SCREENING FOR ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS

B. Uma Reddy

Department of Botany, Gulbarga University, Gulbarga
Karnataka, India, Pin code -585 106
E-mail: drumareddy11@yahoo.co.in

Summary

The crude alcoholic extracts of seeds of *Pongamia pinnata* (L.) Pierre. (Fabaceae), roots of *Tinsospora cordifolia* (Thunb.) Miers. (Menispermaceae), entire plant of *Phyllanthus niruri* L. (Euphorbiaceae) and *Cleome viscosa* L. (Capparaceae) were subjected to phytochemical studies as well as antibacterial activity for the assessment of inhibitory effects of the alcoholic extracts of these plants against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus vulgaris* by agar well diffusion method. The results of the primary investigation revealed the occurrence of few major groups of plant metabolites in these plants are proteins, reducing sugars, saponins, alkaloids, flavonoids, steroids, tannins, phenols and glycosides. Comparatively maximum amount of total phenol was noticed in *Phyllanthus niruri* and moderate in *Pongamia pinnata.* The amount of total protein content in these plants was found to be almost uniform. Qualitative separation of thin layer and paper chromatography methods showed the presence of alkaloid and amino acid bands respectively. The different concentrations of *Pongamia pinnata* seed extract showed higher activity and produced inhibition zones against *Pseudomonas aeruginosa, Escherichia coli* and *Proteus vulgaris. Tinospora cordifolia* showed significant activity against *Pseudomonas aeruginosa* and *Escherichia coli*. Where as, the *Phyllanthus niruri* and *Cleome viscosa* were found to be broad spectrum of potent antibacterial activity against all the five studied pathogens. The results of current study are leading to the conclusion that these plants would serve as sources of novel antibiotic agents.

Key Words: Antibacterial activity, *Pongamia pinnata,Tinsospora cordifolia, Phyllanthus niruri, Cleome viscosa*

Inroduction

Plants are the vital sources of innumerable number of antimicrobial compounds. Several phytoconstituents like tannins, flavonoids, polyphenols etc are effective antimicrobial substances against a wide range of microorganisms (1-2). Although hundreds of plant species have been undertaken to explore the antimicrobial properties, still the vast majority of have not yet been evaluated (3). Considering, the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, systematic investigation was undertaken to screen the local flora for phytochemical studies and antibacterial activity, namely *Pongamia pinnata, Tinospora cordifolia, Cleome viscosa, Phyllanthus niruri.*

***Pongamia pinnata* (L.) Pierre** (Fabaceae) is a deciduous, woody climber. The leaves are heart shaped with pointed leaf tip and dark green. Fruits look like bunch of red cherries. The seeds are crescent shaped. Pongamia seeds and oil is known for anthelmintic. It is useful in rheumatism arthritis, whooping cough, skin ailments. Seed oil is mainly used in cosmetics, in soap making and as a lubricant. It is also used as insecticidal, nematicidal and bactericidal. ***Tinospora cordifolia* (Thunb.) Miers**. (Menispermaceae) A large climber with succulent stems. Leaves 5-10 cm, 5-7 nerved, originated from the base of the base of the leaf margins, petioles slightly shorter than leaves. Flowers minute, male and female separate; male flowers grouped in axils of bracts, female solitary, fruits size of pea, red in colour. It is used as one of the chief ingredients for Ayurvedic preparations used in general debility, dyspepsia, fevers and urinary diseases. Root is a powerful emetic and used for visceral obstruction; its watery extracts is used in leprosy. ***Phyllanthus niruri* L.** (Euphorbiaceae) is erect, annual herb upto 60 cm height, leaves numerous, subsessile distichous, elliptic. Flowers yellowish, capsules are 2.5mm in diameter. It is useful in gastric, ophthalmic disorders etc. ***Cleome viscosa* L.** (Capparaceae) annual herb, branched above, erect, up to 1m. tall. Stem angular, rather densely glandular-hairy. Leaves petiolate, 3–5-foliolate, corymbose ephemeral yellow flowers at the top, Used as anthelmintic, carminative, diaphoretic, rubefacient, stimulant etc (4-6).

Materials and Methods

Collection of Plant Materials: The seeds of *Pongamia pinnata*, roots of *Tinsospora cordifolia,* entire plant of *Phyllanthus niruri* and *Cleome viscosa* were collected from in and around Gulbarga city, Karnataka, India. All these plants were authenticated and the voucher specimens were deposited in Herbarium, Department of Botany, Gulbarga University, Gulbarga, India. Later the respective plant materials of these plants were subjected for surface sterilization using 30% alcohol, and then shade dried for further analysis.

Preparation of Extract: These plant materials were extracted successively with non-polar to polar solvents at 50 - 60°C in a soxhlet apparatus. The different extracts were collected in a separate container and concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) and note down the yield of the crude extracts and recorded the colour, consistency and yield of the extracts. Only the alcoholic (95%) extract of these plant materials were used to screen preliminary phytochemical studies, amino acid and alkaloid separation and for antibacterial studies.

Phytochemical Studies: To screen preliminary phytochemical studies, the alcoholic extracts of these plant materials were used by applying general chemical tests for alkaloids, glycosides, reducing sugars, tannins, steroids, terpenoids, phenols, flavonoids, proteins, saponins, amino acids, etc (7-11). The total amount of protein (12) and phenols (13) were estimated quantitatively from the 500mg of dried powdered plant materials. The qualitative separations of amino acids by paper chromatography, using butanol, acetic acid and water (5:4:1), and visualized the amino acid bands by spraying with Ninhydrin reagent. The alkaloid separation by Thin layer chromatography using benzene and chloroform (7:3), the alkaloid bands were visualized under UV light as well as by spraying with Dragendroff's reagent (7).

Culture Media: The nutrient agar and nutrient broths for antibacterial activity were purchased from HiMedia Laboratories Limited, Mumbai. Streptomycin sulphate (standard reference drug for antibacterial assay) from Nanjing Asian chemicals Co., Ltd. The solvents and other chemicals used were analytical grade.

Collection of microorganisms: The isolated pathogens viz. *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus vulgaris* from clinical samples obtained at the VIMS, Bellary, Karnataka, India, with specific diagnosis of the different human pathologies and enriched in selective media and subjected to identification and characterization. These microbes were maintained by sub-culturing periodically and were preserved at 4°C prior to use.

Characterization: The test organisms were subjected to microscopic examination, Gram's character, motility, cultural and colony characteristics and biochemical test profiles (14).

Antibacterial activity: Here the *in vitro* antibacterial activity was assayed by using agar well diffusion method. The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours, then subjected for optical density of the overnight incubated culture were adjusted to 0.1 at λ_{600} with sterile nutrient broth. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar plate and Sabouraud's dextrose agar plates for bacterial and fungal cultures. The wells were bored with 8mm borer in seeded agar, and then the different concentrations, 2, 4, 6, 8 and 10mg/well of the plant extracts and 0.2, 0.4, 0.6, 0.8 and 1.0mg/well of streptomycin sulphate was added into each separate well.

Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hr. Later, the zone of inhibition was measured and recorded (1).

Statistical Analysis: All the data are expressed as mean ± S.E.M. (standard error of the mean). The significance level was determined using the Student't' test. A *p*-value of <0.05 was considered statistically significant.

Results

The results of the above studies revealed that the alcoholic extracts of these plants showed the presence of major groups of primary and secondary metabolites and also showed the broad-spectrum of antibacterial activity.

Colour, consistency and yield of the extracts: The colour, consistency and yield of the successive extractions of these plant materials revealed differently with different solvent systems. The less to moderate yield of the extracts were noticed with benzene and chloroform. Where as the highest amount of extract was obtained from the alcohol extraction. However, the colour of the benzene and chloroform extracts revealed as yellow to yellowish green with highly viscous and light yellow to dark yellow with gelatinous consistency, respectively. The alcoholic extracts of *P. pinnata* was yellowish, *T. cordifolia* was brown colour, *Phyllanthus niruri* was greenish and *Cleome viscosa* gave dark greenish in colour with sticky or gummy consistency. (Table -1)

Preliminary phytochemical studies: The alcoholic extracts of different plant materials showed to possess both primary and secondary metabolites, namely proteins, carbohydrates (reducing sugars), phenols, saponins, flavonoids, alkaloids, steroids, tannins and glycosides. However, saponins and steroids are absent in *Pongamia pinnata,* steroids are absent in *Tinospora cordifolia,* saponins are absent in *Phyllanthus niruri* (Table - 2)

Quantitative estimations: The different plant materials exhibited comparatively almost equal amount of proteins. Where as, *Phyllanthus niruri* possess comparatively highest amount of total phenols and moderate amount in the roots of *Pongamia pinnata,* but remaining plant materials possess the smaller amount of total phenols (Table-3).

Qualitative separation of Amino acids: These studies revealed the presence of some of the essential amino acids viz lysine, valine, tryptophan, isoleucine, tyrosine and phenylalanine in all the different plant extracts. These were visualized only after spraying with the Ninhydrin reagent, the colourless amino acid bands converted into deep purplish violet colour and then recorded their respective Rf values (table-4).

Qualitative separation of Alkaloids: The thin layer chromatography method of these studies revealed that, the alcoholic extract of *Pongamia pinnata* showed two alkaloid bands at Rf value 0.84 and 0.45, similarly, *Cleome viscosa* also showed two alkaloid bands at Rf values 0.86 and 0.25, *Tinospora cordifolia* possess only one single alkaloid band at the Rf value is 0.17. But *Phyllanthus niruri* did not show any alkaloid band. These alkaloid bands were visualized, when the developed plates were sprayed with Dragendroff's reagent, here the colourless alkaloid bands change into intensive orange colour band (Table-5).

Characterization of Microorganisms: All the above tested bacterial forms are Gram negative and rod shaped, except *Staphylococcus aureus* is Gram positive and perfectly spherical in shape, where as all these bacterial forms are proved to be motile, except *Klebsiella pneumoniae* and *Staphylococcus aureus* are non-motile forms. Results of certain confirmatory biochemical tests of these bacterial forms are also explained in the Table-6. Based on their unique properties, identities of these organisms were confirmed.

Antibacterial activity: The alcoholic extract of *Pongamia pinnata, Tinospora cordifolia, Phyllanthus niruri* and *Cleome viscosa* (2, 4, 6, 8 and 10mg/ well) have shown promising antibacterial activity and effectively inhibited the growth rate of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae* and *Proteus vulgaris*. The different concentrations of *Pongamia pinnata* seed extract showed higher activity and produced inhibition zones against *Pseudomonas aeruginosa, Escherichia coli* and *Proteus vulgaris.* The extracts of *Tinospora cordifolia* showed significant activity against *Pseudomonas aeruginosa* and *Escherichia coli*. Where as, the *Phyllanthus niruri* and *Cleome viscosa* were found to be broad spectrum of antibacterial activity against *P. aeruginosa, E. coli, S. aureus* and *P. vulgaris* pathogens. But *K. pneumoniae, S. aureus* were found to be resistant to *Pongamia pinnata.* Where as, *K. pneumoniae, S. aureus* and *P. vulgaris* were resistant to the extract of *Tinospora cordifolia*. The extracts of *Phyllanthus niruri* and *Cleome viscosa* were proved to be most effective against all the five studied pathogens. The zone of inhibition was increased on increasing the concentration of the extract in the well. This showed the concentration dependent activity (Table-7)

Table-1 Colour and yield of the different solvent extractives of *P. pinnata*, *T. cordifolia*, *P. niruri* and *C. viscosa*

Plants	Colour of the extracts					
	Benzene	Weight (g/100g)	Chloroform	Weight (g/100g)	Alcohol	Weight (g/100g)
Pongamia pinnata (Seeds)	Yellowish green	1.67	Yellowish green	0.33	Colourless	2.33
Tinospora cordifolia (Roots)	Yellow	2.60	Yellow	2.00	Brown	4.00
Phyllanthus niruri (whole plant)	Yellowish green	4.00	Light Yellow	1.93	Green	7.57
Cleome viscosa (whole plant)	Yellowish green	5.00	Yellow	2.64	Dark Green	8.99

Table-2 Distribution of primary and secondary metabolites in *P. pinnata*,, *C. viscosa*, *T. cordifolia* and *P. niruri.*

Chemicals	**Tests**	**Colour/ Precipitate**	**Constituent present**	**Pp (s)**	**Cv (wp)**	**Tc (r)**	**Pn (wp)**
Proteins	Biuret test	Violet or pink colour	Two or more peptide bonds of proteins	+	+	+	+
	Ninhydrin test	Violet to purple	Amino acids and proteins	+	+	+	+
	Xanthoproteic test	Yellow to orange	Nitro-derivatives of aromatic amino acids	-	+	+	-
	Hopkins-Cole test	Violet ring at the junction of two liquids	Indole group of tryptophan	+	+	+	+
	Sakaguchi test	Intense red colour	Guanidine group of arginine and proteins	-	+	+	+
	Sulphur test	Black precipitate	Sulphur containing amino acids	-	+	-	+
	Modified Millon's test	Yellow precipitate	Proteins	+	+	+	+
	Tryptophan test	A reddish violet ring at the junction of two solutions	Tryptophan	+	+	+	+
	Adamkicw's test	A violet colour develops	Tryptophan	+	+	+	+
	Cysteine test	A black precipitate	Cysteine	-	+	-	+

Carbohydrates	Molisch's test	Violet ring at the junction of two liquids	Carbohydrates	+	+	+	+
	Iodine test	Deep blue colour	Starch	-	+	-	+
	Fehling's test	Yellow to brownish red	Reducing sugars	-	+	+	+
	Benedict's test	Yellow, red or green precipitate	Reducing sugars	+	+	+	+
	Barfoed's test			-	+	+	+
	Tromer's test			+	+	+	+
Non-reducing sugars	Benedict's test	No characteristic colour	Non-reducing sugars	-	-	-	-
	Benedict's reagent + NaOH	No characteristic colour	Non-reducing sugars	-	-	-	-
	Mucic acid test	Formation of crystals at the bottom of the test tube	Mucic acid	-	+	+	+
Saponins	Foam test	Honey-Comb like frothing	Saponins	-	+	+	-
	Haemolysis test	Lysis of blood cell	Saponins	-	+	+	-
Alkaloids	Mayer's test	Yellow precipitation	Alkaloids	+	+	+	+
	Wagner's test	Brownish-white precipitate	Alkaloids	+	+	+	+
	Dragendroff's test	Orange precipitate	Alkaloids	+	+	+	+
	Picric acid test (1%)	Deep yellow precipitate	Alkaloids	+	+	+	+
Flavonoids	Flavonoid test	Scarlet colour Cherry red colour	Flavones Flavonoids	+	+	+	+
	Aqueous NaOH test	Yellow colour	Flavonoids	+	+	+	+
	Conc. H_2SO_4 test	Red colour	Flavonoids	+	+	+	+
	Pew's test	Deep purple red, cherry red, pinkish or brownish	Flavones, dihydrochalcones Flavonoids	+	+	+	+

	Shinoda test	Deep-red or magenta colour	Dihydroflavanol, dihydrochalcones and other flavonoids	+	+	+	+
Steroids	Salkowski test	Wine red colour	Steroidal nuclei	-	+	-	+
	Liebermann and Burchard test	Blue-green colour	Steroids	-	+	-	+
Phenols	Ellagic acid test	Muddy yellow, olive brown to deep chocolate colours	Depending on the amount of ellagic acid	+	+	-	+
	$FeCl_3$ test	Intense colour	Phenols	+	+	-	+
Tannins	Gelatin test	White precipitate	Tannins	+	+	+	+
Glycosides	Molisch's test	Reddish-violet ring at the junction of two liquids	Glycosides	+	+	+	+
	Keller-Kiliani test	Reddish-brown ring at the junction of two liquids	Glycosides	+	+	+	+

Note: Pp(s) – *Pongamia pinnata* (seeds); Cv (wp) – *Cleome viscosa* (whole plant); Pn (wp) – *Phyllanthus niruri* (whole plant); Tc (r) - *Tinospora cordifolia* (roots)

Table – 3 Quantitative estimation of primary and secondary constituents of these four plants

Sl No.	Name of the plant sample	Quantity chemical constituents in mg/gm	
		Proteins	Phenols
1	*Pongamia pinnata* (seeds)	2.24 ± 0.01	0.11 ± 0.003
2	*Phyllanthus niruri* (whole plant)	2.45 ± 0.07	0.15 ±0.005
3	*Cleome viscosa* (whole plant)	2.04 ± 0.02	0.08 ±0.003
4	*Tinospora cordifolia* (roots)	2.33 ± 0.04	0.05 ±0.006

Table-4 The qualitative separation of certain essential amino acids in the alcoholic extracts of these plants by paper chromatography

Sl No	Type of Amino acids	Rf value of the amino acids	*Pongamia pinnata* (Seeds)	*Tinospora cordifolia* (Roots)	*Phyllanthus niruri* (whole plant)	*Cleome viscosa* (whole plant)
1	Lysine	0.102	+	+	+	+
2	Valine	0.314	+	+	+	+
3	Tryptophan	0.320	+	+	+	+
4	Isoleucine	0.628	+	+	+	+
5	Tyrosine	0.461	+	+	+	+
6	Phenylalanine	0.628	+	+	+	+

Table-5 The qualitative separation of alkaloids in the alcoholic extracts of these plants by Thin Layer Chromatography.

Name of the test sample	Rf values	Under visible light	Under UV Light	After Dragendroff's reagent spray
Pongamia pinnata (seeds)	0.840	-	Fluorescent green	Orange
	0.450	-	Fluorescent green	Orange
	0.280	-	Fluorescent green	-
	0.120	-	Fluorescent green	-
Tinospora cordifolia (roots)	0.290	-	Fluorescent green	
	0.170	-	Fluorescent green	Orange
Cleome viscosa (whole plant)	0.862	Green	Red	Orange
	0.259	Green	Red	Orange
	0.086	Green	Green	-
Phyllanthus niruri (whole plant)	0.970	Green	Fluorescent green	-
	0.140	-	Fluorescent green	-

Table-6 Microscopic and Biochemical profile of five tested clinical isolates

Microorganisms	Gram Staining	Motility	Structure	Sugar fermentation test	Indole production test	Urease production test	Citrate utilization test	Catalase test	Oxidase test
Klebsiella pneumoniae	Negative	Non-motile	Rod shaped	AG	Negative	Positive	Negative	Negative	Negative
Staphylococcus aureus	Positive	Non-motile	Spherical shaped	A	Negative	Negative	Negative	Positive	Negative
Proteus vulgaris	Negative	Motile	Rod shaped	AG	Positive	Positive	Negative	Positive	Negative
Pseudomonas aeruginosa	Negative	Motile	Rod shaped	Negative	Negative	Positive	Positive	Positive	Positive
Escherichia coli	Negative	Motile	Rod shaped	A	Positive	Positive	Positive	Negative	Negative

Note: AG – Acid and Gas; A –Acid

Table-7 Antibacterial activity of alcoholic extracts of *P. pinnata*, *T. cordifolia*, *P. niruri* and *C. viscose*

Plant materials	Dose mg/well	*Pseudomonas aeruginosa* (mm)	*Escherichia coli* (mm)	*Klebsiella pneumoniae* (mm)	*Staphylococcus aureus* (mm)	*Bacillus* species (mm)
Pongamia pinnata (seeds)	02	05.0 ± 0.30	05.0 ± 0.03	-	-	-
	04	06.0 ± 0.70	05.5 ± 0.30	-	-	02.0 ± 0.60
	06	07.0 ± 0.00	07.0 ± 0.70	-	-	03.0 ± 0.03
	08	08.0 ± 0.03	09.0 ± 0.00	-	-	02.0 ±0.40
	10	11.0 ± 0.30	10.0 ± 0.70	-	-	04.0 ± 0.40
Tinospora cordifolia (roots)	02	02.0 ± 0.40	03.0 ± 0.00	-	-	-
	04	03.0 ± 0.30	07.0 ± 0.30	-	-	-
	06	04.0 ± 0.70	10 .0± 0.30	-	-	-
	08	05.0 ± 0.30	11.0 ± 0.00	-	-	-
	10	07.0 ± 0.30	13.0 ± 0.30	-	-	-

Phyllanthus niruri (whole plant)	02	04.0 ± 0.00	05.0 ± 0.03	07.0 ±0.07	02.0 ± 0.40	04.0 ± 0.00
	04	04.5 ± 0.03	05.0 ± 0.03	07.0 ± 0.07	02.0 ± 0.40	04.0 ± 0.00
	06	05.0 ± 0.30	06.0 ± 0.04	07.0 ± 0.70	03.0 ± 0.50	05.0 ± 0.03
	08	07.0 ± 0.70	07.3 ± 0.01	08.0 ± 0.30	04.1 ± 0.03	06.0 ±0.04
	10	09.0 ± 0.40	08.4 ± 0.30	09.3 ± 0.03	06.1 ± 0.70	08.2 ± 0.03
Cleome viscosa (whole plant)	02	05.0 ± 0.70	05.0 ± 0.07	-	03.0 ± 0.30	-
	04	06.0 ± 0.30	06.0 ± 0.03	-	04.0 ± 0.70	03.0 ± 0.72
	06	07.0 ± 0.40	07.0 ± 0.00	-	05.0 ± 0.03	04.0 ± 0.01
	08	08.5 ± 0.30	08.0 ± 0.01	02.0 ± 0.70	06.0 ± 0.70	04.3 ± 0.03
	10	10.7 ± 0.00	11.0 ± 0.30	05.0 ± 0.36	09.0 ± 0.03	07.2 ± 0.08
Streptomycin sulphate	0.2	11.0 ± 0.00	06.0 ± 0.30	07.0 ± 0.03	11.3 ± 0.30	08.0 ± 0.40
	0.4	14.0 ± 0.30	07.0 ±0.03	09.0 ± 0.70	13.1 ± 0.30	09.3 ± 0.30
	0.6	16.0 ± 0.03	09.0 ± 0.00	10.0 ± 0.00	16.7 ± 0.60	09.7 ± 0.00
	0.8	20.0 ± 0.00	10.0 ± 0.50	12.0 ± 0.00	17.0 ± 0.70	11.0 ± 0.00
	1.0	22.0 ± 0.30	06.0 ± 0.04	19.0 ± 0.07	21.2 ± 0.30	15.0 ± 0.08
Control (dw)	02ml of dw	-	-	-	-	-

Note: dw = distilled water; '- ' = no activity

Discussion

The extracts of these plants were found to be very good source of antibiotics against various bacterial pathogens and exhibited broad spectrum of antibacterial activity. The preliminary phytochemical studies revealed the presence of major groups of primary and secondary metabolites in these plant extracts, indicating that, these chemicals might be responsible for the antibacterial activity. The qualitative separation of amino acid studies revealed the presence of lysine, valine, tryptophan, isoleucine, tyrosine, and phenylalanine in all the different plant extracts, majority of these are acting as precursors for secondary metabolite production. The present study is also evidenced by earlier research studies. Such as Mahesh and Satish (2008) (15) have shown the significant antifungal activity of *Tinospora cordifolia* against *Dreschlera turcica.* Singh *et al.,* (2003) (16) have reviewed the chemical constituents reported from the *Tinospora cordifolia,* belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides, also reviewed the notable medicinal properties reported are anti-diabetic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarithritic, antioxidant, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. Pritee Wagh *et al.,* (2007) (17) have observed the different concentrations of *Pongamia pinnata* has high degree of antimycotic and antimicrobial activity and very effective against *Aspergillus niger, Aspergillus fumigatus, Staphylococcus aureus* and *Pseudomonas aeruginosa* was confirmed by MIC determination and dry-weight method (18). Sudhakar *et al.,* (2006) (19) have shown the broad spectrum of antimicrobial activity of *Cleome viscosa* against *Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa. Phyllanthus niruri* has strong anti-*Candida* activity. Particularly effective against *in vitro* anti-*Candida albicans.* The use of ampicillin is no longer recommended because of the potency of the wide spread resistance to microorganisms (20). The bactericidal property of *Pongamia pinnata* was investigated (21).

Conclusion

All the test samples were proved to have pronounced antibacterial potencies against the Gram positive and Gram negative tested bacterial pathogens. The antibacterial activity of *Pongamia pinnata, Tinospora cordifolia, Phyllanthus niruri* and *Cleome viscosa* plant extracts may be attributed to the various phytochemical constituents present in their crude extracts. The results of the current study are leading to the conclusion that these plants would serve as sources of novel antibiotic agents.

References

1. Yogesh Biradar S, Sheetal Jagatap, Khandelwal KR, Smita Singhania S. Exploring of antimicrobial activity of Triphala *Mashi*- an Ayurvedic fromulation. *eCAM* 2007; 5(1):107-113
2. Brian Leibovitz E, Jennifer Ann Mueller BS. Bioflavonoids and polyphenols: Medical applications. *Journal of Optimal Nutrition* 1993; 2(1):17:35

3. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of Industrial and Medicinal materials Science 1985; 228: 1154-1160.
4. Nadkarni KM, Nadkarni AK. Editors, Indian Materia Medica, Vol-I, third edition, M/S Popular Prakashan Pvt. Ltd., Mumbai, 1976.
5. Kirtikar KR, Basu BD. Editors, Indian Medicinal plants, vol-I, second edition, New Connaught place, International book distributors, 9/3, Dehra Dun, India, 1975
6. Kurian JC. Plants that heal, published by Oriental Watchman Publishing House, Post box-1417, Salisbury park, Pune, India, 2001
7. Harborne JB. Phytochemical methods, A guide to modern techniques of plant analysis, 3rd edition, Springer (India) Pvt. Ltd., New Delhi. 1998; 5-12:124-126.
8. Mohd. Nawagish, Ansar SH, Shoaib Ahmad. Preliminary Pharmacognostical Standardisation of *Lawsonia* inermis Linn. Seeds. *Research Journal of Botany*. 2007; 2(3):161-164.
9. Nooman Khalaf A, Ashok Shakya K, Atif Al-Othman, Zaha El-Agbar, Husni Farah. Antioxidant activity of some common plants. *Turk J Biol* 2008; 32: 51-55.
10. Ogbonnia S, Adekunle AA, Bosa MK, Envuru VNC. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A.Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. *African Journal of Biotechnology* 2008; 7(6):701-705.
11. Sadasivam S, Manickam A. Biochemical methods for Agricultural Sciences. Wiley Eastern Limited, Ansari Road, Daryaganj, New Delhi. 1992: 1-20.
12. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagent. *J. Biol Chem* 1951;193: 265
13. Bray HG, Thorpe WV. In analysis of phenolic compounds of interest in metabolism. *Meth Biochem Anal* 1964; 1: 27-52.
14. Aneja KR. Experiments in Microbiology, New Age International Publications, New Delhi, India, 2002.
15. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens, *World Journal of Agricultural Sciences* 2008; 4 (S): 839 – 843.
16. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). *Indian Journal of Pharmacology* 2003; 35: 83-91.
17. Pritee Wagh, Mahendra Rai, Deshmukh SK, Marta Cristina, Teixeira Durate. Bio-activity of oils of *Trigonella foenum-graecum* and *Pongamia pinnata. African Journal of Biotechnology* 2007; 6(13):1592 -1596
18. Winston David, Maimes Steven. "Adaptogens: Herbs for Strength, Stamina, and Stress Relief," Healing Arts Press. 2007
19. Sudhakar M, Rao ChV, Rao PM, Raju DB. Evaluation of antimicrobial activity of *Cleome viscosa* and *Gmelina asiatica*. *Fitoterapia* 2005; 77 (1): 47- 49
20. Makes M, Torres J, Calzada F *et al.* Antibacterial properties of *Helianthemum glomeratum,* a plant used in Maya Traditional Medicine to treat diarrhea. *Phytotherapy Research* 1997; 11: 128 -131.
21. Baswa M, Rath CC, Dash SK, Mishra RK. Antibacterial activity of Karanj (*Pongamia pinnata*) and Neem (*Azadirachta indica*) seed oil: a preliminary report *Microbios* 2001; 105(412):183-189.