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Antimicrobial efficacy and phytochemical analysis of *Albizia amara* (Roxb.) Boiv. an indigenous medicinal plant against some human and plant pathogenic bacteria and fungi.

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ABSTRACT

The present investigation evaluates the antimicrobial activity of six different solvent extracts and isolated constituents of leaves of *Albizia amara* against a total of 21 microorganisms which consisted of seven human pathogenic bacteria, a phytopathogenic *Xanthomonas campestris* (NCIM 2954) and thirteen seed-borne phytopathogenic fungi. Our result showed that, among the six solvent extracts tested, the chloroform extract showed a higher antibacterial and antifungal activity followed by methanol, ethanol and hydro-methanolic extracts respectively. The chloroform, methanol and ethanol extracts exhibited antibacterial activity with zone of inhibition ranging from 5.25 to 23.75, 6.25 to 23.25 and 7.25 to 22.7 millimeter respectively at 1mg/ml concentration. The minimal inhibitory concentration (MIC) of the chloroform extract ranged from 15µg/ml to 500µg/ml depending upon bacterial species. The most susceptible organism in the present investigation was *Streptococcus faecalis* (NCIM 5025), while the most resistant was *Proteus vulgaris* (NCIM 2027). Highest antifungal activity was observed in chloroform extract followed by methanol extract with percent of inhibition ranging from 30% to 77.4% and 17.4% to 71.7% respectively. The IC_{50} value of chloroform extract ranged from 0.5mg/ml to 5.0mg/ml depending upon fungal species. Among the tested fungi, *Fusarium lateratum* was highly sensitive and *Aspergillus flavus* was least sensitive. Chloroform extract was subsequently fractionated and monitored for antibacterial activity guided assay leading to the isolation of active fraction and was confirmed as alkaloid by further phytochemical analysis. The present study thus confirms antimicrobial property of *A. amara* and also demonstrated the role of *A. amara* used in traditional medicine.

Key words:*Albizia amara*, antimicrobial activity, human and plant pathogenic microbes, alkaloids, MIC and IC_{50} value.

INTRODUCTION

The number of multi drug resistant microbial strains and the appearance of the strains with reduced susceptibility to antibiotics are continuously increasing[1]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased[2]. As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and in some case all, effective antibiotics[3]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic bacteria, need to look alternative strategies for the management of antibiotic resistant bacteria.

Most food are prone to biodeterioration by moulds and other fungi during post harvest processing, transport and storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins[4]. A significant portion of the cereals produced in the world are reported to be contaminated with mycotoxins and other fungal metabolites which are reported to be toxic to man and animals[5]. Pesticides have made great contribution for quick and effective management of plant diseases and microbial contaminations in several agricultural commodities[6]. Seed treatment is the safest and the cheapest means to control seed borne fungal plant diseases and to prevent biodeterioration of grains. A large number of chemical fungicides are being used in the form of dusting, slurry and soaking treatment[7]. Excessive usage of pesticides in agriculture to overcome pre-harvest and post-harvest problem has resulted in many toxic epidemics. It is now realized that chemical fungicides cause serious environmental problems and are toxic to non-target organisms. Reports are available to many phytopathogenic microorganisms having acquired resistance to synthetic fungicides[8]. This seriously hinders the management of diseases of crops and agriculture products[9]. Thus there is an urgent need to search for alternative method for prevention of biodeterioration of grains during storage without any toxicity to the consumer, which are eco-friendly and effective.

The recent reports revealed that many phytochemicals have become lead molecules in various drug and/or pesticide discovery. In the last few years, a number of studies have been conducted in different countries to prove such efficacy of botanicals[2]. The potential of higher plants as a source for new drug and botanical pesticides is thus still largely unexplored. This is also true in India and only a small percentage of plants of this region have been evaluated for antimicrobial activity against plant and human pathogenic microbes. This is the authors screen *in vitro*, a large number of plants for antimicrobial activity against some important plant and human pathogenic microbes, with the ultimate aim of developing plant based formulation for plant and human disease management[3,10,11]. During regular screening *Albizia amara* recorded significant antibacterial and antifungal activity.

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Albizia amara belongs to the family Fabaceae and is globally distributed throughout the tropical and sub tropical regions. The leaves of *A. amara* are used as source for animal fodder and may also be used as firewood. It is folk remedy for curing various diseases *viz.*, dandruff, diarrhea, common cold, wounds and gonnorrhoea[12]. The antibacterial activity of *A. amara* was reported against some human pathogenic bacteria[13]. A scientific and systematic investigation with regard to the various biological activities of this plant is lacking. Thus considering vast potentiality of plants as a source of new chemotherapeutic and fungicidal agents, detailed investigations was conducted to test the efficacy of *A. amara* against some plant and human pathogenic microbes.

MATERIALS AND METHODS

Plant materials

Fresh disease free leaves of *A. amara* were collected from trees growing in the area of southern part of Karnataka, washed thoroughly 2-3 times with tap water and once with sterile distilled water, shade dried, powdered and used for extraction. A authenticated voucher specimen of the plant is deposited in the herbarium of Department of studies in Microbiology and Biotechnology, Bangalore university, Bangalore.

Preparation of hydro-methanolic extract

Fifty grams of shade dried, powdered leaves of *A. amara* was macerated with 250 ml aqueous methanolic extract in the ratio (3:2). The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000*g* for 30 minutes. The supernatant was filtered through Whatman No. 1 filter paper and heat sterilised at 121^{0}C for 30 min. The extract was preserved aseptically in sterile brown bottle at 4^{0}C until further use[14].

Preparation of solvent extract

Fifty grams of shade dried powder of *A. amara* was filled in the thimble and extracted successively with 200 ml of petroleum ether, toluene, chloroform, methanol and ethanol using a soxhlet extractor until colourless extract obtained on the top of the extractor. Each of the solvent extract was concentrated separately under reduced pressure using rotary flash evaporator. After complete evaporation of the solvent each of these solvent extract was weighed, dissolved in DMSO (Dimethyl sulphoxide) and subjected to antimicrobial activity assay. Only chloroform extract, which recorded highest antimicrobial activity, was used for determination of the minimal inhibitory concentration (MIC)[3].

Phytochemical analysis and separation of the active fraction from chloroform extract of *A. amara*

Hydro-methanolic extract and five successive solvent extracts of *A. amara* was tested for the presence of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, carbohydrates, terpenoids, amino acids, proteins and flavanoids[15-17]. The chloroform extract which showed highest antimicrobial activity was further fractionated into four different fractions viz., Acidic (Fraction 1), Basic (Fraction 2), Phenolic (Fraction 3) and Neutral (Fraction 4)[18]. All the four fractions were dried under reduced pressure, dissolved in DMSO and subjected to antibacterial activity assay. The fraction which showed activity was selected for further isolation of the active principle using TLC.

Table 1: Comparison of antibacterial activity of different solvent extracts of leaves of *Albizia amara* and standard antibiotics.

Bacterial Pathogens	Solvent Extracts						Standard antibiotics			
	Hydro Methanolic	Petroleum ether	Toluene	Chloroform	Methanol	Ethanol	Bacitracin (10 Units)	PenicillinG (10 Units)	Polymixin B (300 mcg)	Erythromycin (10 mcg)
E. coli NCIM 2065	10.7±0.3	0±0.0	7.1±0.1	11.5±0.5	10.1±0.1	9.6±0.2	0±0.0	0±0.0	15±0.2	14±0.2
Kl. pneumoniae NCIM 2957	9.7±0.3	0±0.0	8.7±0.6	12.7±0.6	10.7±0.4	10.7±0.4	0±0.0	0±0.0	16±0.3	28±0.4
Pr. vulgaris NCIM 2027	0±0.0	0±0.0	0±0.0	5.25±0.2	6.2±0.3	7.2±0.3	0±0.0	0±0.0	0±0.0	15±0.4
Ps. aeruginosa NCIM 5031	0±0.00	0±0.0	0±0.0	10.2±0.4	10.0±0.4	9±0.4	0±0.0	16±0.3	20±0.3	26±0.2
Salm. typhimurium NCIM2501	9.2±0.3	0±0.0	7.1±0.1	11.7±0.1	10.8±0.1	10.1±0.1	0±0.0	12±0.3	15±0.2	15±0.3
Staph. aureus NCIM 2079	7.2±0.3	0±0.0	8.2±0.3	15±0.0	12.7±0.2	12.0±0.3	28±0.3	32±0.2	22±0.3	22±0.3
Strep. faecalis NCIM 5025	20.2±0.3	0±0.0	16.5±0.5	23.7±0.3	23.2±0.4	22.7±0.2	28±0.2	18±0.2	19±0.4	22±0.3
X. campestris NCIM 2954	0±0.0	0±0.0	7±0.0	10.2±0.2	9.2±0.2	9.0±0.3	0±0.0	0±0.0	16.5±0.2	13±0.2

Data given are the mean of six replicates ± standard error, p > 0.001

Table 2: Antibacterial activity of different fractions and MIC values of chloroform extract of leaves of *Albizia amara*

Bacterial Pathogens	Different Fractions of Chloroform Extract				MIC of Chloroform Extract (µg/ml)
	Acidic	Basic	Phenolic	Neutral	
E. coli (NCIM 2065)	0±0.0	9±0.0	0±0.0	0±0.0	62.5
Kl. pneumoniae (NCIM 2957)	0±0.0	14.5 ±0.5	0±0.0	8±0.0	62.5
Pr. vulgaris (NCIM 2027)	0±0.0	9 ±0.0	0±0.0	0±0.0	500
Ps. aeruginosa (NCIM 5031)	0±0.0	11±0.0	0±0.0	0±0.0	250
Salm. typhimurium (NCIM 2501)	0±0.0	14.5±0.0	0±0.0	0±0.0	125
Staph. aureus (NCIM 2079)	0±0.0	15±0.3	0±0.0	9±0.3	62.5
Strep. faecalis (NCIM 5025)	0±0.0	26±0.0	0±0.0	15±0.0	15
X. campestris (NCIM 2954)	0±0.0	11.5 ±0.5	0±0.0	0±0.0	250

Data given are the mean of six replicates ± standard error at p > 0.001

Isolation of the active compound from basic fraction of *A. amara* by TLC system

The basic fraction which showed highest antibacterial activity was subjected to compound separation by TLC using methanol: ammonium hydroxide (1:0.25(v/v)) as an eluting solvent. The separated bands were identified under iodine vapor and retention factor (R_f) value of the spots were determined[19]. The respective bands were scraped out separately along with silica and dissolved in chloroform and filtered through Whatmann No. 1 filter paper and the filtrate was collected in glass vials and allowed to dry. After complete evaporation of chloroform, all the bands were dissolved in DMSO and subjected to antibacterial activity assay.

Organisms used for antimicrobial assay

Seven human pathogenic bacteria *viz.*, *Staphylococcus aureus* (NCIM No. 2079), *Salmonella typhimurium* (NCIM No. 2501), *Streptococcus faecalis* (NCIM No. 5025), *Escherichia coli* (NCIM No. 2065), *Klebsiella pneumoniae* (NCIM No. 2957), *Proteus vulgaris* (NCIM No. 2027), *Pseudomonas aeruginosa* (NCIM No. 5031) and a plant pathogenic bacteria *Xanthomonas campestris* (NCIM No. 2954) were obtained from National centre of industrial microorganisms, National chemical laboratory, Pune, India, which served as test bacteria for antibacterial activity assay.

A total of thirteen phytopathogenic fungi viz.,*Alternaria brassicola*, *Alternaria geophila*, *Aspergillus flavus*, *Aspergillus tamari*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Curvularia tetramera*, *Fusarium moniliforme*, *Fusarium equiseti*, *Fusarium lateratium*, *Fusarium udum*,*Penicillium chrysogenum* and *Penicillium citrinum*, were isolated from seeds of sorghum, paddy and maize using standard blotter method(SBM) and agar plating method[20], which served as the test fungi for antifungal activity assay.

Antibacterial activity assay

Antibacterial activity of the hydromethanolic extract, five different successive solvent extracts and isolated constituents of *A. amara* were determined by disc diffusion method on the Muller-Hinton agar (MHA) medium[21]. In this method, 5 mm sterilized filter paper discs (Whatmann no. 1) were saturated with sterilized plant extracts and isolated constituents of desired different concentrations. The impregnated discs are then placed on to the surface of MHA medium. The MHA media was pre-inoculated with test bacteria (inoculum size 1 X 10^8 CFU/ml). For each treatment six replicates were maintained. The disc devoid of extract consisting of only dimethyl sulfonoxide (DMSO) served as control. The plates were kept at 4 ^{0}C for 1 hour for diffusion of extract, thereafter the plates were incubated at 37 ^{0}C for 24 hours. After incubation, zone of inhibition if any around the disc was measured in mm (millimetre). Polymixin B (300mcg/disc), Bacitracin (10U/disc), Erythromycin (10mcg/disc) and Penicillin-G (10U/disc) were used as positive reference to determine the sensitivity of each bacterial species tested. The last concentration of chloroform extract showing a clear zone of inhibition was taken as the MIC[22].

Antifungal activity assay

Hydro-methanolic extract and five different successive solvent extracts of *A. amara* were subjected to antifungal activity assay by poisoned food technique[3]. The desired different concentrations of hydro-methanolic extract and solvent extracts were separately added to the Czapeck Dox Agar medium, autoclaved and poured into petridishes (20 ml each) and allowed to cool. After complete solidification of the medium, 5 mm disc of 7 day old culture of the test fungi were inoculated. Four replicates were maintained for each extracts. The petridish containing media devoid of extract containing only DMSO, served as control. The plates were incubated at 26±1^{0}C for seven days. The fungi toxicity of the extract in terms of Percentage Inhibition (%) of mycelial growth was calculated by using the formula,

Percentage Inhibition = dc-dt X 100/dc

Where, dc - Average increase in mycelial growth in control.

dt - Average increase in mycelial growth in treatment

The synthetic fungicides, viz., Blitox and Dithane M-45 which are commonly used for seed treatment were obtained from Bangalore agrochemical market. They were tested at their recommended dosage (2mg/ml) for antifungal activity by poisoned food technique for comparison.

RESULTS

Antibacterial activity assay

The inhibitory activity of the hydro-methanolic extract, five different successive solvent extracts and isolated constituents of leaves of *A. amara* against seven human pathogenic bacteria and a phytopathogenic *X. campestris* is presented in Table 1. Among the six different extracts tested, chloroform extract recorded highest antibacterial activity followed by methanol, ethanol and hydro-methanolic extracts, whereas no significant antibacterial activity was observed in petroleum ether extract. The control DMSO did not inhibit any of the bacteria tested. The chloroform, methanol and ethanol extracts exhibited antibacterial activity with zone of inhibition ranging from 5.25 to 23.75, 6.25 to 23.25 and 7.25 to 22.7 millimeter respectively at 1mg/ml concentration. The most susceptible organism in the present investigation was *Streptococcus faecalis* (NCIM 5025) followed by *Staphylococcus aureus* (NCIM 2079) and *Pr. vulgaris* (NCIM 2027) was found to be most resistant bacteria against all the extracts tested. The present study clearly indicates Gram positive bacteria were more susceptible than Gram negative bacteria. The antibacterial activity of synthetic antibiotics viz., bacitracin, erythromycin, penicillin-G and polymixin B revealed that all the pathogenic bacteria are comparatively susceptible to erythromycin with zone of inhibition ranging from13mm to 28mm. Bacitracin was not active against all the test pathogens except *Staph. aureus* and *Strep. faecalis*. Penicillin-G was not effective on *E. coli*, *Kl. pneumoniae*, *Pr. vulgaris* and *X. campestris*. Polymixin B was not effective on *Pr. vulgaris* The results of the present investigation demonstrate that *E. coli, Kl. pneumonia, Pr. vulgaris and X. campestris* were resistant to bacitracin and penicillin-G. However these bacteria were effectively inhibited by chloroform, methanol and ethanolic extracts of *A. amara*.

The range of MIC of the chloroform extract was 15µg/ml to 500µg/ml depending upon bacterial species. In the present investigation lowest MIC value 15 µg/ml was observed in *Strep. faecalis*, whereas highest MIC value 500 µg/ml was observed in *Pr. vulgaris*. With increasing concentration there was increased antibacterial activity in all the bacterial species. Further separation of chloroform extract exhibited that the inhibitory activity was retained in basic fraction with zone of inhibition ranging from 9.0mm to 26.0mm at 500µg/ml concentration, whereas acidic, phenolic did not show significant antibacterial activity against all tested bacteria and subsequent separation of basic fraction on TLC showed two bands (R_f value 0.29 and 0.52). Activity guided antibacterial assay revealed that band 2 (R_f value 0.52) recorded significant antibacterial activity against all test bacteria.

Antifungal activity assay

The antifungal activity of hydro-methanolic and five different successive solvent extracts of *A. amara* is presented in the Table-3. In hydro-methanolic extract, mycelial growth inhibition was observed against all the test fungi and the extent of mycelia growth inhibition was varied from 68.4% to 89.3% depending on the fungal species tested. In the present investigation it is also interesting to show that all fungi recorded more than 50% of mycelial growth inhibition at 40% concentration. Among the thirteen test fungi, *Fusarium udum* (PI 89.3%) is highly susceptible followed by *F. moniliforme* (PI 87.0%), while *Curvularia tetramera* (PI 68.4%) is least susceptible.

Among the five different successive solvent extracts tested, highest antifungal activity was observed in chloroform extract followed by methanol extract with percent of inhibition ranging from 30% to 77.4% and 17.4% to 71.7% percentages respectively. The IC_{50} value of chloroform extract ranged from 0.5 mg/ml to 5.0 mg/ml depending upon fungal species. The percent mycelial inhibition of two synthetic fungicides viz., Blitox and Dithane M-45 revealed that, among the 13 fungi tested against Blitox, *F. equiseti* (PI 97.2%) was

Table 3: Antifungal activity of hydro-methanolic, different solvents and IC_{50} value of chloroform extract of leaves of *Albizia amara*

Fungal Pathogens	Hydro-methanolic extract (40%)	Solvent Extracts (1 mg/ml) Petroleum ether	Toluene	Chloroform	Methanol	Ethanol	IC_{50} value of chloroform extract(mg/ml)	Synthetic fungicide(2 mg/ml) Blitox	Dithane M-45
Alternaria brassicola	68.5±0.5	28.1±0.8	48.4±0.7	64.3±2.4	47.6±0.7	57.3±1.1	0.5	83.4±0.5	79.1±0.2
Al. geophila	81.4±0.5	34.2±1	50.7±0.5	64.2±0.4	67.1±0	55.7±0.3	0.5	90.9±0.3	68.9±0.3
Aspergillus flavus	69.6±0.5	15±0.5	24.2±1.5	30±0.5	43.9±3.5	32.9±0.2	3.0	92.3±0.3	47.6±0.3
A. fumigatus	81.8±0.5	13.1±1	32.2±0.5	58.5±0.5	71.7±0.5	50.6±0.5	0.5	96.0±0.3	63.1±0.1
A. terreus	75.4±0.5	17.8±1.5	36.4±0.5	38.2±1.5	45.7±0	40.6±1	2.0	95.4±0.2	24.4±0.3
A. tamari	80.4±0.5	5±0.5	15±0.5	37.7±2	50±0.3	28.8±1	3.0	83.5±0.3	78.9±0.3
Curvularia tetramera	68.4±0.3	25.±2.3	42.2±1.1	63.0±0.3	44.8±0.8	56.0±0.3	0.5	94.5±0.3	74.0±0.6
Fusarium moniliforme	87.0±1.5	42.9±1.2	51.4±0.7	57.9±0.1	57.6±0.3	57.9±0.7	0.5	83.8±0.2	58.9±0.3
F. equiseti	83.7±0.5	10.7±0.0	24.6±0.8	67.6±0.6	20.9±0.3	74.1±1.5	0.5	97.2±0.4	69.8±0.8
F. lateratium	82.2±0.5	33.0±1.4	58.8±0.5	77.4±0.3	61.8±0.8	73.7±2.1	0.5	89.6±0.6	69.1±0.1
F. udum	89.3±0.5	0.0±0.0	22.2±1.2	70.4±0.8	17.4±0.8	36.3±1.4	0.5	87.8±0.2	68.3±0.3
Penicillium chrysogenum	76.7±0.5	-2±0.5	24.0±2	42±0.5	30±0.5	28±0.5	3.0	89.0±0.0	72.4±0.3
P. citrinum	81.2±0.5	7.8±0.5	18.1±0.5	31.1±0.5	24.6±0.4	20.8±0.5	5.0	93.9±0.3	63.9±0.3

Data given are the mean of four replicates ± standard error, Analysis of variance (ANOVA) d.f=8 at p > 0.001

Table 4: Phytochemical Analysis of leaves of *Albizia amara*

Phytochemical tests	Hydro methanolic extract	Petroleum Ether extract	Toluene extract	Chloroform extract	Methanol extract	Ethanol extract
Saponins	+	-	-	-	+	-
Tannins	-	-	-	-	+	-
Alkaloids	+	-	-	+	+	+
Cardiac Glycosides	+	-	-	-	+	+
Carbohydrates	+	-	-	-	+	+
Flavanoids	+	-	-	-	+	+
Phlobatannins	-	-	-	-	-	-
Steroids	-	+	+	+	-	-
Terpenoids	+	-	-	-	+	+
Glycosides	+	-	-	-	+	+
Amino acids	-	-	-	-	-	-
Phenolic Compounds	-	-	-	-	-	-
Quinones	+	-	-	-	+	+
Anthraquinones	-	-	-	-	-	-

+ Present, - Absent

highly susceptible and *Alternaria brassicola* (83.4%) exhibited least activity. In case of Dithane M-45, *Al. brassicola* was highly sensitive (79.1%) while *A. terreus* (24.4%) was least sensitive. The antifungal activity of hydro-methanol, chloroform and methanol extracts were almost equivalent to that of synthetic fungicides at 2 mg/ml concentration. The present investigation clearly demonstrated the first time antifungal property of *A. amara* against seed-borne fungi.

Phytochemical analysis

Investigation on phytochemical analysis of different extracts viz., hydro-methanolic, petroleum ether, toluene, chloroform, methanol and ethanol extracts of *A. amara* is presented in the table 4. Phytochemical analysis of chloroform extract which recorded significant antimicrobial activity revealed the presence of steroids and alkaloids. In methanol extract, saponins, tannins, alkaloids, cardiac glycosides, carbohydrates, flavanoids, terpenoids, glycosides and quinones are present.

DISCUSSION

Association of variety of fungi including species of *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Curvularia*, *Drechslera*, causing significant loss in seed quality and nutritional quality of sorghum, maize and paddy have been reported[23-25]. The management of fungal contamination of harvested seeds and grains generally achieved by using fungicides. Chemical fungicides such as copper carbonate, sulphur, organic acid, inorganic mercurial compounds, carboxin, benomyl, captan, thiram, carboxin etc. are generally applied for the management of seed borne fungal diseases and fungal bio-deterioration[26,27]. Even though effective and efficient control of seed borne fungi of seeds can be achieved by the use synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity[28]. The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective. On the other hand spread of multi drug resistant (MDR) strains of bacteria and fungi necessitates the discovery of new class of antibiotics and fungicide that inhibits antibiotic resistant pathogens[29].

Many recent reports revealed that many phytochemicals have become lead molecules in various drug and/or pesticide discovery programs[30]. Raskin *et al*., (2002)[31] reported that rediscovery of the connection between plants and health is responsible for launching new generation of botanical therapeuticals, multicomponent botanical drugs, dietary supplements and functional foods. The World Health Organization (WHO) is encouraging, promoting, and facilitating the effective use of herbal medicine in developing countries for health programs. The potential of higher plants as a source of new drugs is still largely unexplored. Only 5-15% of the higher plants have been systematically investigated for the presence of bioactive compounds. About 1% of the total known medicinal plants species is acknowledged to therapeutic value for human health benefits[32,33]. Varma and Dubey (1999)[34] reported that plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. Considering these the present investigation is an important step in developing plant based drugs and fungicides which are eco-friendly for the management of the pathogenic bacteria and fungi. Considering these, sixty four plants were screened *in vitro* for antibacterial and antifungal activity against some human and plant pathogenic bacteria and fungi. These plants were selected based on traditional medicine knowledge. On the basis of zone/percentage of inhibition, leaves of *A. amara* showed significant antimicrobial activity. The present investigation clearly demonstrated the first time antimicrobial activity of *A. amara* against some important plant pathogenic fungi and bacteria. The results of the present investigation revealed that the antimicrobial activity of chloroform extract was highest followed by methanol extract. All the test bacteria were inhibited by chloroform extract of *A. amara* demonstrating broad spectrum of activity. The present study also clearly indicates that chloroform was the most effective solvent for extracting antimicrobial compound from *A. amara*. Further separation of chloroform extract to isolate and identify the active principle responsible for antimicrobial activity revealed that significant antimicrobial activity was observed in basic fraction. Subsequent separation of basic fraction to isolate and identify the active compounds responsible for antimicrobial activity by TLC revealed that the presence of 2 bands. The result of the present investigation suggests that band-2 (R_f value 0.52) - an alkaloid compound responsible for antimicrobial activity. Further work is going on the identification and characterization of active principle based on Nuclear Magnetic Resonance (NMR), Mass Spectral analysis (MS) and Infra Red (IR) spectral analysis. The present investigation is an important step in developing plant based pesticides and drugs which are eco-friendly for the management of the pathogenic bacteria and fungi and also the development of commercial formulation of botanicals. Further investigations are necessary for developing commercial formulation based on field, animal trails and toxicological experiment.

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