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Antimicrobial Activity of Chloroform Extract of *Aristolochia bracteata* Retz. and analysis of bioactive compounds

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A B S T R A C T

An attempt was made to reveal the bioactive components and functional groups present in the chloroform extract of leaf of *A. bracteata* Retz. through GC-MS and FTIR analysis. Altogether twenty components were detected by GC-MS analysis. FTIR study revealed the presence of various functional groups related to Alcohol, carboxylic acid, alkanes, aldehydes, aromatic acids. The extract showed inhibitory activity against *Vibrio harveyi*, *vibrio vulnificus*, *serratia marcescens*. From this investigation it can concluded that the chloroform extract of leaf of *A. bracteata* Retz. will be the alternative for the treatment of such threat full pathogens.

Introduction

Plants are fundamental source for all other living organisms. During the evolution process plants represent the first stage and they produce the most important materials such as nutrients, fuel, oxygen, etc. Higher plants also play a dominant role in the maintenance of human health by producing many bioactive compounds. Green plants represent a reservoir of effective chemotherapeutants that are easily biodegradable, systemic and non phytotoxic (Verma, 2006).

A large number of medicinal plants are used as alternate medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs.

Aristolochia bracteata Retz. is commonly known as 'Worm killer' in English and 'Aadutheendaapaalai' in Tamil. *A. bracteata* Retz, is used in traditional medicine as gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (8). Root powder is combined with honey and given internally in the case of gonorrhea, boils, ulcers and

other skin diseases (Sankarnarayanan *et al*., 2010). In indigenous system of medicine, it is reported that leaves were used for skin diseases, rheumatism and as analgesic (Manikandar *et al.*, 2006). Hence the present study was undertaken to reveal the antibacterial activity of chloroform extract of *A. bracteata* Retz leaf against some human pathogen, identification of bioactive compounds through Gc- Ms and FTIR analysis.

Materials and Methods

Preparation of Plant Material

Mature leaves of *A. bracteata* Retz, were collected from the agricultural and open fields. Collected materials were washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and powdered.

Extraction of Plant

Five gram of powdered plant sample was extracted with 50ml of chloroform in the round bottom flask using the soxhlet extraction method as per the standard procedure at their boiling points. The residues (extract) obtained were finally dried in the hot air oven and stored at room temperature for further use.

GC-MS and FTIR Analysis

The chloroform extract of *A. bracteata* Retz was mixed with kBr salt, using a mortar and pastle and compressed into a thin pellet. Infrared spectra were recorded on a shimadzu FTIR spectrometer 8000 series, between 4000-400cm^{-1}. GC-MS analysis of chloroform extract of *Aristolochia bracteata* Retz was performed using the equipment Agilent technologies 7890A. The equipment has a DB35-MS capillary standard non-polar column with dimensions of 30mmx0.25mm IDx0.25µm film. The carrier gas Helium was passed through at a flow rate of 1.0ml/min. The injector was operated at 250°c and the oven temperature was programmed as follows 60°c for 15mins then gradually increased to 280°c at 3mins. The constituents were identified after comparison with those available in the computer library (NIST and willey) attached to the GC-MS instrument.

Test Organism

Test organisms were obtained from the doctor's diagnostic centre, Trichy, Tamilnadu, India. The bacterial strains used for testing antimicrobial activity were *Vibrio harveyi, vibrio vulnificus, serratia marcescens*. The bacterial culture were maintained on nutrient agar (Himedia, Mumbai, India) and stored in refrigerator at 4°C for further study. The pathogens were cultured individually on nutrient broth 37°C for 24h, for antimicrobial assay.

Antimicrobial Assay

Antimicrobial activity of crude extract was evaluated by agar well diffusion assay (Bauer *et al.*, 1966). Muller-Hinton agar (Himedia, Mumbai) plates were prepared. After solidification five wells of 6mm were made using well cutter. The test cultures were swabbed on the surface of the solid media using sterile cotton swabs and allowed to dry for 10mins. The crude extracts were dissolved in 1% DMSO (100mg/ml). From the stock solution 10µl, 20µl, 30µl, 40µl, 50µl were taken and loaded in the respective well. Tetracycline (10µl/well) was used as positive control. Negative control was prepared using DMSO solvent. The plates were incubated for 24hrs at 37^{0}C. The activity was evidenced by the presence of a zone of inhibition (mm)

surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the crude extract when compared to the controls.

Results and Discussion

GC-MS analysis of the ethanol extract of leaf of *A. bracteata* Retz. showed the presence twenty bioactive components. Their retention time (RT), molecular formula and concentration (%) were tabulated table 1 and fig 1. Among the twenty identified component few of their activities were listed out in the table 2. Phytol is found to be antimicrobial, anti-inflammatory, anticancer and antidiuretic properties (Sermakkani and Thangapandian, 2012). Similarly sutha *et al*., 2012 observed the antioxitant activity of n-hexadecanoic acid. Previous study by Maruthupandian *et al*., 2011 justified the anti inflammatory activity and anti arthritic activity of 9, 12, 15-Octadecarienoic acid.

Aneesh *et al*., 2013 reported that the ether extract of rhizome of *Nervilia aragoana* contains hexadecanoic acid which has anti inflammatory activity, flavouring agents like pentadecanoic acid, 2-chloroethyl linoleate, isoamyl laureate which is a skin conditioning agent, phthalic acid which is used in neurodegenerative disorders. Similarly in this investigation the chloroform extract of leaf of *A. bracteata* Retz. contains several bioactive components listed in the table 1. The results of the present study coincide with report of Sermakkani and Thangapandian, 2012.

Table.1 Components Detected in the Chloroform Extract of Leaf of *Aristolochia bracteata* Retz

S.No	RT	Name of the component	Molecular formula	Peak area%
1	9.668	Hexanedioic acid, 2-methyl-	$C_6 H_{10} O_4$	3.72
2	10.051	1,6-Octadien-3-ol,3,7-dimethyl	$C_{10} H_{18} O$	2.82
3	10.242	Benzaldehyde,2,5-bis(trimethylsilyl)	$C_{13} H_{22} O_3 Si_2$	2.45
4	12.935	Azidotrimethylsilane	C3H9N3Si	13.72
5	17.316	Phynol,2,4-bis(1,1-dimethylet	$C_{14} H_{22} O$	0.74
6	18.066	2(4H)-Benzofuranone,5,6,7,7a-tetrahyd	$C_{11} H_{16} O_2$	1.12
7	21.568	2,5-Octadecadienoic acid methyl ester	$C_{19} H_{30} O_2$	0.41
8	24.738	2,6,11-Tridecatrien-10-OL,2,6	$C_{16} H_{28} O$	1.91
9	25.688	2(4H)-Benzofuranone,5,6,7,7A-	$C_{11} H_6 O_2$	0.51
10	27.075	8-Quinolinol	$C_9 H_7 NO_2$	-3.86
11	24.385	10,11-Dihydroxy- 3,7,11-trimethyl	$C_{16}H_{28} O_4$	4.26
12	29.568	Heptadeconoic acid, methyl ester	$C_{18} H_{36} O_2$	0.91
13	30.659	13-Hexyloxacyclotridec-10-en-2-one	$C_{18} H_{32} O_2$	2.51
14	31.25	Phytol	$C_{20} H_{40} O$	1.93
15	32.246	Octadeconoic acid, ethyl ester	$C_{20} H_{40} O_2$	0.33
16	32.808	Eicosanoic acid 2-hydroxyethylester	$C_{22} H_{44} O_3$	-0.33
17	33.543	Gingerol	$C_{17}H_{26} O_4$	0.37
18	34.055	1,4-Dioxaspira[4.14]nonadecane	$C_{34}H_{48} N_2O_4$	4.12
19	35.3238	Octadeconoic acid,2 hydroxyethyl ester	$C_{20}H_{40}O_3$	O.43
20	36.281	Bicyclo(3.1.0) hexane- 3-OL,4 methylene	$C_{12} H_{18} O_2$	-0.09

Table.2 Activity of Phyto-components Identified in the Chloroform Extract of Leaf of *A. bracteata* Retz

S. No	Name of the compound	Molecular formula	Activity
1	Phytol	$C_{20}H_{40}O$	Anticancer, Antioxidant Anti inflammatory
2	Octadeconoic acid ,methyl ester	$C_{20}H_{40}O_2$	Anti inflammatory, Hypocholesterolemic, Anti arthritic activity
3	Gingerol	$C_{17}H_{26}O_4$	Anticancer
4	Heptadeconoic acid, metyl ester	$C_{18}H_{36}O_2$	Antioxident, Anti fibrinolytic, Hemolytic and antimicrobial activity

Table.3 FTIR Peak Values of Chloroform Extracts of Leaf of *A. bracteata* Retz

S. No	Peak value	Functional group	Functional group name
1	729.09	C-“H OOP’’	Aromatics
2	1188.15	C-O stretch	Alcohols, carboxylic acids
3	1263.37	C-N stretch	Aromatic amines
4	1379.1	C-H bent	Alkanes
5	1454.33	C-H bent	Alkanes
6	1722.43	C -O stretch	Aldehyde, saturated aliphatic
7	1832.38	O-double bond	Aldehyde
8	2358.94	O-H stretch	Carboxylic acid
9	2858.51	C-H stretch	Alkanes
10	2922.16	C-H stretch	Alkanes
11	3354.21	OH-stretch, H-bonded	Phenol and Alcohols
14	3836.42	O-H stretch	Carboxylic acid

Table.4 Antibacterial Activity of Chloroform Extract of *A. bracteata* Retz. Against Clinical Pathogens

S. No	Name of the Organisms	Zone of inhibition in mm						
		P	N	10µl	20µl	30µl	40µl	50µl
1	*Vibrio harveyi*	9.0±0.3	-	2.2±0.01	2.1±0.03	2.4±0.06	3.0±0.3	7.4±0.03
2	*Serretia marcesence*	8.3±0.01	-	1.1±0.4	1.5±0.04	2.0±0.02	8.2±0.6	6.2±0.02
3	*Vibrio vulnificus*	11.4±0.05	-	2.0±0.2	2.1±0.02	3.0±0.06	5±0.5	7.0±0.05

Values are means of three replicates, ± Standard deviation
Positive control (P): Tetracycline
Negative control (N): DMSO

Fig.1 GC-MS Chromotogram of Chloroform Extract of Leaf of *Aristolochia bracteata* Retz**.**

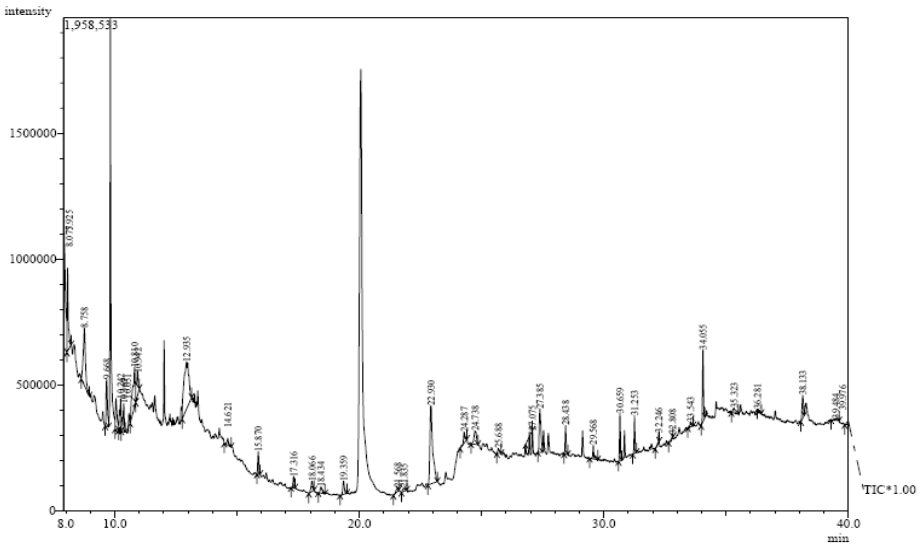
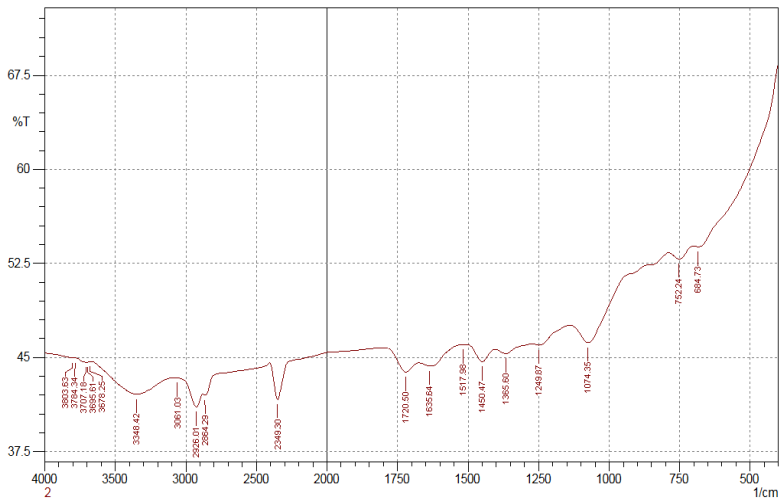


Fig.2 FTIR Spectrum of Chloroform Extract of Leaf of *A. bracteata* Retz.



Sivakumar and Dhivya, 2015 screened some bioactive compounds from leaves of *Cordia monoica* Roxb. which includes Phytol acetate, n-hexadecanoic acid, neophytadiene, neopentyl hydroxyl acetate and nonacosane. It has been proved that these components have several applications like antioxidant, anticancer and anti-inflammatory properties (Chella perumal *et al*., 2014).

The result obtained through FTIR analysis of leaf extract of *A. bracteata* Retz. clearly indicates the presence of Alcohol, carboxylic acid, alkanes, aldehydes, aromatic acids and phenols (table 3 and fig 2). Similar result was observed by Starlin *et al.*, 2012.

Antimicrobial activity of various extracts of medicinal plants is due to bioactive components had been already shown (Gopinath and Prakash, 2013). The presence of OH group in methanolic extract of medicinal plants is responsible for microbicidal activity (Ashokkumar and Ramaswamy, 2013). Chloroform extract of *A. bracteata* Retz. leaf was used to study the antimicrobial activity against three clinical pathogens *Vibrio harveyi, Serretia marcesence, Vibrio vulnificus* and the results were given in table 4. From the results it was noted that the chloroform extract showed maximum inhibition against *S. marcesence* (8.2±0.6 mm) followed by *V. harveyi* (7.4±0.03 mm) and *V. vulnificus* (7.0±0.05 mm).

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