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Medicinal Value of Bioactive Compounds in the (*Aristolochia bracteolata*) Leaves Extract Using GC-MS

[1]*G. Gnanamuthu and* [2]*M. Jeevitha*

[1]Department of Zoology, Pope's College (Autonomous),
Sawyerpuram, Thoothukudi, Tamil Nadu, India
[2]Department of Botany, Pope's College (Autonomous),
Sawyerpuram, Thoothukudi, Tamil Nadu, India

Abstract: *Aristolochia bracteolata* is an important medicinal herb and it is an original of Indian subcontinent and has become naturalized in the tropical and sub-tropical areas around the world. The plant is usually gathered from the wild and is used locally in traditional medicine. It is sometimes cultivated for medicinal use in India. The whole plant was used as a purgative, anti-pyretic and anti-inflammatory. It also possesses a potent anti-allergic activity and has pronounced antibacterial and antifungal activities as well as antioxidant activity. Based on the above information the present study, *Aristolochia bracteolate* leaves were collected from in and around Thoothukudi and then powered. The different extracts (aqueous and acetone) of *Aristolochia bracteolate* leaves were prepared by soxhlet apparatus. The phytochemical compounds of these leaves were screened qualitatively in aqueous and acetone extract, the present study showed the presence of phytochemical compounds such as flavanoids, carbohydrates, amino acids and protein were present in both aqueous and acetone extract, whereas Phylobatannis, Volatile oil and Hydrolysable tannins present only in aqueous extract, saponin, tannins phenol, alkaloids, steroids, terpenoids and glycosides were present only in acetone extract. The phytochemicals like phenol, steroids, volatile oils and hydrolysable tannins were absent in both aqueous and acetone extract. The compound were analysed in the acetone extract using GC-MS. The test organisms were obtaining from Microbial type culture collection (MTCC) Center, Chandigarh. Antimicrobial activity of two different solvent extracts of *Aristolochia bracteolate* were analyzed against the *Escherichia coli, Staphylococus aureus, Pseudomonas aeruginosa, Salmonella typhi* and *Bacillus subtilis* by disc diffusion method. Organisms were placed on the inoculated plates, incubated and observed for zone of inhibition. Finally, it is concluded that screening for photochemical of *Aristolochia bracteolate* showed that the presence of certain important bioactive compounds in GC-MS analysis and exhibited good antimicrobial activity against pathogenic bacteria which indicating the potential of this plant as source of functional ingredient that can be used in pharmaceutical industries so as to develop it as a potent antimicrobial drug.

Key words: *Aristolochia bracteolate* · Phytochemicals · GC-MS · Functional ingredient · Antimicrobial activity

INTRODUCTION

Medicinal plants occupy a distinct place in the life of human, right from the primitive till today. Use of plants as a source of medicine has been inherited and is an important component of health care system in India. India has more than 3000 years of medicinal heritage based on medicinal plants. Medicinal plants are widely used by all sections of the population either directly as folk remedies or indirectly in the preparation of modern pharmaceuticals. India is endowed with a rich wealth of medicinal plants; microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. Plants produce a diverse extent

Corresponding Author: G. Gnanamuthu, Department of Zoology, Pope's College (Autonomous), Sawyerpuram, Thoothukudi, Tamil Nadu, India.

of bioactive molecules, making them wealthy source of various types of medications. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine [1].

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, anti-analgesic, anticancer, antiviral, antimalarial, antibacterial and antifungal activities of the medicinal plants are due to the presence of the above-mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very much helpful for the manufacturing of new drugs. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [2].

Aristolochia is a large plant genus with over 500 species, belongs to the family *Aristolochiaceae*. In the indigenous system of medicine, the plant was used for the treatment of skin diseases, inflammation and purgative. Root extract was accounted to have anti-bacterial activity. *Aristolochia* species has been used extensively in the traditional Chinese medicine. Its diverse biological functions include hypertension relief, leukocyte enhancement, rheumatism relief, edema therapy, as well as analgesic and diuretic effects. It is a perennial climber shrub with woody base stocks. Leaves simple, alternate, entire, with undulate margins, acute; flowers greenish white, in auxiliary cymes; fruits rounded oblong, 6 chambered contain numerous winged compressed seeds [3].

A. bracteolata is traditionally used as an analgesic, anti-scorpion and anti-snake. It is also used in the treatment of tumors, malaria and for fevers [4]. The whole plant was used as a purgative, anti-pyretic and anti-inflammatory. It also possesses a potent anti-allergic activity and has pronounced antibacterial and antifungal activities as well as antioxidant activity. It was reported to contain alkaloids, triterpenoids, steroids and sterols, flavonoids, tannins, phenolic compounds and cardiac glycosides [5, 6].

The ethanolic extract of the shade dried leaves of *A. bracteolata* was evaluated anti-inflammatory activities in wistar rats by using the carrageen an induced left hind paw edema method. Significant reduction of edema volume was observed in the drug treated group when compared with the standard and untreated control. Antioxidant investigation of the ethanol extract along with its two successive fractions using nitric oxide and 1, 1-diphenyl-2 picryl hydrazyl (DPPH)-induced free radical assay methods showed good free radical scavenging activity, thereby supporting its anti inflammatory properties[7].

The aqueous extract of leaves of *A. bracteolate* exhibited antiulcer activity in rats. The antiulcer activity of *A. bracteolata* was evaluated against ethanol induced and pylorus ligation induced models, at two different dose levels of 400 and 800 mg/kg/body wt/day. The activity was compared with standard drug Ranitidine. Pre-treatment with the extract resulted in a significant decrease of the ulcerated area. The volume and acidity of the gastric juice decreased in the pre-treated rats. Among the two dose assessed, 800 mg/kg was found to have the significant activity than the lower dose [8].

A. bracteolata leaves were subjected to antibacterial activity on disc diffusion method against *Bacillus subtilus, Lactobacillus plantarum, Escherichia coli, Staphylococcus aureus, Streptococcus faecalis* and *Pseudomonas aeruginosa*. The leaves of *Aristolochia bracteolata* Retz were extracted with petroleum ether, chloroform and alcohol. Alcoholic extract showed significant antibacterial activity as compared to that of other extracts [9]. Negi [10] investigated the antibacterial activity of *Aristolochia bracteolata* root extracts. Powdered Roots of *A. bracteolata* were extracted with ethyl acetate, acetone, methanol and water for 8 hours each using a Soxhlet extractor. Antibacterial activity of dried ex- tracts was evaluated by the pour-plate method against a few Gram positive and Gram-negative bacteria. All the crude extracts showed a broad spectrum of antibacterial activity among which ethyl acetate extract was found to be the most effective. This study shows the potential for replacement of synthetic preservatives by the use of natural extracts. According to another study Kavitha [11] the different extracts (Aqueous, methanol and chloroform) of this plant were effective against the bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Shigella flexneri*, *Proteus vulgaris* and the fungal strains like *Aspergillus niger*, *Aspergillus terreus*, *Penicillium notatum* and *Rhizopus stolonifer*. Among the three extracts, methanol extract was found to have the significant activity followed by the chloroform extract against certain bacteria. Water extract

did not have any activity against bacteria. Antifungal activity assessment indicated that the tested fungal strains are more susceptible to aqueous extract followed by methanol extract and chloroform extract. the ethanolic extracts of *A. bracteolata* was studied Antifungal activity using disc diffusion method and was found to have highest activity at minimum concentration. The study justify that the bioactive principles present in the extracts may be responsible in the treatment of ringworm infection. It was reported that Ethanolic extract effective against *Trichiophyton rubrum* and *Microsporum canis* [12].

Invitro antiplasmodial activity against *Plasmodium falciparum* 3D7 (chloroquine sensitive) and Dd2 (chloroquine resistant and pyrimethamine sensitive) was investigated by Ramasubramania Raja [13]. It was found that extract of *A. bracteolata* exerted activity on *P. falciparum* strain 3D7 with an IC50 less than 5 µg/mL. Phytochemical analysis indicated that the most active phase contained terpenoids and tannins and was devoid of alkaloids and saponins. The effect of plant extracts on lymphocyte proliferation showed low toxicity to the human cells [14]. Another studies [15] shows that the plant extract of *A.bracteolata* has potent antimalarial activity(*in-vitro*) against schizonts maturation of *Plasmodium falciparum*, the major human malaria parasite. The whole plant extracts of *Aristolochia bracteolate* produced 100% inhibition of the parasite growth at concentration ? 50 µg/ml. The two most active plants showed the presence of sterols, alkaloids and tannins.

The antimicrobial activity of *A. bracteolata* was investigated by Parekh *et al*. The antimicrobial assay was done by both the agar disc and agar well diffusion method against six medically important microorganisms viz. *Bacillus subtilis, Staphylococcus subfava, Alcaligenes fecalis, Proteus mirabilis, P. aeruginosa* and *Candida tropicalis*. According to the study, the methanol extract was found to be more effective than the aqueous extract Parekh and Chanda [16]. It has been found that the aristolochic acid from the root of *Aristolochia bracteolata* possess significant antimicrobial activity. Aristolochic acid I was isolated from the methanolic and ethyl extract extracts of *Aristolochia bracteolata* and conformed through IR, NMR & MS. The percentage purity of aristolochic acid I was determined by UV & HPLC method. Antibacterial activity of extracts of *A. bracteolata* and the isolated compound was determined by disc diffusion method. Microbial assay of isolated compound (Aristolochic acid I) from ethyl acetate & ethanol extracts were shown good antimicrobial activity and the zone of inhibition of both at higher concentration 50µg/ml was similar with the standard aristolochic acid [17].

The ethnobotanical knowledge base for treatment of cuts and wounds which includes a usage of plants/plant extracts/decoctions or pastes, methods employed by tribals and folklore practices prevailing in India have been analysed [18].

The ethanol extract of the leaves of *Aristolochia bracteolata Lam.* was studied for its effect on wound healing in rats, using incision, excision and deadspace wound models, at two different dose levels of 400 and 800 mg/kg/body wt /day. The plant showed a definite, positive effect on wound healing, with a significant increase of the level of two powerful antioxidant enzymes, super oxide dismutase and catalase, in the granuloma tissue [19]. Based on the above information the present study has been choosing to analyse the phytochemicals and identification on active compounds in the *Aristolochia bracteolata* leaves extract against the some pathogenic bacteria.

MATERIALS AND METHODS

Collection of Plant Material: The fresh, healthy and disease free leaves of *Aristolochia bracteolate* were collected from Tuticorin, Tamilnadu, India and their identification was confirmed with the help of herbarium specimens The sample specimen was identified based on the taxonomical characteristics with help of flora presidency of madras and flora of the Tamilnadu carnatic.

Preparation of Leaves Powder: The collected plant leaves were washed thoroughly using water and transferred to the laboratory. The plant material was shade dried for three days, after drying plant material powdered with the help of mixer grinder. Same procedure was followed for both leaf extractions for acetone extraction. The fine particles were separated and stored container and used for further analysis.

Preparation of Aqueous Extract: Fifty grams of powdered leaves of *Aristolochia bracteolate* were macerated with 10 ml of sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 120°C for 30 min. The extract was preserved aseptically in a in a brown bottle at -4°C until further use.

Phytochemical Screening: Different qualitative chemical tests can be performed for its chemical composition using standard procedure in order to identify the constituents as described [20-22].

Test for Saponin: Two ml of the filtrate was mixed with five ml of distilled water and shaked vigorously for a stable persistent froth. The frothing was mixed with three drops of olive and shaken vigorously and observed for the formation of emulsion [23].

Test for Terpenoids: Five ml of extract was mixed in two ml of chloroform and three ml concentrated sulphuric acid was carefully checked to form a layer. A reddish brown colour was formed at the interface to show positive results for the presence of terpenoids (Salkowski test).

Test for Flavanoids: Five ml of diluted ammonia solution was added to the protion of the aqueous filtrate of leaf extract followed by addition of concentrated sulphuric acid. A yellow colour was observed in extract which indicates the presence of flavanoids. The yellow coloration disappeared on standing.

Test for Amino Acids: Two ml of extract was taken in a test tube and added one ml of ninhydrin solution. It was heated at 50°C for one minute. The violet color indicates the presence of amino acids [24].

Test for Phylobatannins: Deposition of a red precipitate occurs when one ml of aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid and it was taken as evidence for the presence of phylobatannins.

Test for Volatile Oil: Two ml of extract was taken in a test tube and added 0.1 ml of diluted HCl. The appearance of white precipitate indicates the presence of volatiles oil (it disappears immediately).

Test for Tannins: About 0.5 ml of powder sample was boiled in 20 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brown green or blue-black coloration for positive result.

Test for Phenol: Two ml of extract was taken in a test tube and added five ml of Fehlings solution A and it was heated at 50°C for one minute. The red precipitate indicates the presence of phenol [25].

Test for Glycosides: Five ml of extract was added with two ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with one ml of concentrated sulphuric acid. A brown ring may appear below the brown ring, a greenish ring may from just gradually throughout thin layer [26].

Test for Carbohydrate: One ml of each Fehling solutions, A and B was added to the two ml of the plant extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The appearance of a brick red precipitate indicates the presence of carbohydrate.

Test for Steroids: About 0.5 ml of extract was dissolved in 3 ml of chloroform and filtered. To the filtrate, concentrated H_2SO_4 was added to form a lower layer. Reddish brown colour was taken as positive for the presence of steroids.

Test for Hydrolysable Tannins: Two ml of extract was taken in a clean test tube and mixed with 2 ml of ammonia solution. The formation of emulsion indicates the presence of hydrolysable tannins.

Assay of Antimicrobial Activity: Twenty grams of powdered plant material was mixed with 100 ml of ethanol solvent. The extracts prepared in succession from powdered root material, by soxhlet method, the extract preparations were done by Baker [27]. The collected extracts were stored in a vial of further studies.

Disc Preparation: Sterile empty antibiotic discs (6 mm diameter) are to be prepared from Whatman No. 1 filter paper and the discs are sterilized by autoclave at 121°C. After sterilization moisture in the discs are dried on hot air oven at 50°C. 20 mg of dried crude extract will be dissolved in 1 ml of 20%. DMSO (Dimethyl sulphoxide). From this stock solution 10μl of respective solvent extracts are to be added to the disc (0.2mg/disc) individually and aseptically. After drying they will be used for screening the antibacterial activity [28].

Microorganisms Used: Bacteria causing infectious diseases both in animals and humans are to be used in the present study. Both gram positive and negative bacteria, such as *Escherichia coli, Enterobacter aerogenes, Salmonella spp, Staphylococus aureus, Klepsiella pneumonia* and *Bacillus subtilis* were used and they are collected from the Microbial Type Culture Collection (MTCC) at Chandigarh, India.

Assay of Antibacterial Activity: Antibacterial activity test was carried out following the modification of the method originally described by Bauer [29]. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The prepared sample was placed on the each petriplates and also placed control and standard (Ciprofloxacin) discs. The plates were incubated at 37°C for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

GC-MS Analysis of Samples: GC-MS plays key role in the analysis of unknown components of plant origin GC-MS ionizes compound and measures their mass numbers. Ionization method includes EI (Electro ionization) and CI (chemical ionization) typically, the CI method is used. The EI method produces ions by colliding thermal electrons emitted from a filament with sample has molecules. This method provides high stability in ionization and the obtained mass specta show good reproducibility. The quantitative analysis as well qualitative with GC-MS, in which only ions Specific to the compounds are measured, is highly selective method without interfering components.The GC-MS analysis was performed in a gas chromatograph (Perkin-Elmer, Auto system XL) linked to a mass spectrometer (Turbo mass) available at the Jawaharlal Nehru University New Delhi, India. An aliquot of 2 Ml of extract was injected into the PE-5MS column of 20mm0.18 mm internal diameter 0.18 mm film thickness glass capillary column using the following temperature programme: initial oven temperature of 40°C for 5 minutes, increasing to 100°C at a rate of 7°C for 6 minutes and then to 250°C at a rate of 7°C for 9 minutes. The injector temperature was maintained at 260°C. The interface temperature was 250°C. Helium was used as a mobile phase at a flow rate of 1.1 mL/min. Mass spectral detection was carried out in electron ionization mode by scanning at 20 to 650 (m/z). Finally, unknown compounds were identified by comparing the spectra with that of the National Institute of Standard and Technology library. The total time required for analysing a single sample was 45 minutes. A blank was run after every five samples.

Statistical Analysis: The present results are expressed in the mean ± standard error and the data were compiled using SPSS statistical software 10th version and subjected to analysis of variance (ANOVA) with post hoc comparison (One – way) [30].

RESULTS

The present study is carried out to identify, screening and know the antimicrobial activity of *Aristolochia bracteolate* leaves against selected pathogenic bacteria. The phytochemical compounds were also analyzed using GC-MS and the results are tabulated.

Screening of Phytochemical Compounds: The phytochemical compounds of *Aristolochia bracteolate* were qualitatively analyzed. Further, the present study showed the presence of phytochemical compounds such as flavanoids, carbohydrates, amino acids and protein were present in both aqueous and acetone extract, whereas Phylobatannis, Volatile oil and Hydrolysable tannins present only in aqueous extract, saponin, tannins phenol, alkaloids, steroids, terpenoids and glycosides were present only in acetone extract. The phytochemicals like phenol, steroids, volatile oils and hydrolysable tannins were absent in both aqueous and acetone extract. (Table 1, Fig. 1).

Antimicrobial Activity of *Aristolochia bracteolate*: The antimicrobial activities of *Aristolochia bracteolate* leaves extract were analyzed against *Escherichia coli, Staphylococus aureus, Pseudomonas aeruginosa, Salmonella typhi* and *Bacillus subtilis.*in acetone and aqueous extract. In the present study, the maximum level of inhibition showed in the standard disc ciprofloxacin. But the acetone extracts of *Aristolochia bracteolate* leaves are the maximum level of zone of inhibition showed in the pathogenic bacteria *Pseudomonas aeruginosa, Salmonella typhi* and *Bacillus subtilis* when compared to aqueous extracts, standard and control. In aqueous extracts, there is no more variation occur in the level of zone of inhibition showed in the pathogenic bacteria like *Escherichia coli, Staphylococus aureus* compared to acetone extracts (Table 3 & 4).

GC-MS Study: GC-MS study revealed that nearly eighteen active compounds like Cyclo pentane propnoic Acid, Hydrazine Carbothioamide, 1-Methyl-3-Piperidine Methanol, 2-Propane, 1-(4-Pyridinyl), 2-Propane, 1-amine, 1, 2-Dithiane, Toluene, Thiirane, Ethane, Isocyanato, Cyclo Heptane, 2-Pentyne-1-ol, 1-Propenylaziridine, Cyclobutanone, 2-methyl-2-oxiranyl, Benzyl Benzoate, 2, 3 Hexadiene, 2-methyl, Phthalic Acid, 1, 2-Bebzene Dicarboxdylicacid, Butyl 2-Ethylhexylester and Iso phytol were present, among those 4H Pyran- 4 one, 2, 3-dihydroxy- 6-methyl and 2-Hexadecen-1-OL, 3, 7, 11, 15-Tetramethyl were present in maximum level when compared to the other compounds (Table 2, Fig. 1).

Soxhlet apparatus

A B C D E F G H I J K L

Phytochemical screening

A - Saponins
B - Tannins
C - Phenol
D - Steroids
E - Terpenoids
F - Flavanoids
G - Amino acid and Protein
H - Carbohydrate
I - Phylobatannins
J - Volatile oil
K - Hydrolysable tannins
L - Glycosides

Fig. 1: GC-MS analysis of *Aristolochiabracteolate* leaves extract

Table 1: Preliminary phytochemical studies on various extract of *Aristolochiabracteolate*

S.No.	Phytochemical constituents	Reagent used	Aqueous extract	Acetone extract
1	Saponin	H_2O	-	+
2	Tannins	Ferric chloride	-	+
3	Phenol	Ferric chloride	-	+
4	Alkaloids	Picric acid	-	+
5	Steroids	Acetic anhydride	-	+
6	Terpenoids	Chloroform, H_2SO_4	-	+
7	Flavanoids	NaOH, Dil. HCl	+	+
8	Proteins	Ninhydrin solution	+	+
9	Carbohyrates	Felling solution	+	+
10	Phylobatannis	HCL	+	-
11	Volatile oil	NaoH, HCL	+	-
12	Hydrolysable tannins	Ammonia	+	-
13	Glycosides	Glacial Acetic Acid, Ferric Chloride, H2SO4	-	+

Table 2: Bioactive compounds identified in acetone extract of the *Aristolochiabracteolate* leaves

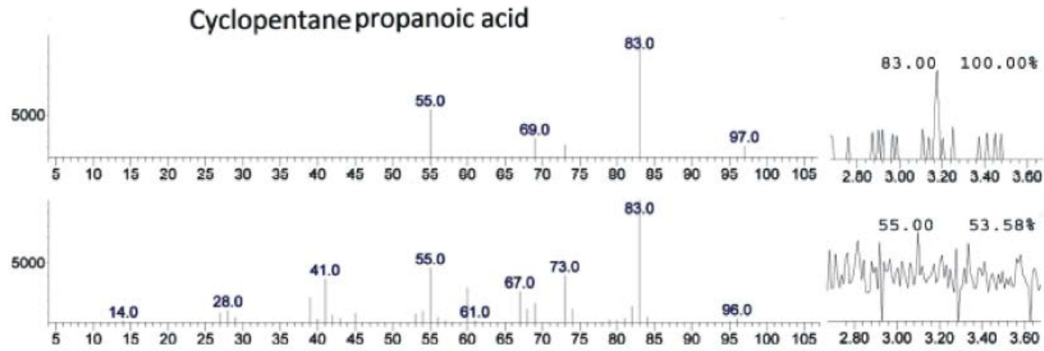
Time (Mins)	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Nature Compound	Area Percentage (%)
3.174	Cyclopentanepropanoic acid	C8H1302	141	Acid	2.34
3.609	Hydrazine carbothioamide	C2H7N3S	105	Amine	0.90
3.939	1-Methyl-3-piperidine methanol	C7H15NO	129	Alcohol	2.89
5.283	2-propane, 1-[4-pyridinyl	C13H14N2	198	Ketone	1.13
5.718	2-propane, 1-amine	C3H6N	56	Amine	1.04
6.294	1, 2-Dithiane	C4H8S2	120	Alkane	1.92
6.994	Toluene	C6H5CH3	92.1	Alkane	1.20
7.590	Thiirane	C2H45	60.1	Alkane	1.02
7.959	Ethane, Isocyanato	CH3CH3, CNO	57	Alkane	0.85
8.138	Cyclo Heptane	C7H15NO	96	Alkane	0.43
8.256	2-pentyne-1-ol	C5H80	84	Alkane	0.43
8.791	1-propenylaziridane	C5H9N	83	Pryidine	o.45
9.339	Cyclobutanone, 2-methyl-2-oxiranyl	C7H10O2	126	Ketone	2.34
9.887	Benyl Benzoate	C6H5CH2	212	Acid	12.36
10.64	2, 3-Hexadiene, 2-methyl	C7H12	96	Alkene	5.82
11.35	Phthalic Acid	C8H6O4	166	Acid	5.86
11.81	1, 2-Benzene Dicaboxylicacid, Butyl 2-Ethylexylester	C20H30O4	334	Acid	5.94
13.20	Iso phytol	C20H40O	296	Phytol	52.97

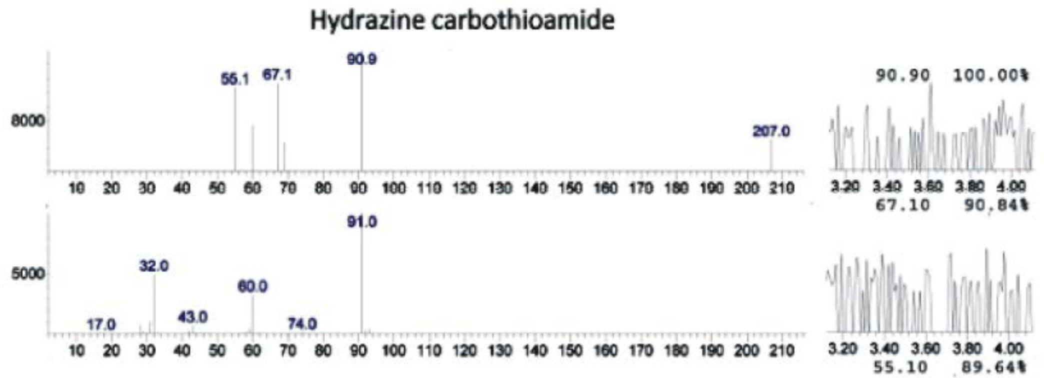
Table 3: Antimicrobial activity of aqueous extract of *Aristolochia bracteolate leaves* against pathogenic bacteria

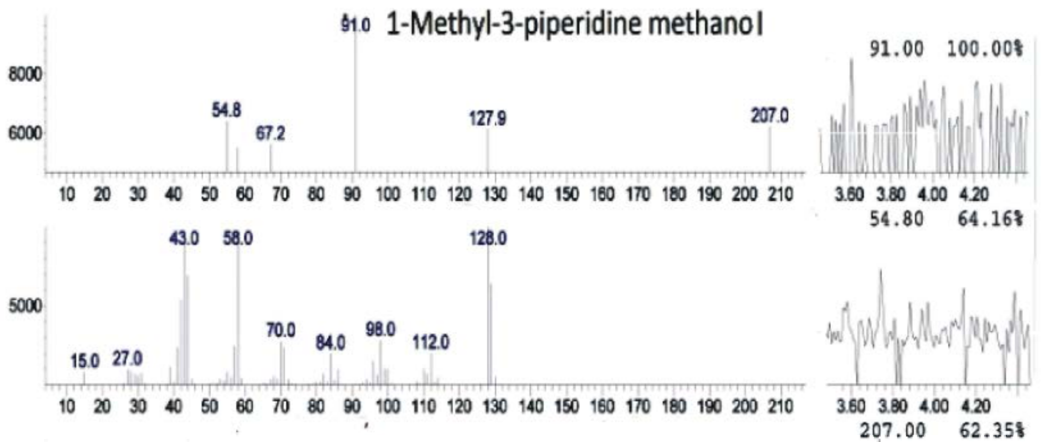
		Concentration of Sample					
S.No.	Name of the Organisms	Control	10 mg	20 mg	30mg	40 mg	50 mg
1	*Escherichia coli*	.	0.8	1.2	1.8	2.3	3.1
2	*Staphylococus aureus*	-	0.6	0.9	1.2	1.9	2.6
3	*Pseudomonas aeruginosa*	-	3.4	3.9	4.2	5.3	7.1
4	*Salmonella typhi*	-	2.7	2.9	3.4	4.2	5.5
5	*Bacillus subtilis*	-	2.2	2.6	3.9	5.1	6.3

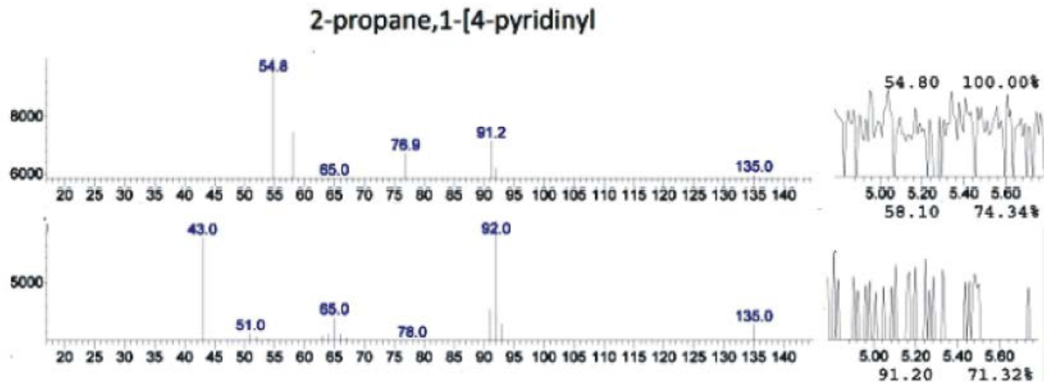
Table 4: Antimicrobial activity of Acetone extract of *Aristolochia bracteolate leaves* against pathogenic bacteria

		Concentration of Sample					
S.No.	Name of the Organisms	Control	10 mg	20 mg	30mg	40 mg	50 mg
1	*Escherichia coli*	.	2.8	3.2	3.8	4.2	4.4
2	*Staphylococus aureus*	-	1.5	2.5	2.8	2.9	3.3
3	*Pseudomonas aeruginosa*	-	13.4	17.4	18.2	25.3	27.4
4	*Salmonella typhi*	-	12.2	15.7	19.3	22.4	25.8
5	*Bacillus subtilis*	-	11.7	12.6	13.6	15.2	16.3

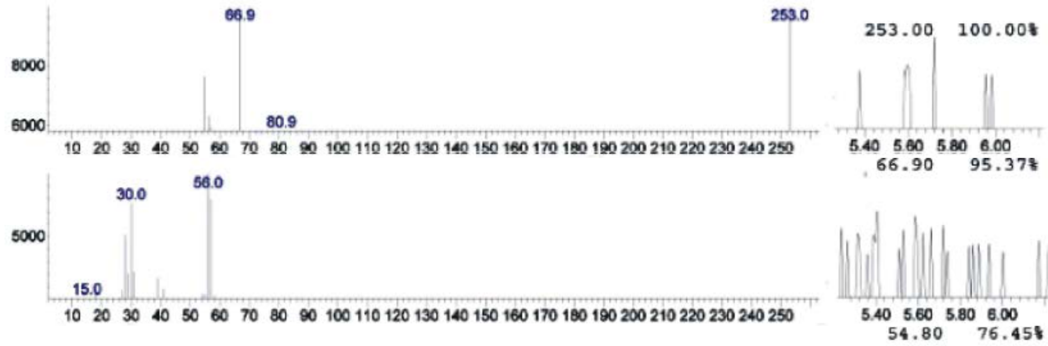

Cyclopentane propanoic acid
83.0
55.0
69.0
97.0
83.00 100.00%
41.0
28.0
14.0
61.0
67.0
73.0
96.0
55.00 53.58%


Hydrazine carbothioamide
55.1
67.1
90.9
207.0
90.90 100.00%
67.10 90.84%
91.0
32.0
60.0
17.0
43.0
74.0
55.10 89.64%

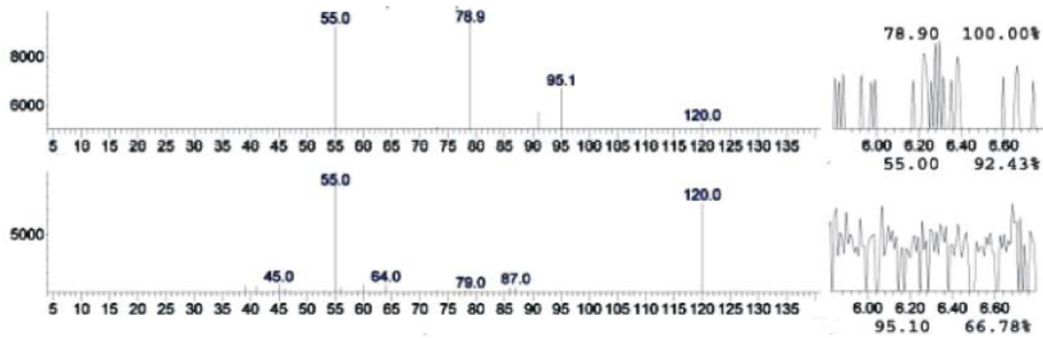

1-Methyl-3-piperidine methanol
91.0
54.8
67.2
127.9
207.0
91.00 100.00%
54.80 64.16%
43.0
58.0
128.0
15.0
27.0
70.0
84.0
98.0
112.0
207.00 62.35%


2-propane,1-[4-pyridinyl
54.8
65.0
76.9
91.2
135.0
54.80 100.00%
58.10 74.34%
43.0
92.0
51.0
65.0
78.0
135.0
91.20 71.32%

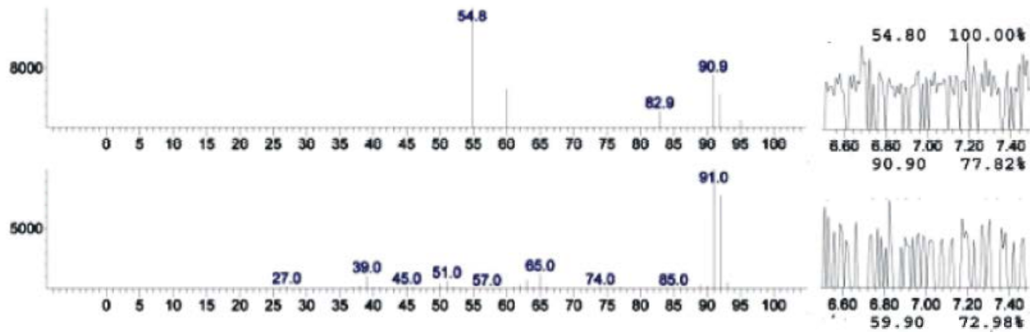
2-propane,1-amine



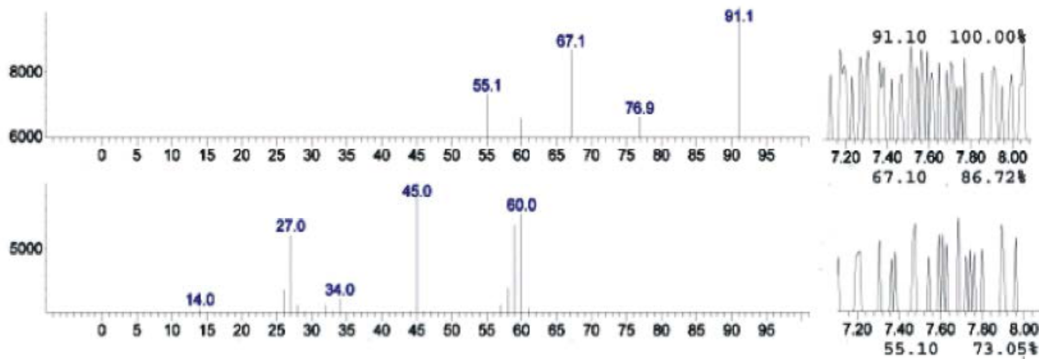
1,2-Dithiane



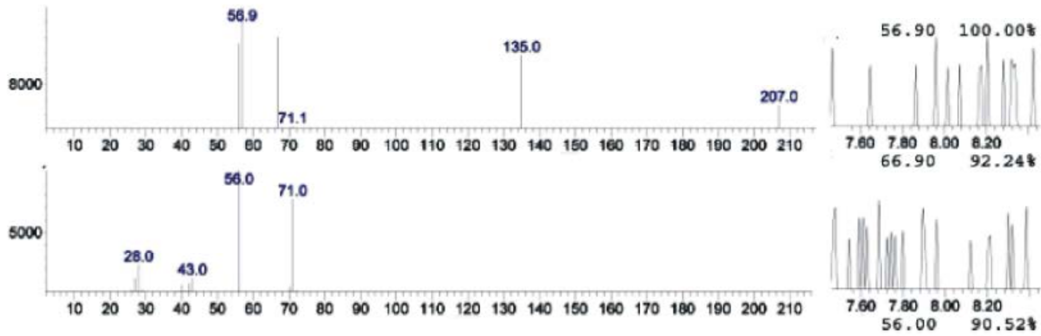
Toluene



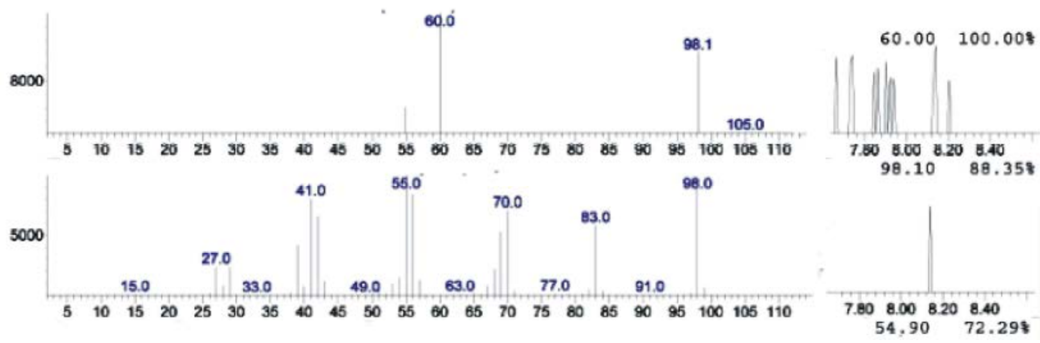
Thiirane



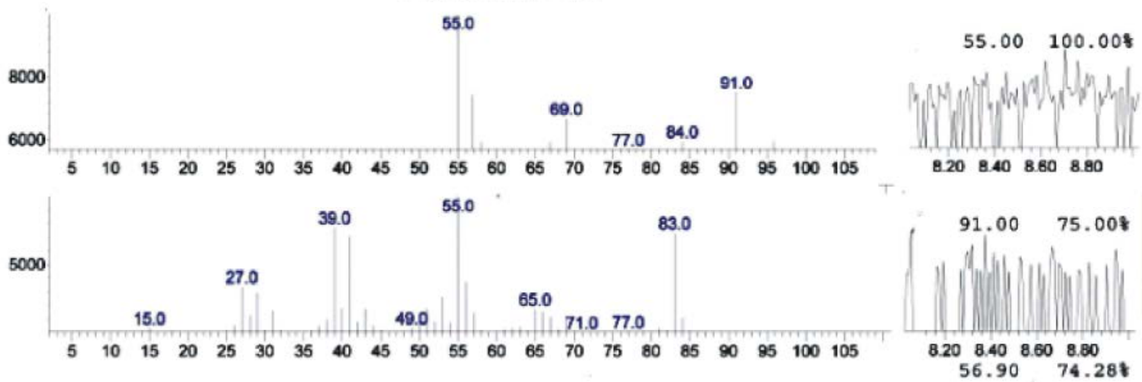
Ethane,Isocyanato



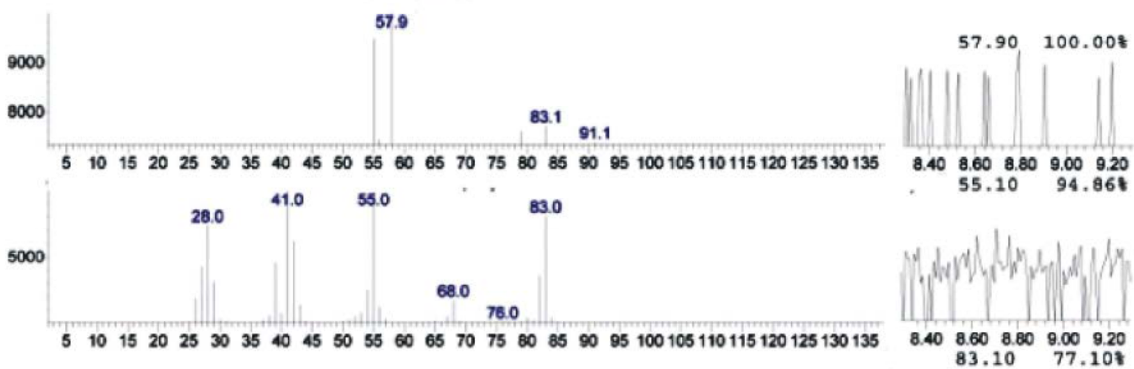
Cyclo Heptane



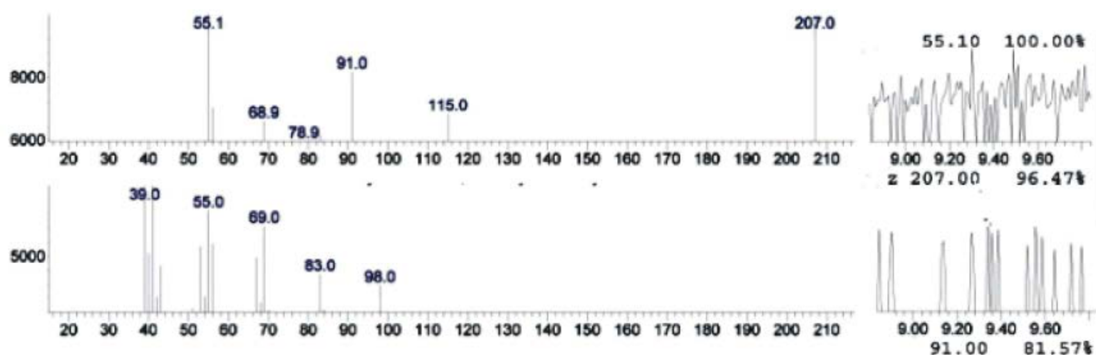
2-pentyne-1-ol



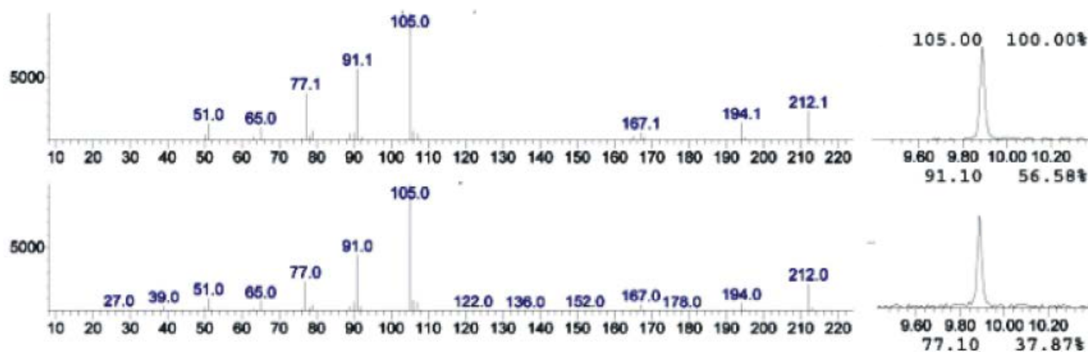
1-propenylaziridane



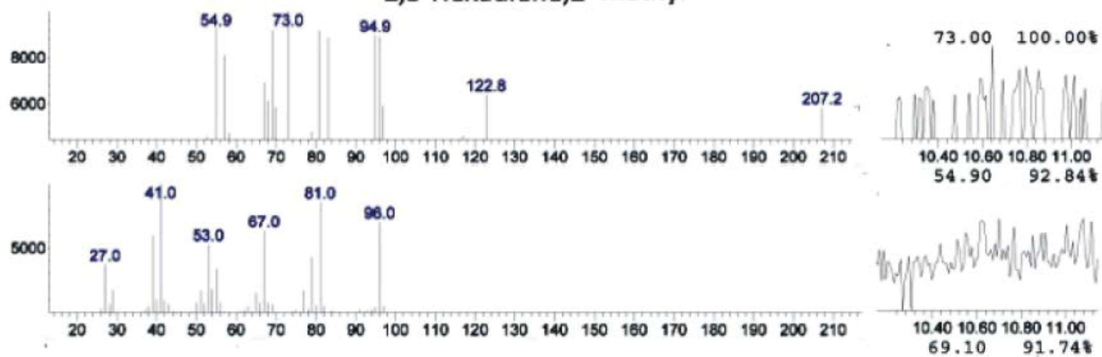
Cyclobutanone, 2-methyl-2-oxiranyl



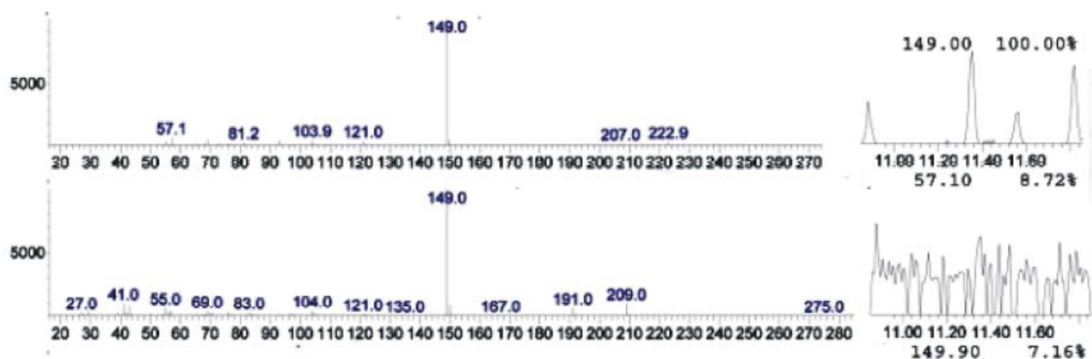
Benyl Benzoate



2,3-Hexadiene,2- methyl



Phthalic Acid



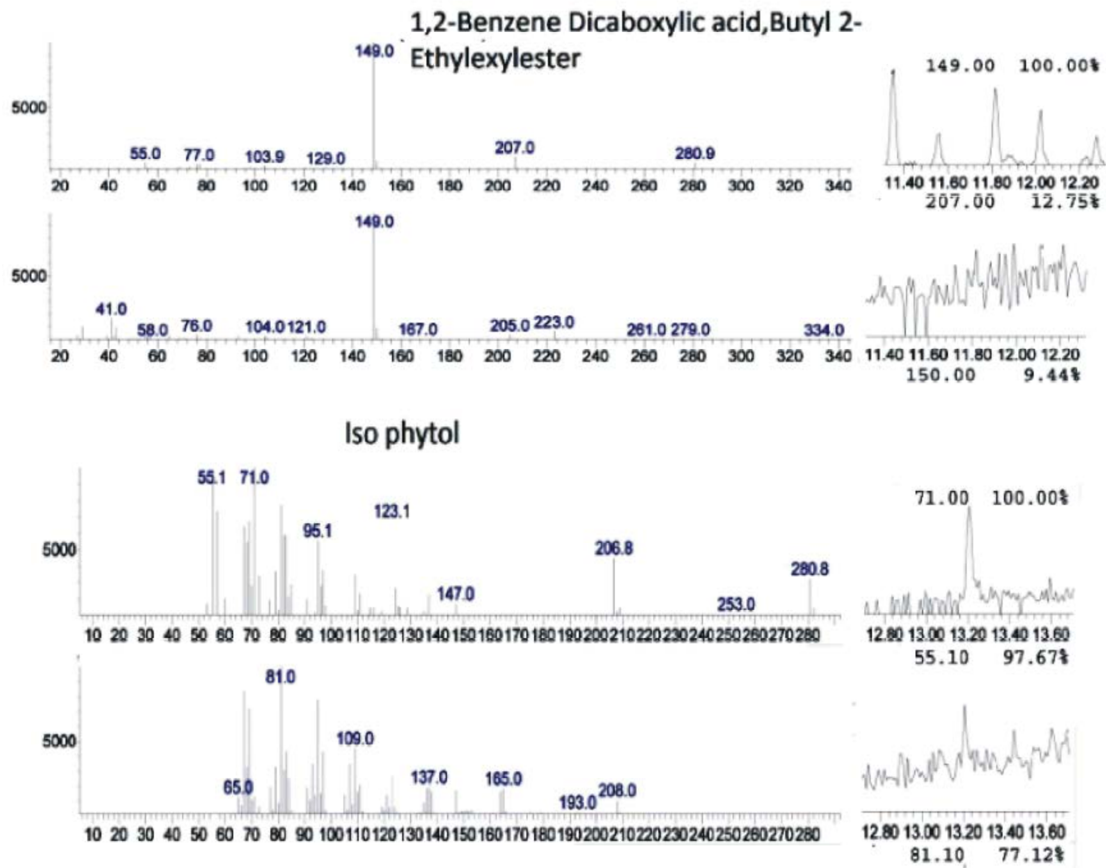
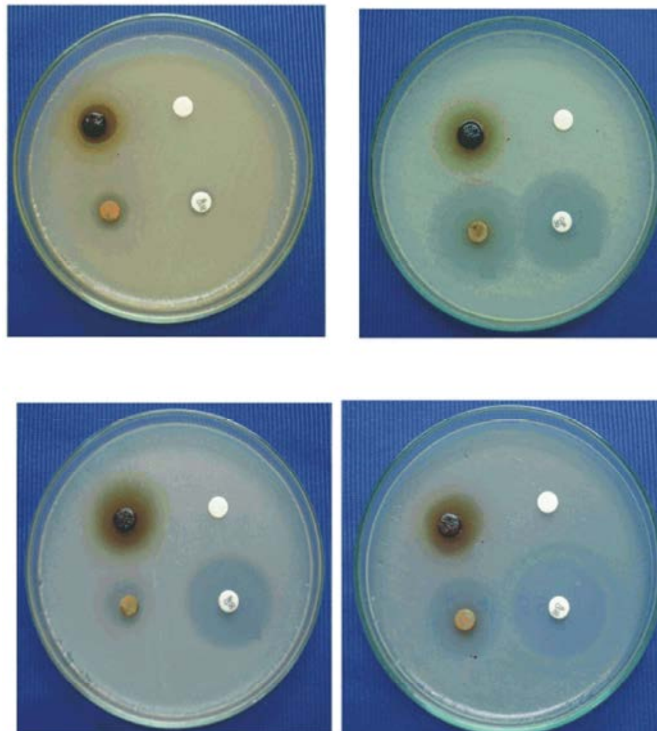


Fig. 2: Antimicrobial Activity of *Aristolochia bracteolate* Leaves Against Pathogenic Bacteria



DISCUSSION

In the present study, the phytochemical screening, identification of active compounds and antimicrobial activity were analysed against some pathogenic bacteria in the *Aristolochia bracteolate* leaves extract. The investigated results were discussed theoretically in this chapter.

In the present study, the phytochemical compounds of *Aristolochia bracteolate* were qualitatively analyzed. Further, the present study showed the presence of phytochemical compounds such as flavanoids, carbohydrates, amino acids and protein were present in both aqueous and acetone extract, whereas Phylobatannis, Volatile oil and Hydrolysable tannins present only in aqueous extract, saponin, tannins phenol, alkaloids, steroids, terpenoids and glycosides were present only in acetone extract. The phytochemicals like phenol, steroids, volatile oils and hydrolysable tannins were absent in both aqueous and acetone extract. Others study the phytochemical screening carried out on the *Aristolochia bracteata* leaf extracts. Phytochemical compounds present were found to be carbohydrates, flavonoids, saponins, alkaloids, steroids and proteins in both the extracts, fixed oils, fats, gums, mucilage and volatile oils were not detected in both the extracts. The variation in type of phytochemicals present in different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvents to dissolve into solution specific type of phytochemicals as reported by Yusha [31]. Tannins bind to proline rich proteins and interfere with the protein synthesis [32].

Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [33].

The phyto constituents like alkaloids, saponins and glycosides were reported to have various biological functions which include anticancer, anti-inflammatory and antimicrobial activities. Phenolic compounds which are commonly found in both edible and inedible plants are reported to have multiple biological effects, including antioxidant activity 15 and promotion of health benefits [34]. Alkaloids which are one of the largest groups of phytochemicals which has human based on toxicity against cells of foreign organisms and it may responsible for the antimicrobial activity of herbal extract. Saponin, which is one of the active constituents involved in plant disease resistance because of its antimicrobial activity [35]. Traditionally, saponins are subdivided into triterpenoid and steroid glycoside. Tannins are phenolic compound which act as primary antioxidants or free radical scavengers [36].

Herbal drugs contain unique constituents which differ from one herb to another, hence the type and extent of their medicinal property also differs [37, 38]. Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts will be different [39]. These results indicated that, the different extracts of the two plants under study exhibited antibacterial activity and among the various extracts, benzene and methanol extracts have shown better activity as compared to other extracts. The phytochemical analysis of the different extracts from the leaf and stem of both the plants revealed the presence of important phytochemicals. Further work on the types of phyto-constituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes.

Plants produce a diverse range of bioactive molecules making them rich source of different types of medicines. Various techniques are employed for their investigation which includes bioassays for chemical screening and their evaluation for presence of biological activities. Isolation of pure pharmaceutically active constituents from plants remains a long tedious process. Chemical screening is performed to target isolation of new or useful type of constituents having potential activities. This procedure enables recognition of known metabolites in extracts in the earliest stages of separation and thus economically very important. To characterize the bioactive compounds several techniques were used among the bioactive compounds several techniques were used among which chromatographic techniques were extensively used.

In the present study, the maximum level of inhibition showed in the standard disc ciprofloxacin. But the acetone extracts of *Aristolochia bracteolate* leaves are the maximum level of zone of inhibition showed in the pathogenic bacteria *Pseudomonas aeruginosa, Salmonella typhi* and *Bacillus subtilis* when compared to aqueous extracts, standard and control. In aqueous

extracts, there is no more variation occur in the level of zone of inhibition showed in the pathogenic bacteria like *Escherichia coli, Staphylococus aureus* compared to acetone extracts (Table 3 & 4). Panthi and Chaudhury [40] reported that presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative. The zone of inhibition against bacterial pathogens ranged between 42 – 19mm in methanolic extract and there is no activity found in aqueous extract. The maximum activity (42mm) was recorded from 200mg of methanolic extract of *A. bracteata* against *Streptococcus pyogenes* followed by 38mm against *Pseudomonas aeruginosa* and minimum (19mm) against *P. aeruginosa* at 50mg level. The antifungal activity of both the extract of *A. bracteata* leaf extracts were determined against four fungal strains and recorded in Fig. 2. The zone of inhibition against fungal pathogens ranged between 18 - 10mm in methanolic extract and there is no inhibition in aqueous extract. The maximum activity (18mm) was recorded from 200mg of methanolic extract against *Rhizopus indicus* and minimum (10mm) by the above fungus at 50 mg level. There was no activity observed in aqueous extract against the tested fungus. Our findings are in accordance with the observations of Ravindra [41] who proved that highest antifungal activity was observed with methanolic extract of *Capparis pepiaria* against the tested fungal strains.

In the present study, the eighteen active compounds like Cyclo pentane propnoic Acid, Hydrazine Carbothioamide, 1-Methyl-3-Piperidine Methanol, 2-Propane, 1-(4-Pyridinyl), 2-Propane, 1-amine, 1, 2-Dithiane, Toluene, Thiirane, Ethane, Isocyanato, Cyclo Heptane, 2-Pentyne-1-ol, 1-Propenylaziridine, Cyclobutanone, 2-methyl-2-oxiranyl, Benzyl Benzoate, 2, 3 Hexadiene, 2-methyl, Phthalic Acid, 1, 2-Bebzene Dicarboxdylicacid, Butyl 2-Ethylhexylester and Iso phytol were present, among those 4H Pyran- 4 one, 2, 3- dihydroxy- 6-methyl and 2-Hexadecen-1-OL, 3, 7, 11, 15-Tetramethyl were present in maximum level when compared to the other compounds (Table 2, Fig. 1) also studied [41].

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human disease because they contain numerous active constituents of therapeutics value. In recent years, ethnobotanical and traditional uses of natural compounds especially of plant origin received much attention as generally believed to be safe for human use. Through screening of literature available on *A. bracteata* depicted the fact that it is a popular remedy among the various ethnic groups. It is interesting to note that crude extract and aqueous extracts of root and leaf of *A. bracteata* have been screened for some pharmacological activities and anti-angeogenic, analgesic, anti-inflammatory and antioxidant, antibacterial, antiplasmodial, antimicrobial activity and trypansocidal activity. Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. In future study the isolated principle needs to be evaluated in scientifically animal model and clinical trial to understand the molecular mechanism of action, in search of lead molecules from natural resources. As the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from *A.bracteolata* should be emphasized for the control of various diseases.

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