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Original Article

PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL STUDIES ON LEAF EXTRACTS OF *ALTERNANTHERA BRASILIANA* (L.) KUNTZE

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ABSTRACT

Objectives: The present study was to study the phytochemical and antibacterial properties of *Alternanthera brasiliana* leaf extracts using three different solvents (hexane, chloroform and methanol) against human pathogenic microorganism.

Methods: Preliminary phytochemical screening of leaf extracts (in different solvents) of *A. brasiliana* were qualitatively tested using standard procedures. Thin Layer Chromatography (TLC) was performed to detect the phytochemical compounds present in *A. brasiliana*. Antibacterial activity of crude extract was determined by disc diffusion method. Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were also performed for the hexane extract.

Results: The preliminary qualitative phytochemical screening of hexane extracts revealed the presence of alkaloids, phenols, flavonoids, saponins, tannins, phytosterols and carbohydrates. In chloroform and methanolic extracts, all the tested phytochemicals were present except flavanoids and saponins. Alkaloids were not present in the chloroform extract. TLC analysis revealed the presence of seven compounds with R_f values ranging from 0.173 to 0.933. Promising antibacterial activity was observed in the hexane extract with an inhibition zone of 22 mm against *E. coli.* Chloroform and methanolic extracts showed negligible antibacterial activities.

Conclusion: The results revealed that the hexane leaf extract of *A. brasiliana* alone exhibited potential antibacterial activity specifically against pathogenic *E. coli* (MTCC 729).

Keywords: Antibacterial activity, *Alternanthera brasiliana, E.coli,* Phytochemical, Flavanoid.

INTRODUCTION

Emergence of pathogenic microorganisms that are resistant or multiresistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs [1]. In addition, high cost and adverse side effects commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, immunosuppression etc. are the major burning global issues in treating infectious diseases [2, 3]. According to the World Health Organization (WHO), about 80 % of the people in developing countries rely primarily on medicinal plants for their primary health care [4].

Although pharmacological industries had produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at an alarming rate and multi drug resistant microorganisms have exacerbated the situation [5]. In the present scenario, there is an urgent and continuous need to explore and develop cheaper and effective plant based drugs with better bioactive potential with lesser side effects.

Alternanthera brasiliana (L.) Kuntze, Amaranthaceae is an evergreen, perennial herb, native to tropical and sub-tropical regions of Australia and South America. It is widely used by rural communities as medicinal agent to cure different disease, such as inflammation, wound healing, analgesic, antitumor activity, immunomodulator and lymphocyte proliferation. With the influence of different kind of lights, *A. brasiliana* produce compounds with possible analgesic action [6] and were used against cough and diarrhoea in Brazilian popular medicine [7]. The genus *Alternanthera* was reported to contain amino acids, flavone glycosides, steroids, saponins [8], lipids [9] and vitamins [10]. The genus finds potential claims in the viral diseases [11] as an immunomodulator [12], protection against cancer [13], antimalarial [14] and in the treatment of diarrhoea [15]. The inflorescence of *A. brasiliana* is used for the treatment of colds and grippes, headaches and as an expectorant [16]. The leaves are used as antipyretic agent and the roots are used against diarrhoea [16]. The aim of this study was to investigate the presence of phytochemicals and to evaluate the antibacterial activity of *A. brasiliana* leaf extracts against five pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant material

Fresh and healthy leaves of *A. brasiliana* were collected from Cherthala, Alappuzha district, Kerala state, India. The plant material was identified by Dr. Shaji P.K., Scientist, Environmental Resources Research Centre (ERRC), P.B. No. 1230, P.O. Peroorkada, Thiruvananthapuram, Kerala state, India. The plant materials were initially cleaned, dried under shade and then pulverized to coarse powder in an electric grinder. The powder was then stored in airtight bottles for further studies.

Chemicals

All the solvents used for the extraction process were of analytical grade and procured from SD Fine Chemicals, Mumbai, India. Ciprofloxacin (10 µg/ml) discs, Amphotericin B (10 µg/ml), Mueller Hinton agar and Nutrient agar medium were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India and TLC Silica gel 60 F_{254} plates were procured from Merck, Germany.

Preparation of extracts

The shade dried leaf powder was subjected to cold extraction using three solvents in the increasing order of polarity (hexane, chloroform and methanol). The final filtrate of each of the extract was concentrated using a rotary vacuum evaporator (IKA, RV 10 digital, Germany). The extracts collected were evaporated to dryness and stored in a vial for further analysis.

Qualitative phytochemical screening

Preliminary phytochemical screening for the various secondary metabolites in the different solvent leaf extracts of *A. brasiliana* was qualitatively tested using standard procedure of Brain and Turner [17].

Phytocompound analysis using TLC

Thin layer chromatography of *A. brasiliana* hexane leaf extract was performed on a pre activated Silica gel GF_{254} plate. 10 µl (25 mg/ml) of sample was applied at the center of the plate about 1 cm from the bottom. They were allowed to develop in a TLC chamber at 25°C which was already saturated with standardized mobile phase (toluene: ethyl acetate, 8.5:1.5 v/v) in an ascending manner. After development, solvent front was marked using pencil and then the plates were allowed to dry by using air dryer. The developed plates after saturation in iodine chamber were analyzed to calculate R_f value using the formula:

$$Rf\ value = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Bacterial strains and growth medium

Gram negative bacteria such as *E. coli* (MTCC 729), *Pseudomonas aeruginosa* (MTCC 4676), *Klebsiella pneumonia* (MTCC 432) and a gram positive bacteria *Bacillus subtilis* (MTCC 619) were used for this study and these microorganisms were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. Bacterial strains were grown and maintained on Muller-Hinton Agar medium. The cultures were maintained at 4ºC and sub cultured frequently in respective agar slants.

Antibacterial assay

Disc diffusion method

The antibacterial sensitivity assay was carried out by disc diffusion method [18]. The different solvent extracts of *A. brasiliana* were tested against the above mentioned bacterial strains. The test bacterial cultures were evenly spread over Mueller Hinton agar plates using a sterile cotton swab. The sterile discs (6 mm in diameter) were impregnated with 100 µl of different plant solvent extract solution (100 mg/ml) and placed in the inoculated agar. The plates were then incubated at 37°C for 24 to 48 hours. After incubation, the results were observed and the zones of inhibition thus developed were measured with the scale to the nearest in mm. Ciprofloxacin served as a positive control and respective solvents served as a negative control. The experiment was done in triplicates and the mean values were presented.

Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC was determined by macro dilution method using serially diluted plant extracts according to the NCCLS protocol [19]. The plant extract showed promising inhibition zones in crude antimicrobial assays were used for MIC. The extracts were dissolved in dimethy sulfoxide (DMSO) and subsequent two-fold serial dilutions were performed in the culture medium to get concentrations from 10 mg/ml to 0.019 mg/ml (10 tube dilutions). The microbial suspension of 50 µl was added to the broth after 24 hours of incubation, MIC was recorded as the lowest concentration that limited the turbidity of the broth to <0.05 at the absorbance of 600 nm. All tubes from the MIC experiments that showed no visible turbidity were serially diluted and spread onto agar plates for viable cell counting. The plates were incubated for 24 to 48 hours. MBC was then recorded as the lowest concentration that killed at least 99.99% of the initial number of bacteria.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary qualitative phytochemical screening of hexane, chloroform and methanol extracts indicated the presence of alkaloids, phenolic groups, flavonoids, saponins, tannins phytosterols and carbohydrates (Table 1). Phytochemicals or secondary metabolites usually occur in complex mixtures that differ among plant organs and stages of development [20, 21]. Knowledge of the phytochemical constituents present in *A. brasiliana* will be very useful for the maximum exploitation of this plant in medicine. The hexane leaf extract showed the presence of phytochemical substances such as, phenols, flavanoids, alkaloids, tannins, phytosterols, saponins, carbohydrate. Phenolic compounds detected in this plant might be responsible for the antibacterial property. Phenols are the important plant derived bioactive compounds with antioxidant [22] and antibacterial activities. Flavanoids and saponins were not found in chloroform and methanolic extract (Table 1).

Table 1: Qualitative phytochemical analysis of *A. brasiliana* leaf extract

	Alternanthera brasiliana leaf extracts		
Phytoconstituent	**Hexane**	**Chloroform**	**Methanol**
Phenols	++	+	+
Flavanoids	+	–	–
Alkaloids	+	–	+
Tannins	+	+	+
Phytosterols	+	++	+
Saponins	++	–	–
Carbohydrate	++	++	++

"Presence (+), absence (-) and abundant (++)"

Phytocompound analysis using TLC

Phytocompound analysis using TLC was found to be effective in solvent system toluene: ethyl acetate (8.5:1.5 v/v). Accordingly about seven compounds were observed on the TLC plate. The R_f value obtained by different compound separated in TLC analysis are given in Table 2.

Table 2: R_f value of separated compounds on TLC

Distance traveled by the solvent front (cm)	Distance traveled by the compound (cm)	R_f value
7.5	1.3	0.173
7.5	1.6	0.213
7.5	2.2	0.293
7.5	2.6	0.346
7.5	4.4	0.586
7.5	5.5	0.733
7.5	7	0.933

Disc diffusion assay

Antibacterial activity of *A. brasiliana* leaf extracts tested against selected microorganisms is recorded in Table 3. Among the three leaf extracts tested for bioactivity, hexane extract only showed significant antibacterial activity (22 mm) against *E. coli.* Whereas the control drug ciprofloxacin showed an inhibition zone of 32±1.15 against *E. coli.* The activities of other solvent extracts are given in Table 3. According to the size of zone of inhibition, Bauer classified the antimicrobial activity into three i.e. resistant (less than 7), intermediate (7-9 mm), sensitive (10 mm or more) [18, 23]. The chloroform extracts showed poor antibacterial activity because its zone of inhibition was less than 7.

MIC and MBC

The most sensitive organism in this investigation was *E. coli*, against which most of the plant extracts showed inhibition zones and the best activity observed in hexane leaf extract, with an inhibition zone of 22 mm. So, MIC and MBC value were evaluated for the hexane extract. The lowest MIC value of 0.625 mg/ml was observed against *E. coli* indicating a significant antibacterial property and the MBC value were determined as 1.25 mg/ml which was found to be higher when compared to MIC value (Table 4). This means that a greater concentration of the drug is necessary to inhibit the bacterial culture.

In the present study, only hexane extract of *A. brasiliana* leaf showed higher antibacterial activity particularly against *E. coli* supporting its ethnomedicinal record as antibiotics, while other solvent extract was not as effective against bacteria which shows that the bioactive

compounds were extracted in the non polar solvent rather than in polar solvents.

Moreover, only limited studies exist showing the antibacterial efficacy of *A. brasiliana.*

Table 3: Antibacterial disc diffusion assay of *A. brasiliana* leaf extract

Microorganisms	Zone of inhibition (in mm)			
	Hexane	Chloroform	Methanol	Ciprofloxacin (5µg/ml)
B. subtilis	3.33 ± 0.47	4 ± 0.81	4.3 ± 0.4	26 ± 0
E. coli	22.33 ± 1.24	6.6 ± 0.94	7 ± 1.63	32 ± 1.15
P. aeruginosa	4.3 ± 1.24	6 ± 0.81	8.6 ± 0.47	30 ± 0
K. pneumoniae	4.3 ± 1.24	5 ± 0	7.6 ± 0.4	28 ± 1.52

Table 4: MIC and MBC values of hexane extract

Organism	MIC	MBC
E. coli	0.625± 0 mg/ml	1.25± 0 mg/ml

Therefore the present study is an additional report for the existing literature to show the antibacterial activity of this plant especially against *E. coli*, which is the most opportunistic pathogen showed increased resistance towards many antibiotics.

CONCLUSION

It was concluded that the hexane extract of *A. brasiliana* showed potential antibacterial activity specifically against pathogenic strain of *E. coli* (MTCC 729). The phytochemical studies of hexane extract indicated the presence of all the seven phytochemical constituents tested, which might be responsible for its bioactivity. Moreover our literature survey concluded that only limited studies were carried out by researchers regarding the biological activity of this plant. Thus, this study gives an insight and preliminary information to take this plant as a source to find out antibacterial agent particularly against multi drug resistant *E. coli*.

CONFLICTS OF INTEREST

The authors have none to declare.

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