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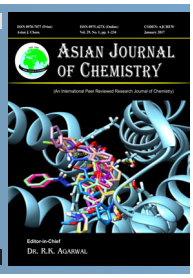

Phytochemicals of *Anthurium andraeanum* and its Pharmacological Applications

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The present study involves phytochemical screening and analysis of medicinal plant. Plant parts of *Anthurium andraeanum* (root, stem, leaf, flower and seed) were subjected to screen phytochemical constituents and the results revealed that this plant possess the phytochemicals such as phlobatannins, steroids, phenols and saponins. The plant parts were extracted individually and subjected for antimicrobial activities. Result revealed that this sample was effective against various pathogenic bacteria. The bioactive compound was purified by using silica gel chromatography and thin layer chromatography. The high performance liquid chromatography purified sample was found to be effective against the tested cell lines (Hep G2, A549 and MCF7). Gas chromatography and mass spectrophotometer analysis revealed the presence of anticancer compounds such as 2,3-dihydro-benzofuran and stigmasta-5,20(22)-dien-3-ol.

Keywords: *Anthurium andraeanum*, Antibacterial, Anticancer.

INTRODUCTION

The emergences of multidrug-resistant bacterial pathogens have threatened the antibacterial therapy [1]. These resistant bacterial infections often lead to longer length of stays in hospitals, higher cost of treatment and great care and may lead to death [1,2]. The most common drug-resistant bacteria include, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (ESBL-KP), β-lactamase-producing *Escherichia coli*, vancomycin resistant *Enterococcus* [3-5]. The Infectious Diseases Society has listed these pathogens as notorious, hence effective antimicrobials are urgently required against these pathogens [4]. Effective drugs against these pathogens are highly limited and doctors are forced to use the drugs such as colistin, which are associated with numerous side effects to the patients [1]. Therefore, it is an urgent need to search the alternatives that can be effective in the treatment of these highly pathogenic bacterial infections. The applications of plant extracts for antimicrobial therapy have been registered to be promising remedies in olden days in Ayurveda, Chinese medicine, Unani and Arabic medicine [4]. The number of drug resistant microbial pathogens and the appearance of strains with less susceptibility to common antibiotics are increasing in recent years. The increase of these pathogens attributed to indiscriminate use of broad-spectrum antibiotics, intravenous catheters, organ transplantation, immuno-suppressive agent and ongoing epidemics of HIV infection [6,7]. In addition, countries like India, synthetic drugs are not only very expensive and inadequate for the treatment of diseases but also have side effects and adulterations. Therefore, there is a need to search new molecules from the plants against these drug resistant pathogens strategies to control microbial infections.

Approximately 60 % of drugs presently used for the treatment of cancer have been identified from natural products [8] and the plants have been the important source. These include Taxus diterpenes, vinca alkaloids, Podophyllum lignans and Camptotheca alkaloids. Presently, more than 16 plant-derived compounds were tested in clinical trials. Of which, 13 drugs are in phase I and II clinical trial and three compounds were in phase III clinical trial [9]. Among the anticancer compounds, flavopiridol, which was isolated from the Indian tree *Dysoxylum binectariferum* and meisoindigo, which was isolated from the Chinese plant *Indigofera tinctoria*, were shown to exhibit potent anticancer effects with lesser toxic effect than that of conventional drugs [9]. Medicinal plants are the very common alternative for the treatment of cancer treatment around the world [10]. The main objective of this study is to investigate the phyto-chemical compounds of *Anthurium andraeanum* and its pharmacological applications.

EXPERIMENTAL

Anthurium andraeanum is one of flowering plants from the Araceae family. This species is native to Ecuador and Colombia. This plant was collected at Kanyakumari, Tamilnadu, India. The whole plant was transported to the laboratory and the different parts of this plant (root, stem, leaf, flower and seed) were dried (air drying) for several days and powdered using a mixer grinder. The powdered sample was stored in an airtight container.

Preparation of plant extracts: The powdered *Anthurium andraeanum* plant parts were extracted with aqueous, acetone, dimethyl sulfoxide, chloroform and ethanol.

Aqueous extraction (Maceration method): 5 g of powdered material of *Anthurium andraeanum* (root, stem, leaf, flower and seed) were taken for maceration with 100 mL of double distilled water for 2 h on a rotary shaker. Further, the extract was filtered by a muslin cloth and Whatman filter paper (No. 1) and then concentrated by evaporation on water bath. The extract was further dried and used for further studies.

Soxhlet extraction: The plant parts (5 g each) was extracted for 6-8 h with 500 mL of organic solvent (acetone, dimethyl sulfoxide, chloroform and ethanol) by hot continous percolation method using a Soxhlet apparatus. After extraction, the solvents were concentrated using a vacuum evaporator and remaining water was further removed by evaporation on a hot water bath to yield crude extract.

Qualitative analysis for phytochemical components from *Anthurium andraeanum*: The extracted plant parts were subjected to phytochemical screening. 1 g of the ethanolic extract again reconstituted in 20 mL of ethanol and was subjected to phytochemical screening for the evaluation of various groups of compounds. All plant parts were extracted after shade drying and powdering. The screening methods for the identification of phytochemicals were according to the method of Guevarra *et al.* [11]. Each extract was filtered and this process was repeated until all soluble compounds had been successfully extracted. Then, the extract from each part of the plant evaporated to in vacuum drier and further dried using hot-air oven. Then a part of the residue was used to screen for plant constituents. The phytochemical constituents such as carbohydrate, protein, amino-acids, vitamin-C, chloride, tannin, alkaloids, flavanoids, phlobatannin, steroids, phenolic compounds and saponin were screened.

Test microorganisms: The bacterial strains such as *Bacillus cereus* (MTCC 1133)*, Escherichia coli* (MTCC584), *Klebsiella pneumonia* (MTCC 9544), *Staphylococcus aureus* (MTCC 9886), *Aspergillus niger (MTCC*1785), *Aspergillus fumigatus (MTCC*-3376) and *Penicillium* sp. (*MTCC* 5108) were obtained from Microbial Type Culture Collection and Gene Bank (*MTCC*), Institute of Microbial Technology, Chandigarh, India. These organisms were maintained on Mueller-Hinton Agar medium.

Bacterial inoculums: Stock culture was maintained at 4 °C on nutrient agar slants and potato dextrose agar slants. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to the nutrient broth medium (g/L) (peptic digest of animal tissue, 5.0; beef extract, 1.5; yeast extract and 1.5; sodium chloride, 5.0). The inoculated bacterial cultures were incubated at 37 °C for 18 h and were used as the inoculum.

Inoculum preparation for fungal strains: The selected fungi were grown at 30 ± 2 °C on potato dextrose agar. The spores of the fungus were further collected from cultures on agar plates after 7 days of incubation. The concentration of sporangial suspension was estimated using a cell counting chamber and the spore concentration was adjusted to 2×10^6 spores/mL [12].

Culture media: Mueller-Hinton Agar (Hi-Media, India) was prepared according to the instruction given by the manufacturers, autoclaved and dispensed at 20 mL per plate in Petri dishes. These petri plates were incubated overnight to ensure the sterility of the plates before use.

Antimicrobial bioassay: For fungal screening, suspension of fungal isolates were made in sterile saline (0.8 % Sodium chloride) and adjusted to (10^8 cfu/mL (Macfarland standard) [13]. The bacterial inoculum was diluted appropriately and used for antibacterial screening assay. 0.1 mL of the root extract was dropped into appropriately labeled well. The inoculated plates were incubated for 24 h for antibacterial bioassay and 72 h for antifungal bioassay. Antimicrobial activity was then determined by measuring the diameter of inhibitory zones (mm) produced after incubation around the colony.

Isolation of novel compound using column chromatography: Silica gel was used as stationary phase for column chromatography. The highly active fraction of the root extract was further fractionated using silica gel chromatography employing a step elution (hexane 100 %, hexane 75 %: ethyl acetate 25 %, hexane 50 %: ethyl acetate 50 % hexane 25 %: ethyl acetate 75 %). The fractions were collected on the basis of colour changes. One active fraction was selected, dried and frozen at -80 °C.

Thin layer chromatography: The purified compound with the concentration of 1 mg/mL was spotted on the thin layer chromatography plate (Merck, Bangalore, India) and dried. Chloroform and methanol (19:1) mixture was poured into the TLC chamber to a level few centimetres above the chamber bottom. The TLC plate was immersed such that sample spots are well above the level of mobile phase but not immersed in to the solvent. Then the plates were removed and allowed to dry. The sample spots were visualized and the bands were scraped off individually from the TLC plates, transferred into separate vials and 1 mL acetonitrile was added to the sample. The sample was sonicated for 0.5 h and left at room temperature overnight. The active fraction was further subjected to high performance liquid chromatography (HPLC) analysis.

Reverse phase HPLC purification of anticancer molecule: Isolation of the molecule was performed using a reverse phase HPLC system (Cyberlab, USA). C-18 column was applied using acetonitrile: water (65:35) as mobile phase. The solvents and samples applied in this system were sonicated for 0.5 h prior to HPLC. 10 µL of the TLC purified sample was injected in HPLC column and eluted with an isocratic elution continued for 20 min (flow rate of 1 mL/min at 215 nm).

GC-MS analysis: GC-MS for ethanolic extract of root of *Anthurium andraeanu* was carried out at Department of Biotechnology, Indian Institute of Science, Chennai, India.

Determination of *in vitro* antiproliferative effect of purified compound from the root extract on cultured Hep G2 cells, A549 and MCF-7: Lung carcinoma cell lines (A549), breast cancer cell lines (MCF-7) and hepatic carcinoma cell lines (Hepg2) were purchased from NCCS, Pune, India and were maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10 % FBS (Invitrogen) and grown at 37 °C in 5 % CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, GERMANY). The cells were trypsinized (500 µL of 0.025 % Trypsin in PBS/0.5 mM EDTA solution (Himedia) for 2 min and passaged to T flasks in complete aseptic conditions. The purified compound was added to grown cells at a final concentration of 6.25, 12.5, 25, 50 and 100 µg/mL from a stock of 1 mg/mL and incubated for 24 h. The percentage difference in viability was determined by standard MTT assay after 24 h of incubation.

MTT assay: MTT Assay is a colorimetric assay, which measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by the action of an enzyme mitochondrial succinate dehydrogenase. MTT enters into the mitochondria of the cells where it is reduced to an insoluble, coloured formazan product. The tested cells are then solubilized using dimethyl sulfoxide and released formazan was measured at 540 nm. The cells were further washed with 1X PBS and then added MTT solution (30 µL) to the culture. It was then incubated at 37 °C for 3 h and MTT was removed by washing with 1x PBS. About 200 µL of DMSO was added to the culture and incubated at room temperature for 30 min and the lysis activity was determined by forming colour. The solution was transferred to centrifuge tubes and centrifuged at 14,000 rpm for 10 min. The optical density was read at 540 nm using DMSO as reagent blank in a micro plate reader (ELISASCAN, ERBA).

RESULTS AND DISCUSSION

Phytochemical screening: In the present study the phytochemical components were screened from the plant *Anthurium andraeanum*. The phytochemicals such as carbohydrates, proteins, tannins, alkaloids, flavanoids, phlobatannins, steroids, phenols and saponins are observed in this plant (Table-1). Phytochemicals are the active constituents of medicinal plants. The phytochemical constituents showed that this plant is rich of phlobatannins, flavonoids, phenoilics and alkaloids. These phytochemicals have antioxidant, analgesic, anti-inflammatory and anti-cancer properties [14].

Antimicrobial activity: The MTCC strains used were *Bacillus subtilis* (MTCC 1133), *Escherichia coli* (MTCC584), *Klebsiella pneumonia* (MTCC 9544), *Staphylococcus aureus* (MTCC 9886), *Aspergillus Niger (MTCC*1785), *Aspergillus fumigatus (MTCC*-3376) and *Penicillium* sp. (*MTCC* 5108). The extracts from stem, leaf, flower and root extract showed antimicrobial activity against atleast one of the selected test isolates. The extract of root presented high activities and they were able to inhibit the growth of tested bacteria. These extract showed highest activity against antibiotic resistant *S. aureus* (Table-2). The extracts also showed antimicrobial activity against the tested *B. cereus*, *E. coli* and *A. fumigatus*. Each of the extract used in the present study displayed potent anti-

TABLE-1
QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL COMPOUND FROM THE ROOT EXTRACT OF *Anthurium andraeanum*

	Solvent				
	Aqueous	DMSO	Ethanol	Acetone	$CHCl_3$
Carbohydrates	+	+	+	+	+
Protein	+	+	+	+	+
Amino acid	+	+	+	+	+
Vitamin C	–	–	+	–	–
Chloride	+	+	–	–	–
Tannins	–	–	+	+	+
Alkaloids	+	+	+	–	–
Flavanoids	+	+	+	+	–
Phlobatannins	–	–	+	+	–
Steroids	+	+	+	–	+
Phenol	–	–	+	–	+
Saponins	–	–	+	+	+

TABLE-2
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *Anthurium andraeanum*

Test organism	Solvent				
	Aqueous	DMSO	Acetone	$CHCl_3$	Ethanol
			Stem		
E. coli	–	8	8	13	13
K. pneumonia	–	–	–	11	12
S. aureus	–	–	–	–	–
B. cereus	–	–	–	11	10
A. fumigatus	–	–	–	–	9
A. niger	16	–	–	22	10
Penicillium sp.	–	–	–	16	11
			Leaf		
E. coli	–	–	13	11	12
K. pneumonia	–	–	11	10	11
S. aureus	–	9	11	11	12
B. cereus	–	–	11	12	13
A. fumigatus	–	–	–	–	–
A. niger	–	–	–	–	–
Penicillium sp.	–	–	–	–	–
			Flower		
E. coli	–	11	–	14	12
K. pneumonia	–	8	–	11	11
S. aureus	–	–	–	–	–
B. cereus	–	–	10	–	11
A. fumigatus	28	17	17	25	20
A. niger	–	–	–	–	15
Penicillium sp.	–	–	–	–	–
			Root		
E. coli		–	10	–	10
K. pneumonia	11	10	10	–	–
S. aureus	–	10	12	16	17
B. cereus	10	10	–	–	12
A. fumigatus	–	14	0	12	15
A. niger	–	10	13	13	12
Penicillium sp.	–	13	10	–	10
			Seed		
E. coli	–	–	14	10	11
K. pneumonia	10	11	11	11	10
S. aureus	10	10	–	12	11
B. cereus	–	10	22	10	12
A. fumigatus	–	–	10	10	13
A. niger	–	10	19	11	11
Penicillium sp.	–	10	–	10	11

microbial activity against the selected bacterial strains tested. But, the differences were observed between the antibacterial activities of the plant parts. These differences are mainly due to the fact that the chemical composition of these extracts as the secondary metabolites of plant parts has many effects including antifungal and antibacterial properties [15-17].

Thin layer chromatography: In this study, the crude compound was fractionated with various solvents and the active fraction was separated on TLC plates. A big spot was developed on the TLC plate which was further used for HPLC purification.

High performance liquid chromatography: In the present study, the TLC purified sample was loaded on a analytical HPLC and results were observed in Fig. 1.

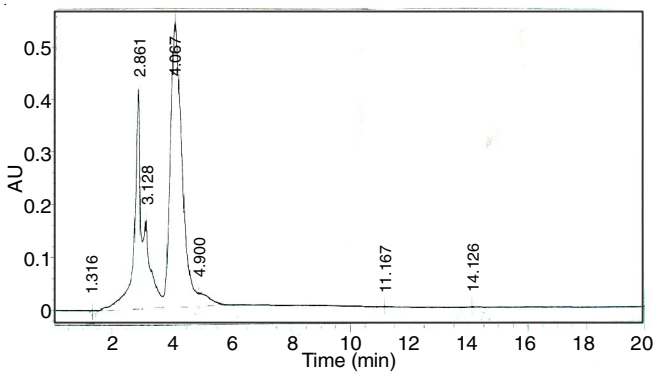

Fig. 1. High performance liquid chromatography (HPLC) analysis of compound from *Anthurium andraeanum*

Anticancer activity: The anticancer effect of root extract was determined using MTT assay. The present finding indicated the percentage inhibition of HepG2, A549 and MCF-7 cell lines (Fig. 2). Acridine orange and ethidium bromide staining was used to differentiate the normal cells and apoptotic cells. The normal cells show green colour and apoptotic cells are orange in colour. Cytotoxic effect is one of the important properties of anticancer molecules. In this study, the purified compound from the root extract of *Anthurium andraeanum* was effective against the cell lines, Hep G2, A549 and MCF-7, respectively. Among the cell lines, the purified compound was very effective against the cell lines MCF-7 (Fig. 3). This result was in accordance the observations made previously. Solowey *et al.* [18] extracted novel compounds from the plants, *Urtica membranacea*, *Origanum dayi* post and *Artemesia monosperma* and were promising against various cancer cell lines. The present finding suggested the presence of active compounds in the root extract, which are potential for development of effective anticancer agent. The GC-MS spectrum analysis confirmed the presence of components with different retention times as described in Fig. 4. Many bioactive compounds were identified from the GC-MS analysis, including, 2,3-dihydro-benzofuran and stigmasta-5,20(22)-dien-3-ol.

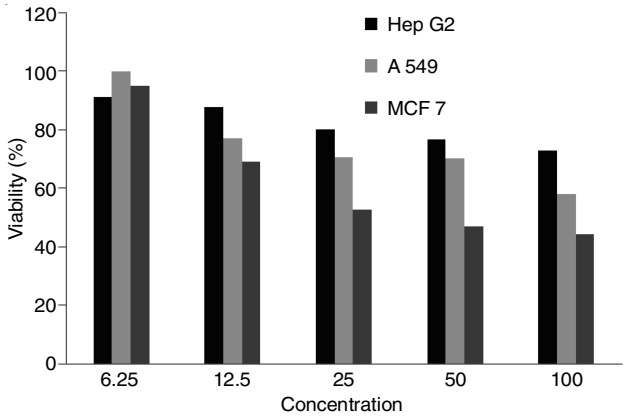

Fig. 3. Percentage viability of Hep G2, A 549 and MCF 7 cell lines after the exposure with the root extract of *Anthurium andraeanum*

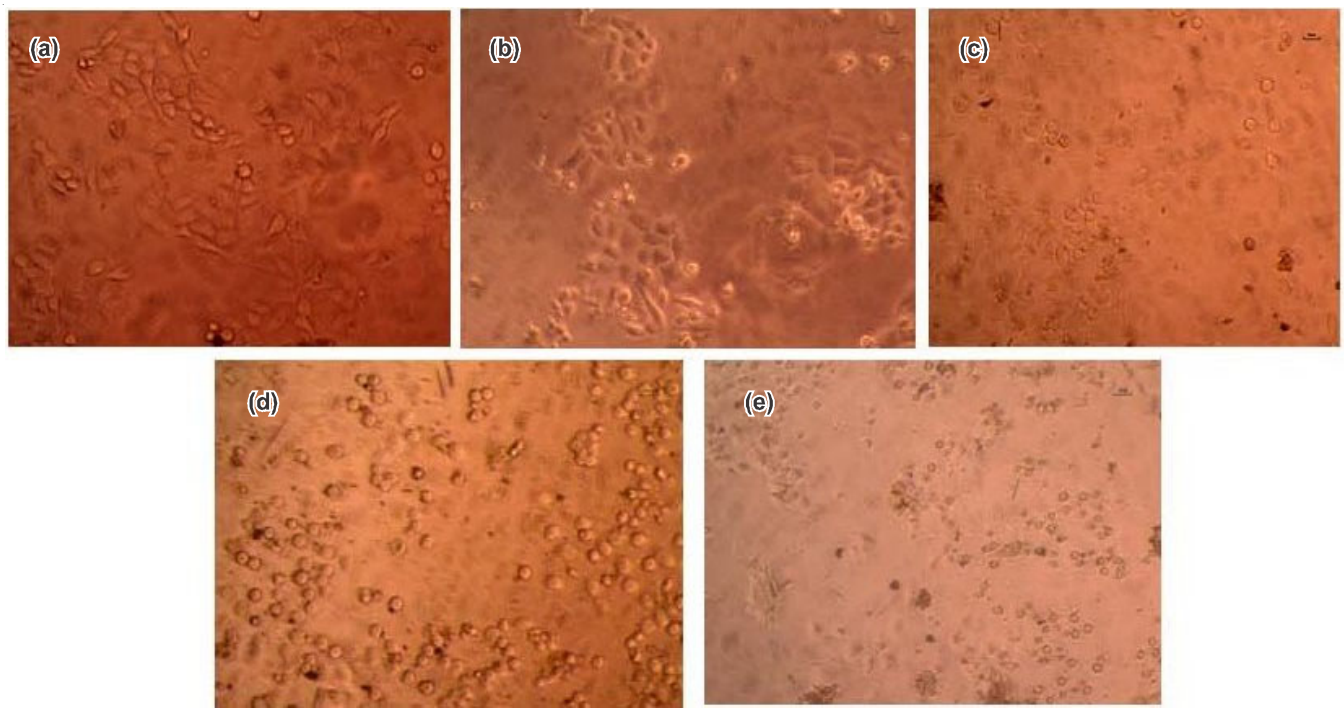

Fig. 2. Anticancer activity of root extract of *Anthurium andraeanum* at various concentrations of compound (a = 6.25 µg, b = 12.5 µg, c = 25 µg, 50 µg and 100 µg)

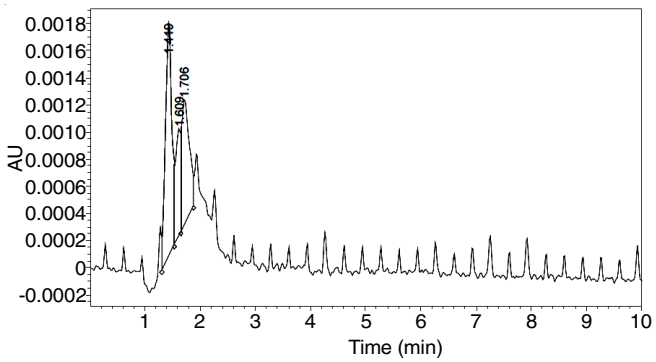

Fig. 4. GC-MS chromatogram of ethanolic extract of root from *Anthurium andraeanum*

Conclusion

In this study, the antimicrobial and anticancer activity was registered from the medicinal plant, *Anthurium andraeanum*. The root extract of this plant showed potent activity against various human pathogens. The purified compound showed anticancer activity against various cell lines. Further studies are important to explore other parameters relates to antimicrobial and anticancer efficiency.

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