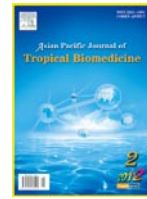




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(12)60321-9 ©2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Screening for antibacterial principle and activity of *Aerva javanica* (Burm .f) Juss. ex Schult.

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ARTICLE INFO

Article history:

Received 22 June 2012
Received in revised form 5 July 2012
Accepted 7 August 2012
Available online 28 August 2012

Keywords:

Aerva javanica
Antibacterial activity
MIC
HPTLC
Bioautography

ABSTRACT

Objective: To investigate the antibacterial principle and activity of *Aerva javanica*, a medicinal plant. **Methods:** Crude extracts of different parts of *Aerva javanica* were made with hexane, chloroform and methanol. Phytochemical analysis of the crude extracts was done by following the standard methodology, and antibacterial activity was evaluated by inhibition zone and MIC values. Crude extracts were resolved through HPTLC and the antibacterial activity of the separated compounds was evaluated by bioautography. **Results:** The yields of crude extracts made from different plant parts varied both with plant part and solvent. Methanolic extracts of leaf and flower have shown a wide range of phytochemicals and more antibacterial activity. HPTLC separation of extracts coupled with bioautography studies revealed that apigenin followed by rutin and kaempferol has shown antibacterial activity against more number of bacteria. **Conclusions:** The present study supports the use of *Aerva javanica* in the traditional medicine, and it can be used against bacterial infections.

1. Introduction

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care^[1]. They have many advantages such as safety, efficacy, cultural acceptability, better compatibility with human body and lesser side effects. Ancient literatures also mention herbal medicines for age-related diseases like memory loss, osteoporosis, diabetic wounds, immune and liver disorders, *etc* for which no modern medicine or only palliative therapy is available. Life saving and essential drugs from medicinal plants such as morphine, dioxin, aspirin, emetine, and ephedrine were introduced into modern therapeutics several centuries ago. Namdeo stated that about a quarter of all prescribed pharmaceuticals in advanced countries contain compounds that are directly or indirectly derived from plants^[2]. Today, there is a wide spread interest in drugs derived from plants, and it is reported that green medicine is safe and dependable.

Research in herbal medicine and isolated drug discovery need to be continued, considering the treatment of new emerging disease such as SARS, bird flu and not to mention AIDS. There would be requirements of scientific evidence to confirm the folklore practices. One such evidence comes from investigations on antimicrobial activity of selected plants using contemporary antimicrobial tests. Knowledge of the phytochemical constituents is essential in the investigations of the actual effectiveness of the plant in medicine^[3]. In nature, besides physical stresses, plants are exposed to a plethora of hostile microorganisms and naturally they have developed an arsenal of weapons to counter the attacks of microbial invasions^[4]. Apart from preexisting general defense constituents, a wide variety of specific new compounds are synthesized at post infection stage. Obviously, they are expected to contain a variety of antimicrobial compounds^[5]. Some of these anti-microbial compounds in plants may be exploited to against bacterial diseases in man.

In developing countries, low-income people such as farmers, people of small isolated villages and native communities which have no access to modern medicine depend on traditional medicines and use many native plants for the treatment of common diseases. An extensive survey and interaction with local ethanopharmacologists, herbal

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Foundation project: It is supported from UGC New Delhi, under SAP (DRS) (No F.3-23/2009 (SAP)-II).

drug sellers and rural native healers revealed that the native plant *Aerva javanica* (*A. javanica*) is routinely and widely used for the treatment of various ailments of humans and live-stock. Naturally, it tempted us to verify the traditional wisdom of local community in using this plant as herbal drugs especially its antimicrobial properties.

The plant *A. javanica* (Burm.f)Juss. ex Schult. (vernacular names : Telugu–Tella Buraga, English–Kopak Bush, Hindi–Safed shamli, Gujarati–Safed Shimlo, Marathi–Shamil, Bengali–Swet shimul, Kannada–Dudi, Tamil–Panchu) belonging to Amaranthaceae family is an erect perennial herb and widely distributed in the various parts of the world. It is native to Africa and also found in some Asian countries. In traditional medicine, the herb (Figure 1) is used as diuretic, diabetic demulcent and decoction of plant is used to ameliorate swellings and urinary disorders. Powders of the plant are applied externally to treat ulcers in domestic animals. The seeds are used to relieve head ache and also rheumatism. In Ayurveda the leaves, seeds and roots are used for treatment of kidney stones, and as astringent[6]. To the best of our knowledge no reports are available on the antibacterial activity of *A. javanica* plant. The present study therefore was undertaken to evaluate the antibacterial activity to verify the authenticity of its use in traditional medicine and also to project the plant as a source of potential antibacterial agents.

2. Material and methods

Fresh parts of the plant free from diseases were collected and brought to the laboratory in sterile polyethylene bags and washed thoroughly 2–3 times with running tap water and then once with sterile water, shade dried for two weeks, subsequently ground into fine powder using mechanical grinder and motor driven grinding mill. The powder was used for extraction of crude extracts with organic solvents.

2.1. Method of extraction

Sequential extraction method was employed to extract the plant powders using different polar solvents starting from non polar to polar namely hexane, chloroform and methanol[7]. 100 g of dried powder of plant material (leaf, flower, stem and root) was soaked in 300 mL of respective solvent in an air tight bottle separately and kept in an electric shaker at room temperature for 72 h. The resultant suspensions were first filtered through a double layered muslin cloth and then with Whatman No.1 filter paper and each filtrate were collected into conical flask, separately. Above extraction procedure was repeated thrice and the extract was concentrated under reduced pressure and low temperature using rotary evaporator. The dried extracts were stored in refrigerator until used for the assay.

2.2. Phytochemical analysis of extracts

Phytochemical analysis of the extracts was carried out as per the standard methods[8–10].

2.3. Antimicrobial assay

The microbial strains used in the present study were obtained from National Chemical Laboratory. The bacterial strains comprised: *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* NCIM 2719, *Proteus vulgaris* NCTC 8313, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas putida* ATCC 12842, *Salmonella typhimurium* ATCC 23564, *Bacillus cereus* ATCC 12842, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29737 and *Staphylococcus epidermidis* ATCC 12228.

The bacteria were maintained in Mueller–Hinton Agar. Inocula were prepared by adding an overnight culture of the organisms in nutrient broth to obtain an OD₆₀₀ of 0.1. The cultures were allowed to grow until they obtained the McFarland standard 0.5 (approximately 10⁸ CFU/mL). The suspensions were then diluted to 1:100 in nutrient broth to obtain 10⁶ CFU/mL. 30 mL of sterile molten agar medium was seeded by organisms (about 2 mL according to McFarland's standard), in semi hot conditions (40 °C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on the agar medium using sterile borer and 0.1 mL of the extracts at 100 mg/mL concentration in DMSO was added to respective bores. 0.1 mL of the standard streptomycin at a concentration of 100 µg/mL was used as standard. The Petri plates seeded with organisms, containing extracts and the standard were kept in a refrigerator at 4 °C for 1 hour to facilitate the diffusion of the extracts and the standard in to the media. After diffusion, the Petri plates were incubated at (37±1) °C for 24 h in an incubator and later the zone of inhibition was observed and measured using a scale[11].

2.4. Minimum inhibitory concentration (MIC)

MIC was determined by agar dilution method[11]. In this study, the extracts were taken at different concentrations ranging from 0.156 to 5.0 mg/mL. It involved a series of six tubes for each test compound against each strain. To the first assay tube, 1.9 mL of seeded broth was transferred and then 0.1 mL of test extract of 50 mg/mL was added to it and mixed thoroughly. To the remaining five assay tubes, 1 mL of seeded broth was transferred. From the first tube 1 mL of the content was pipetted out into second test tube and this was mixed thoroughly. This twofold serial dilution was repeated up to sixth tube.

2.5. Statistical analysis

Diameter of zone of inhibition (excluding well diameter)

resulted from replicates were expressed as mean \pm standard deviation (SD). The data were analysed by one-way analysis of variance (ANOVA). *P* value < 0.05 was considered as significant and mean values were compared by using Least Significant Difference test using computer software, SAS system for windows (version 8).

2.6. Thin layer chromatography (TLC)

TLC was performed on analytical plates coated with silica gel (TLC-grade; Merck India) using an appropriate mobile phase. The loaded TLC plate was carefully placed in the TLC chamber with the sample line towards the bottom. The plate whose top was leaned against the jar wall was adjusted to sit on the bottom of the chamber and in contact with the developing solvent (solvent surface was below the extract line). The TLC chamber was covered and the plate was allowed to remain undisturbed. When the solvent front reached three quarters of the length of the plate, the plates were removed from the chamber and the position of the solvent front was immediately marked. Then plates were allowed to air dry until solvent was removed and then placed in iodine chamber for visualizing spots. The R_f values of resolved compounds were calculated.

2.7. High performance thin layer chromatography (HPTLC)

Qualitative analysis was performed with the help of HPTLC instrument (Camag, Muttenz, Switzerland) which consisted TLC scanner connected with a PC running Win CATS software under MS Windows NT; Linomat V sample applicator and a photo documentation system, Camag, Reprostar III^[12,13].

2.8. Spotting of samples

The chromatographic separation was performed by streaking the extracts in the form of narrow bands of 6 mm length on the precoated silica gel 60 F254 aluminum TLC plate (5 cm \times 10 cm), at a constant application rate of 150 μ L/s and gas flow of 10 s/ μ L was employed with help of Camag 100 μ L syringe connected to a nitrogen tank: using a Camag Linomat V. The space between three bands was kept 15 mm. 5 μ L of 1% concentration solution from each of three extracts (methanol, chloroform and hexane) was placed as a spot.

2.9. Plate development and chromatographic conditions

After spotting, the plate was subjected to linear ascending running up to a distance of about 90 mm in a suitable solvent system, in Camag Twin Trough glass chamber, which was saturated with the same solvent system at room temperature just 10 minutes prior to development.

2.10. Scanning of plate

TLC plates were dried in flowing air at room temperature. Densitometric scanning was carried out using Camag TLC Scanner III between wavelength of 254 & 366 nm with a slit dimension of 6.00 mm \times 0.30 mm, scanning speed of 20 mm/s, and data resolution at 100 μ m/step. The source lamps for radiation were deuterium and tungsten lamps. All remaining measurement parameters were left at default settings. The chromatograms were integrated and regression analysis and statistical data were generated using Win CATS evaluation software (version 1.4.2.8121).

2.11. Bio-autography

Plant extracts (5.0 μ L) were applied at 2.5 cm from the base of precoated TLC plate (60 F254). After drying, the TLC plates were developed in a suitable mobile phase and run in duplicate, one set was used as the reference chromatogram and other set for bio-autography.

The reference TLC plates were then developed and visualized under visible and UV light at 254 and 366 nm^[14]. The chromatograms so developed were placed in a TLC chamber (10 cm \times 20 cm) filled with bacterial culture (at 10⁶ CFU/mL dilution in molten Mueller Hinton agar) to get a uniform coat of bacterial culture over the TLC plates. After the solidification of the medium, the TLC plates were incubated overnight at 37°C. Bioautograms developed were sprayed with 1% aqueous solution of 2, 3, 5, triphenyl tetrazolium chloride and incubated at 37°C for four hours. Inhibition zone indicated the presence of active compounds^[15].

3. Results

3.1. Extraction and preliminary phytochemical analysis of plant extracts

The yields of extractions made from different plant parts *viz.* leaf, stem, root and flower with hexane, chloroform and methanol sequentially varied with plant part and also polarity of the solvents. In most of the cases, the amount of residues extracted with methanol was higher when compared to other two solvents and in case of plant parts, leaf and flower yielded more amounts of extracts.

The phytochemical analysis of methanolic extraction of leaf of revealed the presence of alkaloids, glycosides, steroids, terpenoids, resins and cardiac glycosides (Table 1). The chloroform fraction of leaves revealed the presence of flavonoids, glycosides, phenols, saponins, tannins, cardiac glycosides and carbohydrates but negative results were obtained for alkaloids, steroids, resins and terpenoids. In hexane extract, alkaloids, steroids, terpenoids and cardiac glycosides were present in moderate concentrations. Hexane fraction of flower revealed the presence of only tannins and

Table 1.
Phytochemical analysis of extracts of leaf, stem, flower and root of *A. javanica*.

Phytochemical constituents	Hexane				Chloroform				Methanol			
	Leaf	Flower	Stem	Root	Leaf	Flower	Stem	Root	Leaf	Flower	Stem	Root
Flavonoids	–	–	–	–	++	+	+	+	++	+++	++	++
Alkaloids	–	–	–	–	–	+	+	–	–	++	+	–
Glycosides	–	–	–	–	+	+	+	+	++	++	+	++
Steroids	++	++	++	++	–	+	–	+	+	++	–	++
Phenols	–	–	–	–	+	+	+	+	++	++	++	++
Terpenoids	+	–	+	++	–	–	–	+	–	–	–	–
Saponins	–	–	–	–	+	+	+	+	+++	++	++	++
Resins	–	–	–	–	–	+	+	–	+	+	+	+
Tanins	–	+	–	–	+	–	+	–	++	++	+	–
Cardiac glycosides	++	–	–	–	+	+	+	+	++	+	++	++

– Absent; + Trace amount; ++ Moderate amount; +++ High amount.

Table 2.
Antimicrobial activity of extracts of *A. javanica* (leaf, flower, root and stem extracts).

Test organisms	Hexane				Chloroform				Methanol				Streptomycin (100 mg/mL)
	L	F	S	R	L	F	S	R	L	F	S	R	
<i>Escherichia coli</i>	10	9	–	10	8	10	8	10	14	12	–	12	18.0±0.03
<i>Enterobacter aerogenes</i>	–	10	–	10	–	10	–	–	12	13	–	10	20.0±0.03
<i>Klebsiella penumoniae</i>	14	10	–	10	8	16	8	9	15	13	–	14	14.0±0.33
<i>Proteus vulgaris</i>	–	–	–	–	–	–	–	–	–	10	–	10	18.0±0.33
<i>Pseudomonas aeruginosa</i>	10	10	–	10	8	18	8	12	16	13	10	13	14.0±0.33
<i>Pseudomonas putida</i>	–	–	–	–	–	–	–	–	13	11	–	14	18.0±0.33
<i>Salmonella typhimurium</i>	–	10	8	12	12	14	–	11	13	16	8	14	18.0±0.33
<i>Bacillus cereus</i>	14	11	8	–	9	16	10	–	11	13	8	–	20.0±0.33
<i>Bacillus subtilis</i>	8	12	–	15	8	16	–	13	12	12	–	15	16.0±0.33
<i>Staphylococcus aureus</i>	8	–	–	8	8	14	–	–	12	9	–	–	15.0±0.33
<i>Staphylococcus epidermidis</i>	10	12	–	12	–	16	–	10	12	14	–	12	20.0±0.33

Inhibition zones are the mean including (6 mm) bore diameter; –ve control DMSO NA; – NA, +ve control as streptomycin at 100 µg/mL; extract conc 100 mg/mL, L: Leaf, F: flower, S: Stem and R: Root.

Table 3.
Minimum inhibitory concentration of extracts of *A. javanica* against different bacterial species.

Strain used	Hexane				Chloroform				Methanol				Streptomycin (100 mg/mL)
	L	F	S	R	L	F	S	R	L	F	S	R	
<i>Escherichia coli</i>	1.250	1.250	–	2.500	< 5.000	2.500	2.500	5.000	2.500	5.000	–	1.250	0.005
<i>Enterobacter aerogenes</i>	–	2.500	–	> 2.500	–	5.000	–	–	1.250	2.500	–	2.500	0.005
<i>Klebsiella penumoniae</i>	0.625	1.250	–	> 2.500	< 2.500	0.325	2.500	5.000	1.250	5.000	–	0.625	0.005
<i>Proteus vulgaris</i>	–	–	–	–	–	–	–	–	–	–	–	2.500	0.012
<i>Pseudomonas aeruginosa</i>	2.500	5.000	–	> 2.500	2.500	0.325	0.625	2.500	2.500	5.000	5.000	1.250	0.005
<i>Pseudomonas putida</i>	–	1.250	–	–	–	–	–	–	2.500	–	–	1.250	0.005
<i>Salmonella typhimurium</i>	–	1.250	< 5.0	1.250	1.250	1.250	–	> 5.000	2.500	1.250	> 2.500	0.625	0.012
<i>Bacillus cereus</i>	1.250	1.250	< 5.0	–	2.500	0.625	2.500	–	1.250	1.250	2.500	–	0.005
<i>Bacillus subtilis</i>	2.500	0.325	–	0.625	5.000	0.625	–	1.250	5.000	0.625	–	0.625	0.005
<i>Staphylococcus aureus</i>	2.500	–	–	> 5.000	5.000	1.250	–	–	2.500	–	–	–	0.005
<i>Staphylococcus epidermidis</i>	1.250	0.625	–	0.625	–	0.625	–	< 2.500	2.500	1.250	–	1.250	0.025

No activity, L– leaf, F–flower, S–stem, R– root; values are expressed in mg/dL.

steroids, however, it was negative for flavonoids, alkaloids, glycosides, saponins and resins. The chloroform extract showed the presence of flavonoids, alkaloids, glycosides, trace amounts of steroids, phenols, saponins, resins and cardiac glycosides whereas methanol fraction was positive for flavonoids and phenolic acids, alkaloids, glycosides, saponins and tannins, steroids in high concentration. In case of stem extractions, hexane fraction revealed the presence of steroids and terpenoids; chloroform fraction consisted the

flavonoids, alkaloids, glycosides, phenols, resins, tannins and cardiac glycosides. Methanol fraction of stem revealed the presence of high concentrations of flavonoids, glycosides and phenols but trace amounts of resins and alkaloids. The phytochemical screening of methane fraction of root revealed the presence of flavonoids, glycosides, steroids, phenols, saponins and carbohydrates. Chloroform fraction revealed the presence of the moderate concentration of flavonoids, glycosides steroids, saponins and tannins and

Table 4. Antibacterial activity of selected compounds as shown by bioautography.

Strain used	Rutin	Luteolin-O glycoside	Kaempferol	Tricin	Apigenin	Quercetrin	Azealatin	Chlorogenic acid	Hyperoside
<i>Escherichia coli</i>	+	-	+	-	+	-	-	-	-
<i>Enterobacter aerogenes</i>	-	-	+	-	-	+	-	-	+
<i>Klebsiella penumoniae</i>	+	-	+	+	+	+	-	+	+
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	+
<i>Pseudomonas aeruginosa</i>	-	+	+	-	-	-	-	-	-
<i>Pseudomonas putida</i>	+	-	-	-	+	-	-	+	-
<i>Salmonella typhimurium</i>	++	+	-	-	++	-	-	-	-
<i>Bacillus cereus</i>	+	-	-	+	-	-	-	-	-
<i>Bacillus subtilis</i>	-	+	++	-	++	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	+	-	+	-	-	+
<i>Staphylococcus epidermidis</i>	-	+	-	-	+	-	-	-	-

-: No activity,+:moderate activity ++: High activity.

hexane fraction resulted only in steroids and terpenoids.



Figure 1. *A. javanica* (Burn.f) Juss. ex. Schult.

3.2. Antibacterial activity of extracts

Results pertaining to antibacterial activity of extracts from different parts of the plant with hexane, chloroform and methanol are presented in Table 2 and Figure 2. A critical perusal of the table revealed that for all the three solvents, flower followed by leaf and then root extracts, exhibited antibacterial activity. Stem extracts showed activity against few bacteria and even that activity was not significant. The extracts were equally effective against gram-positive as well as gram-negative bacteria. It is interesting to note that in some cases even the crude extracts like chloroform extracts of flower, methanolic extracts of leaf and flower have shown more or/equal activity with the standard streptomycin (at 100 μ g/mL). Comparatively, methanolic extracts have shown more antibacterial activity than the hexane and chloroform. The reason may be that methanol being polar it

sequestered more number of compounds from different plant parts. Among the bacterial species, *Proteus vulgaris* was resistant to most extracts followed by *Pseudomonas putida*. Interestingly, the very closely related species *Pseudomonas aeruginosa* was susceptible to all the extracts except hexane stem extracts.

The MIC values of different extracts varied both with the solvent and plant part (Table 3). Among all, methanolic extracts of root exhibited low MIC values, followed by methanolic leaf extracts and hexane extracts of leaf and flower. MIC values varied from bacterial species to species. With few exceptions, the MIC values of all extracts were found to be less for gram-positive bacteria. In some cases the MIC values were as low as 0.33 as in the case of hexane flower extract against *Bacillus subtilis*. In any case, the MIC values of all extracts were far below the MIC values of standard streptomycin. The inconsistency between the values of cup method and MIC values may be because of interfering factors present in the crude extracts.

A comparison between the phytochemicals present and antibacterial activity showed relationship between the variety of phytochemicals present in the extract and antibacterial activity. For instance, the methanolic extract displayed a large number of phytochemicals and so also wide spectrum of antibacterial activity. At this stage it is difficult to predict as to what type of chemical constituents show antibacterial activity and against which organisms. The antibacterial activity presented by a crude extract may be due to synergistic or antagonistic activity of the different chemical constituents present in it. Hence, separation and subsequent screening of individual constituents is essential.

3.3. Fractionation of extracts by HPTLC & identification of compounds

After the preliminary screening of phytochemicals and antimicrobial activity of different parts, promising extracts *viz*, methanolic extracts of leaf and flower were further evaluated by HPTLC analysis for identifying the important phytoconstituents (Figure 3). Methanolic extract of flower

was resolved by using a mobile phase ethyl acetate: acetic acid formic acid and water (10: 1.1:1.1:2.6). This fraction got separated into compounds with R_f values ranging from 0.05–0.84. The major peaks observed at R_f values 0.53 and 0.72 were at peak positions of 6 and 8, respectively and have occupied 38.85% and 39.04% among the 10 compounds. The compounds that have occupied these peaks were identified as rutin and hyperoside by comparison with standard compounds^[16]. Apart from these major compounds,

two more compounds with R_f values 0.74 and 0.59 were identified as luteolin–o–glycoside and chlorogenic acid. The chromatograms were scanned under 254 nm and 366 nm (Figure 4). The densitogram finger printing obtained from the chromatogram was further scanned for 3D image to determine the overlapping of compounds at similar R_f values (Figure 5). However, there were no such overlapping compounds at same R_f values.

Methanolic fraction of leaf gave a good resolution with

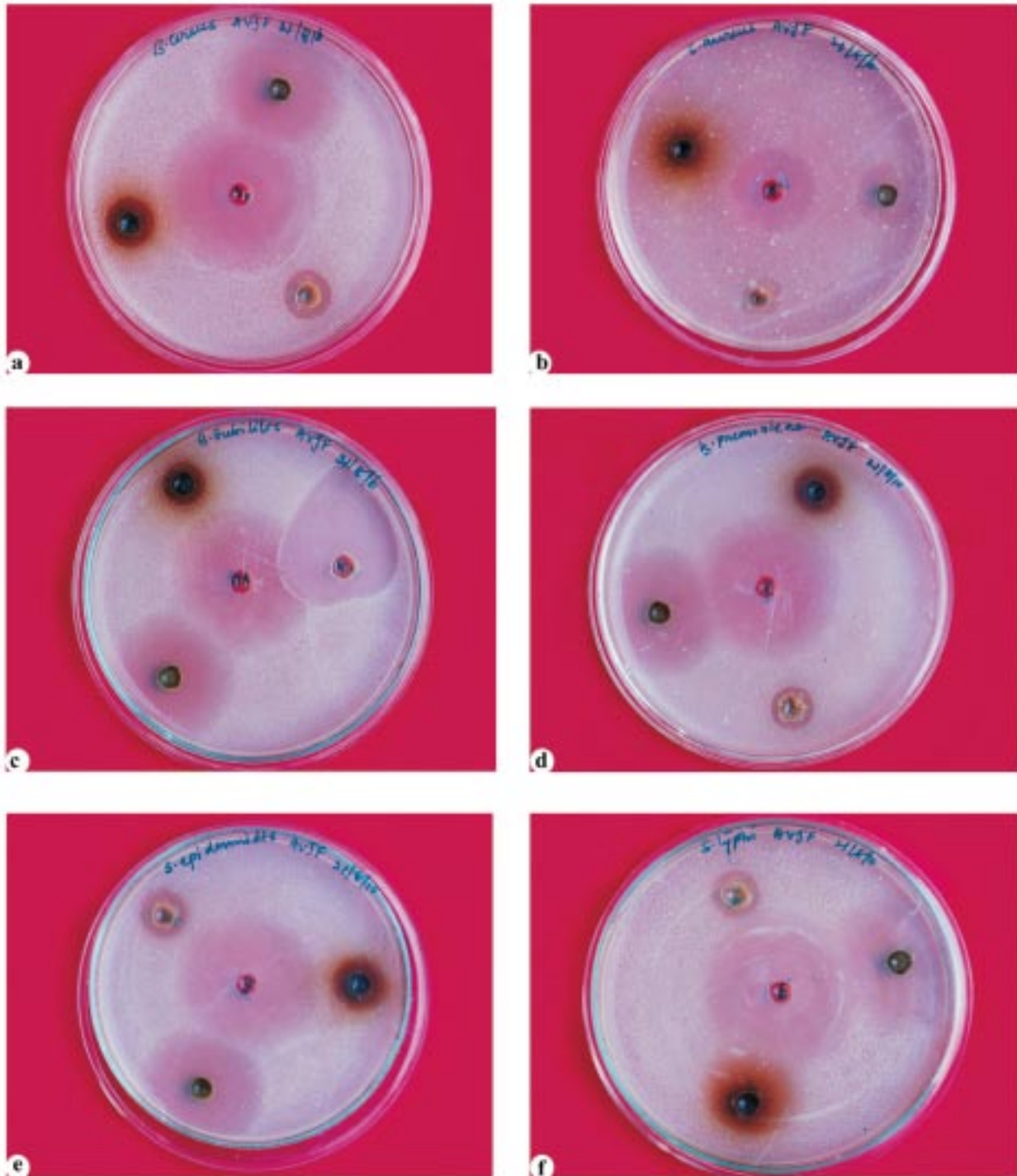


Figure 2. Antibacterial activity of *A. javanica*.

a: *Bacillus cereus*; b: *Staphylococcus aureus*; c: *Bacillus subtilis*; d: *Klebsiella pneumoniae*; e: *Staphylococcus epidermidis*; f: *Salmonella typhimurium*.

the mobile phase concentration HCl: acetic acid: water in the ratio 4:1:5 v/v. The fluorescence detected in UV after derivatization of the chromatogram at 254 nm and 366 nm

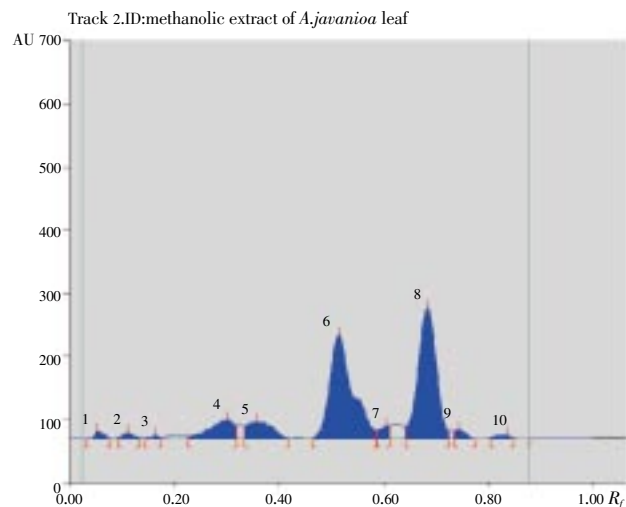


Figure 3. Densitogram of methanolic extract of *A. javanica* leaf using ethyl acetate: acetic acid: formic acid: water (10:1.1:1.1:2.6 v/v) at 254 nm.

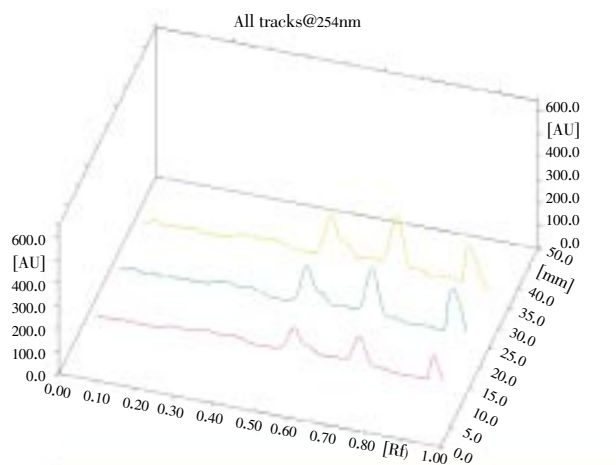


Figure 5. All tracks of methanolic extract of *A. Javanica* leaf, absorption/reflectance mode at 254 nm.

resolved compounds from flower extract ranged between 0.22–0.83. Maximum finger prints were occupied by second and eighth of area with 22% and 20.10%, respectively, which were identified as apigenin and other as unknown. Other major compounds were quercetin, azaleatin, kaempferol and triclin at R_f values at 0.49, 0.55, 0.72. HPTLC profile of terpenoids has been chosen to reveal the diversity of existing at biochemical level in *A. lantana*[12].

3.4. Bio-autography of fractionated compounds

Based on the HPTLC finger printing, methanolic extract of leaf and flowers were run with normal TLC with suitable mobile phases. Later, they were screened against 24 hours old broth culture of test bacteria to determine their antimicrobial activity (Table 4 & Figure 6). It was evident from the results that apigenin followed by rutin and

confirms the presence of flavonoids. The extracts were run and compared with standard R_f values that further confirmed the presence the presence of flavonoids. The R_f value of the

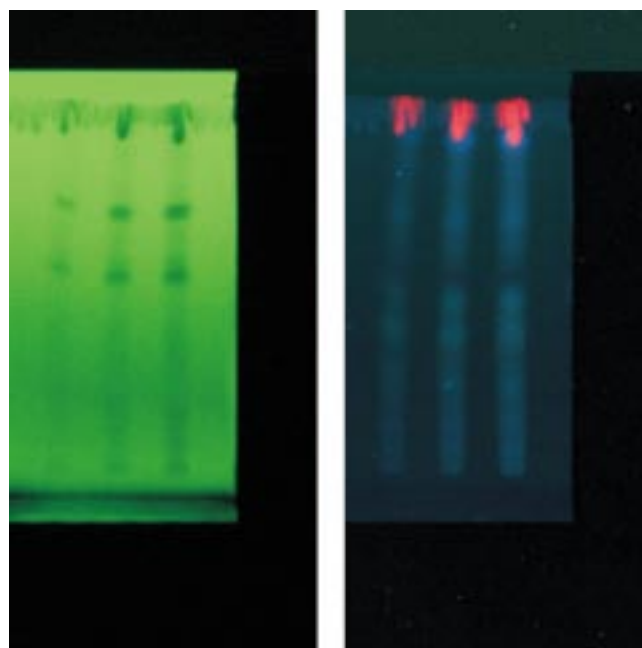


Figure 4. Video image at 254 nm and 366 nm.

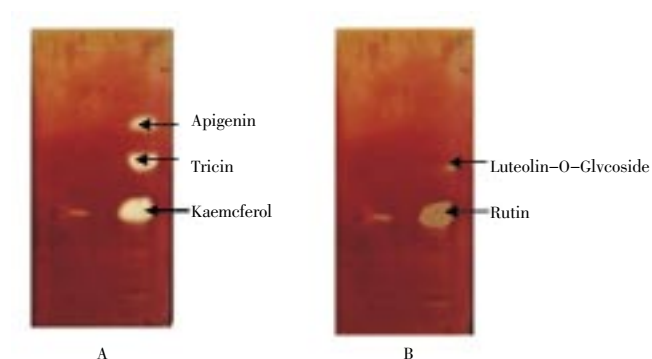


Figure 6. Bio-assay of methanol extract of *A. Javanica* (leaf). A: *Klebsiella pneumoniae*; B: *Salmonella typhimurium*.

kaempferol have shown antibacterial activity against more number of bacteria. However, the relative activity varied with the compound. Quercetin, triclin and chlorogenic acid have shown activity against few bacteria only. Azealatin did not show any activity against tested bacteria. The differential activity of compounds may be partly due to the amount of compound present in the extract. The sensitivity of bacteria also varied with the compound. In general gram-negative bacteria are more sensitive than the gram-bacteria. *Klebsiella pneumoniae* was inhibited by seven out of nine compounds tested, whereas *Bacillus cereus* was inhibited by triclin only.

4. Discussion

Very few reports on the antibacterial activity of *A. javanica*

are available^[17,18]. Reddy and Reddy have reported the antimicrobial activity of leaves of *A. javanica* extracted with petroleum ether, ethyl acetate and methanol. The crude methanolic leaf extracts showed significant antibacterial and antifungal activity. However, hexane extracts did not show any activity. Ahsan Sharif *et al*^[17] have isolated six natural products from the whole plant of *A. javanica*. Similar to our results, they have also detected isoquercetrin, apigenin and kaempferol and their antibacterial activity.

Thus, this study supports the use of *A. javanica* in the traditional medicine to cure certain infectious disease. Methanolic fractions of leaves and flower are more effective than chloroform and hexane extracts. The antibacterial activity of *A. javanica* appears to be due to the presence of compounds like luteolin-*o*-glycoside, rutin, apigenin, tricrin and kaempferol.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research work was carried out through partial financial assistance received from UGC New Delhi, under SAP (DRS) (No F.3–23/2009 (SAP)–II),. Thanks are also due to Head, Department of Microbiology, Kakatiya University, Warangal (India) for facilities and encouragement.

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