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Research Article

SCREENING OF ANTIBACTERIAL ACTIVITY AND PHYTO-COMPOUND STUDIES OF Aduthodavasicaneesvasicanees

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ABSTRACT

Medicinal plants contribute in human health care system. Most of the plants utilized by village people are folk medicine. Now we are turned into medicinal plant analysis of active compounds and conservation aspect. The medicinal plants are used in traditional treatments to cure variety of diseases for thousands of year. The aim of this study is to identify such plants with antibacterial efficacy for controlling human pathogens.The present study describes the phytochemical profile and antimicrobial activity ofAduthodavasicaneesvasicanees. For the present investigation the ethanol extract of the leaves of Adutodavasicanees were tested for their antimicrobial activity and further subjected to qualitative analysis and GC-MS analysis to find out the nature of the compounds responsible for the antimicrobial activity. The anti-bacterial and anti-fungal activities were assessed by measuring the diameter of the inhibition zones of the extract against the bacterial strains such as Escherichia coli,Salmonella paratyphi, Vibrio cholera, and Staphylococcusaureus andfungal pathogens namely Aspergillusniger, Aspergillusfumigatus, Candida albicans. The extract has a marked sensitivity towards Staphylococcus aureus, with 28.0 mm at concentration 1000 µg/ml respectively against the standard which has 25.0 mm of inhibition efficiency and the extract has a marked sensitivity towards Candida albicans, with 19.0 mm at concentration 1000 µg/ml respectively against the standard which has 26.0 mm of inhibition efficiency at a concentration of 50 mg/ml. The phytochemical screening revealed the presence of various secondary metabolites such as alkaloids, carbohydrates, pseudo tannins, chlorogenic acids, steroidal glycosides, saponins and flavonoids . GC-MS analysis revealed that the ethanol extract contains 1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-,(E)- (20.64%), Trifluoroacetyl-lavandulol (6.18), 2,3-Diamino-2-cyanosuccinonitrile (2.58), Tetrazolo[1,5-b]1,2,4-triazine,5,6,7,8-tetrahydro-, 7-dimethyl-(3.28%), Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3- propanediyl ester(67.24%), 3-Butyn-2-ol(0.08%) to be the molecules responsible for the antimicrobial activity of Aduthodavasicaneesvasicanees. It was concluded that plant extract can be used as a preservatives against the human pathogens.

Keywords: Medicinal plants, Phytocompounds, Anti-bacterial activity, Anti-fungal activity, Aduthodavasicaneesvasicanees and Pathogens.

INTRODUCTION

In developing countries, the frequency of life-threatening infections were caused by pathogenic microorganisms has led to increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients1. The historical point, plants have been used as an important source of natural products for human health. All over the world, the antimicrobial properties of plants have been investigated by a number of studies and many of them have been used as therapeutic alternatives because of their antimicrobial properties2 and they contains secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections3,4. Despite the existence of potent antibiotic and antifungal agents, resistant or multiresistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs. It is therefore necessary that the search for newer antibiotic sources be a continuous process. Plants are the cheapest and safer alternative sources of antimicrobials[5, 6].

MATERIALS AND METHODS:

Plant collection:

The plant leaves used in this study were collected in and around Trichy District. The collected samples were brought into the laboratory carefully stored in sterile polythene bags and used for the further study.

Sterilization of Plant Materials:

The disease free and fresh plants were selected for this investigation. Then, surface sterilized with 0.1% mercuric chloride and alcohol for few seconds. Again the plant materials were washed thoroughly thrice with distilled water.

Preparation of Plant Extracts:

Two grams of sterilized plant leaves were kept in the 10 ml of ethanol. Then these were grind with the help of mortar and pestle. The grinded plant material was subjected to centrifugation, for 10-15min (at 10,000rpm). Again, it was filtered through whatmann No. 1 filter paper. The supernatant was collected and stored for further antimicrobial screening purposes.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Chemical tests were also conducted on the aqueous extract of each plant sample and also of the powdered form of the plant samples by using standard methods of Harborne and Edeoga.[7,8]

GAS CHROMATOGRAPHY - MASS SPECTRUM STUDY (GC-MS)

The components of test sample were evaporated in the injection port of the GC equipment and segregated in the column by adsorption and absorption technique with suitable temperature programme of the oven controlled by software. Different components were eluted from the column based on the boiling point of the individual components. The GC column was heated in the oven between 60 to 270°C. The time at which each component eluted from the GC column was termed as Retention Time (RT).

Interpretation of mass spectrum (GC□MS) was conducted using database of National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library. The retention time, molecular weight, molecular formula and composition percentage of the sample material was recorded.

Micro-organisms tested:

Four bacterial species were collected from Microbial Type Culture Collection (MTCC), from the Institute of Microbial Technology (IMT), Chandigarh in Punjab, for the study. The Microbial strains used were Escherichia coli MTCC 443, Salmonella paratyphi; Vibrio cholerae; 441 and Staphylococcus aureusMTCC 87.

Aspergillusfumigatus, Aspergillusniger and Candida albicanswere also collected from Microbial Type Culture Collection, the Institute of Microbial Technology, Chandigarh in Punjab, India.

Preparation of Microbial Inoculum:

The young microbial inoculum culture was prepared and used during the research period. The nutrient broth (NB) was prepared and poured into several tubes. Then these tubes were sterilized. The pure microbial cultures were collected from the institute (either solid or liquid medium) and inoculated in the tubes by using inoculation loops. The tubes were incubated and the cultures were used for the experiments.

MEDIA PREPARATION

Composition of Nutrient Agar Medium

Peptone - 5gm

Beef extract - 3gm

NaCl - 5 gm

Agar - 15gm

Distilled water - 1000ml

pH - 6.8

Maintenance of Microorganisms

The test bacteria's were maintained in Nutrient Agar (Himedia Laboratories Pvt. Ltd., Mumbai) and test fungi were maintained in Potato Dextrose Agar (PDA) slants (Himedia Laboratories Pvt. Ltd., Mumbai). The microbial cultures were subcultured and the cultured strains were allowed to grow one week for fungi and two days for bacteria and they were stored at 5°C for further studies.

ANTI-MICROBIAL TESTING:

Disc Diffusion Method[9,10]:

The freeze dried extract was reconstituted with DMSO to obtain a stock solution of 50 µg/ml ,250 µg/ml, 500µg/ml and 1000µg/ml. Nutrient agar (Hi Media Laboratories Pvt. Ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective microbial

strains. Discs of (6 mm were punched from Whatman No.1 filter paper. Upto 10 µl of each concentration of the extract were respectively introduced in the discs using sterile automatic pipettes. The discs were allowed to dry at room temperature for 2 hours and were placed at equidistance in each of the plates using a sterile forceps. The plates were incubated to 37°C for 24 hours. The control antibiotic Kanamycin (10 µg) and Nystatin were used for gram-positive bacteria and fungi (Hi Media Laboratories Pvt. Ltd. Mumbai). Diameters of the inhibition zones were measured. The anti-microbial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract.[11,12].

RESULTS AND DISCUSSION:

Preliminary phytochemical screening:

Phytochemical screening of the extract of the leaves of Aduthodavasicaneesvasicaneesrevealed the presence of alkaloids, carbohydrates, pseudo tannins, chlorogenic acids, steroidal glycosides, saponins and flavonoids (table-1).

Table – 1: Qualitative analysis of Phytochemical constituents in the ethanolic extract of the leaves of *Aduthodavasicaneesvasicanees*

Phytochemical constituents	Extract
Alkaloids	++
Carbohydrates	+
Saponins	-
Tannins	-
PseudoTannins	+
Chorogenic acid	+
Anthocyanin	-
Steroidal Glycosides	+
Saponins Glycosides	+
Flavonoids	+
Flavones	-
Phenols	-
Coumarin	-
Anthracene Glycoside	-

(+)= Detected; (-) = Not detected

GC-MS Analysis:

The GC separated compounds are identified from the recorded mass spectra by comparison with the mass spectra from the database of National Institute of Standard and Technology. Aduthodavasicaneesvasicanees showed 6 peaks indicating the presence of 6 chemical constituents (Figure - 1).

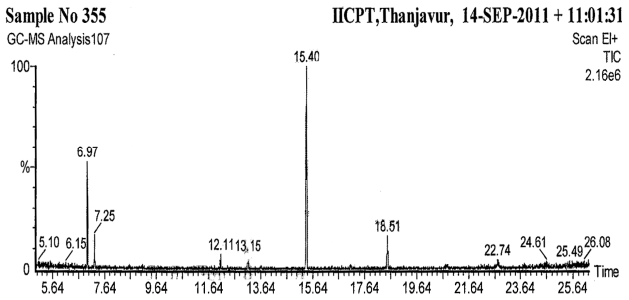


Figure – 1: GC-MS for *Aduthodavasicanees* Leaves

The 6 active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the ethanolic extract of Aduthodavasicanees. are presented in Table - 2. On comparison of the mass spectra of the constituents with the NIST library the 3 predominant constituents were characterized and identified. The structure and nature of the compound are presented in Table – 3.

Anti-microbial Studies

Anti-bacterial Assay in Aduthodavasicaneesvasicanees

Disc Diffusion Method

The sensitivity pattern of the four selected antibacterial strains (Escherichia coli, Salmonella paratyphi, Vibrio cholera and Staphylococcus aureus)used for the study are shown in Table - 4. The most pronounced activity with inhibition zones is shown at a concentration of 1000 µg/ml against the corresponding bacteria. The extract has a marked sensitivity towards Salmonella paratyphiand Staphylococcus aureuswith inhibition zones 26.0 mm and 28.00 mm at concentration 1000 µg/ml respectively against the standard which has 40.0 mm and 25.0 mm of inhibition efficiency respectively.

Anti-fungal Assay in Aduthodavasicaneesvasicanees

Disc Diffusion Method

The sensitivity pattern of the three fungal pathogens (Aspergillusniger, Aspergillusfumigatus, Candida albicans) used for the study are shown in Table - 5. The most pronounced activity with inhibition zone is shown at a concentration of 1000 µg/ml against the corresponding fungus. The extract has a marked sensitivity towards Candida albicans, with 19.0 mm at concentration 1000 µg/ml respectively against the standard which has 26.0 mm of inhibition efficiency at a concentration of 50 mg/ml.

Table – 2: Phytocomponents identified in the ehanolic extract of the leaves of *Aduthodavasicaneesvasicanees*by GC-MS study

S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area%
1	6.97	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-,(E)-	$C_{15}H_{24}$	204	20.64
2	7.25	Trifluoroacetyl-lavandulol	$C_{12}H_{17}F_3O_2$	250	6.18
3	12.11	2,3-Diamino-2-cyanosuccinonitrile	$C_5H_5N_5$	135	2.58
4	13.15	Tetrazolo[1,5-b]1,2,4-triazine,5,6,7,8-tetrahydro-, 7-dimethyl-	$C_5H_{10}N_6$	154	3.28
5	15.40	Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3- propanediyl ester	$C_{16}H_{30}O_4$	286	67.24
6	18.51	3-Butyn-2-ol	C_4H_6O	70	0.08

Table – 3: The Structure and nature of the predominant compounds

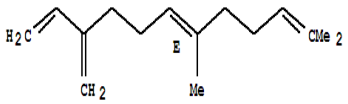
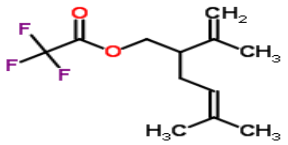
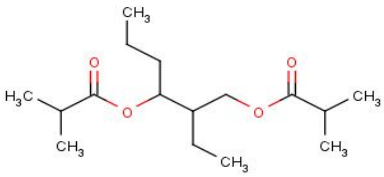
S. No.	Name of the compound	Structure	Nature
1	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-, (E)-		Alkene compound
2	Trifluoroacetyl-lavandulol		Alkaloid
3	Propanoic acid, 2-methyl-, 2-ethyl-1-propyl-1,3- propanediyl ester		Ester compound

Table – 4: Effect of Anti-bacterial Activity of *Aduthodavasicanees by* Disc Diffusion Method

Name of the extract	Concentration of the extract µg/ml	Zone of inhibition (mm)			
		Gram positive bacteria		Gram negative bacteria	
		Escherichia coli	*Salmonella paratyphi*	*Vibrio cholera*	*Staphylococcus aureus*
Ethanol	250	16	18	15	21
	500	20	19	20	24
	1000	23	26	25	28
Kanamycin	10 mg/ml	20	40	30	25

Table – 5: Effect of Anti-fungal Activity *Aduthodavasicanees*by Disc Diffusion method

Name of the extract	Concentration of the extract µg/ml	Zone of inhibition (mm)		
		Aspergillusniger	*AspergillusFumigatus*	*Candida albicans*
Ethanol	550	10	13	13
	250	12	14	15
	500	14	16	17
	1000	16	18	19
Nystatin	50 mg/ml	25	25	26

CONCLUSION:

It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective as antimicrobial agents. Usually medicinal plants contain several phytochemical compounds, which are very much necessary to control the growth of the micro organisms. The extract has a marked sensitivity towards Staphylococcus aureus, with 28.0 mm at concentration 1000 µg/ml respectively against the standard which has 25.0 mm of inhibition efficiency and the extract has a marked sensitivity towards Candida albicans, with 19.0 mm at concentration 1000 µg/ml respectively against the standard which has 26.0 mm of inhibition efficiency at a concentration of 50 mg/ml. In this work Aduthodavasicanees leaf extract has good activity against antibacterial strains namely Salmonella paratyphi and Vibrio Cholera and antifungal strain namely Aspergillus fumigates.

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