol extract of *A. bettzickiana* have great potential as antimicrobial agent against tested pathogenic bacteria and fungi. The present study reveals the presence of different phytochemical contents in the five different extracts of *A. bettzickiana*. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

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