Afr. J. Traditional, **Complementary and Alternative Medicines**
www.africanethnomedicines.net

Research Paper

ISSN 0189-6016©2008

ANTIBACTERIAL ACTIVITY OF SOME FOLKLORE MEDICINAL PLANTS FROM SOUTH INDIA

M. Jaya Prakash Goud[1*], A. Komraiah[2], K. Narasimha Rao[1], A.Ragan[2], V.S.Raju[2], M. A. Singara Charya[1]

[1]Department of Microbiology, Kakatiya University-506009, India
[2]Department of Botany, Kakatiya University-506009, India
***E-mail : jp_muthyala@yahoo.co.in**

Abstract

Antibacterial activity and phytochemical tests of eight whole plant methanol extracts belonging to family Euphorbiaceae were evaluated. In agar well diffusion assay the diameter of inhibition zones ranged from 3-13 mm. *Phyllanthus emblica* showed maximum activity of 13 mm. The MIC and MBC observed were 30-140 mcg/ml and 40-160 mcg/ml, respectively. *P. piscatorum* and *P. emblica* showed the lowest MIC (30 mcg/ml), *P. emblica* the lowest MBC (40 mcg/ml) and thus an effective inhibitor of the tested bacteria. Alkaloids, saponins and tannins were detected in 7 out of 8 tested plants.

Key words: Plant extracts, Antibacterial activity, MIC, MBC, Phytochemical tests

Introduction

Finding healing powers in plants is an ancient idea. There is an evidence that Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyhock (Stock, 1988) as medicines. These plants are still widely used in ethnomedicine around the world. It is estimated that there are 250,000 to 500,000 species of plants on earth . A relatively small percentage (1 to 10%) of these are used as food. It is possible that even more are used for medicinal purposes (Moerman, 1996).

Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). The increase in resistance of microorganisms due to indiscriminate use of commercial antimicrobial drugs encouraged scientists to search for new antimicrobial substances from various sources including medicinal plants (Karaman et al., 2003). Another driving factor for the renewed interest in past 20 years has been the rapid rate of plant species extinction.

Around 12,000 plant secondary metabolites of antimicrobial importance have been isolated. These compounds fall in one of the major groups of compounds like phenols, quinones, flavonoids tannins, terpenoids, alkaloids and other mixtures (Schultes, 1978).

Infectious diseases account for high proportion of health problems in the developing countries (Sashi et al, 2003). In India, about 2500 species of plants are used for medicinal purposes and about 90% of the medicinal plants provide raw materials for the herbal pharmaceuticals, which are collected from wild habitats. The rich knowledge base of countries like India in medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programmes in the pursuit of discovering novel drugs (Rajasekharan and Ganeshan, 2002).

An antimicrobial compound arylnaphthalide lignan Justicidin B was isolated from *Phyllanthus piscatorum* (Gertsch et al., 2004). Antimicrobial acivities have been repored from *Bridelia ferruginea* (Kabir et al., 2005;

Akinpelu, 2000), *Mallotus phillipensis* (Taylor et a.l., 1996), *Phyllanthus emblica* (Panthi and Chaudhary, 2006), *Phyllanthus polyphyllus* (Ngemenya et al, 2006) and *Bridelia* species (Irobi et al 1994). However, several plants are used in India in the form of crude extracts without scientific evidence of efficacy (Ahmed et al., 1998). At this juncture, it is of interest to determine the scientific basis for the traditional use of these medicinal plants. The aim of the present study is to reveal the antimicrobial properties and phytochemicals of 8 selected medicinal plants through *in vitro* investigation.

Materials and Methods

Plant Materials

Eight whole plant materials (Table 1) of the family Euphorbiaceae were collected locally or either procured from local traditional healers claiming their efficacies. Their botanical identities were determined and authenticated. Samples were deposited in the Botany Department Herbarium of Kakatiya University. The whole plants were oven dried at 60^0C for one week, and powdered and stored in airtight containers. 10 g of each of the powdered plant materials were extracted in a soxhlet extractor containing 40 ml of 80% methanol. The resulting extracts were evaporated under reduced pressure.

Phytochemical tests

Methanolic extracts of the plants were qualitatively analyzed. Tannins, phenols and steroids were tested as described by Gibbs (1974). Alkaloids, ellagic acids, iridoids, lignans, methelene dioxy compounds, triterpenoids were tested by standard procedures (Trease and Evans, 1989).

Bacterial cultures

Four Gram positive bacteria, *Bacillus stearothermophilus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus leuteus*, and four gram negative bacteria, *Salmonella typhi*, *Enterobacter aerogens*, *Proteus mirabilis* and *Proteus vulgaris* were used for bioassay. The pure strains were obtained from microbial type culture collection and gene bank (MTCC), Institute of microbial Technology, Chandigarh, India. The organisms were maintained on agar slopes at 4^oC and sub cultured for 24hr before use.

Bacterial susceptibility testing

The Agar plate well–diffusion method was used as described by Desta (2005). A standardized inoculum 1-2 $\times 10^7$ cfu/ml 0.5 MC Farland standards was introduced onto the surface of sterile agar plate, and evenly distributed by using a sterile glass spreader. Simultaneously, 8 mm wells were cut from the plate using a sterile cork borer. 70 µl of extract at a concentration of 50 mcg/ml was introduced into each well. The agar plates were incubated aerobically at 37^oC. After 24hr, the inhibition zones were measured with a ruler and compared with the control well containing only methanol. 30 mcg/ml of ampicillin served as control.

Determination of MIC and MBC

MICs and MBCs of the extracts were determined as described by Kabir et al. (2005). MICs of the extracts were determined by diluting them to various concentrations ranging from 10 to 200 mcg/ml. Each volume of each extract and nutrient broth were mixed in a test tube and 0.1 ml of standard inoculum (1-2 $\times 10^7$ cfu/ml) was added to each tube. Control tubes were maintained simultaneously. The tubes were incubated aerobically at 37^0C for 24 hrs. The lowest concentration of extract that produced no visible bacterial growth (no turbidity) when compared with control tube was regarded as MIC. MBC was determined by sub-culturing the test dilution onto a fresh agar plate (without extract) and incubated for 24 hr. The highest dilution that yielded no single bacterial colony was taken as MBC.

Statistical analysis

All the tests were conducted in triplicates. The data of all the parameters were statistically analyzed and expressed as mean ± S.D.

Results and Discussion

The profile of eight medicinal plants used in this study is shown in Table 1.

Tests were conducted for the presence of phytochemicals in all of these methanolic extracts (Table 2). Alkaloids, phenols and tannins were detected in all the extracts except that of *P. Emblica*. *P .piscatorum* and *P. flaccidus* which were positive to 9 and 8 phytochemical tests respectively. These results are in parallel to the earlier studies conducted on terpens , alkaloids , lignans , flavonoids and tannins in *Phyllathus* species (Vongvanich et al, 2000; Houghton, 1999; Lin et al, 1995). The results of the phytochemical tests on *B. ferruginea* confirms the studies on the same by Kabir, where he reported the presence of alkaloids, tannins, saponins and flavonoids (Kabir et al.,

Table -1: Profile of Eight medicinal plants used

Plant species	Activity	Voucher number
Breynia ramnoids	Anti-convulsant, sedative, Antibacterial	APE 11
Bridelia montana	Antibacterial, skin cleanser	APE 12
Bridelia ferruginea	Antibacterial, Antifungal, Anti-spasmatic	APE 13
Mallotus phillipensis	Urolithotriptie, Antibacterial	APE 14
Phyllanthus emblica	Antidiabetic, antioxidant, antibacterial, antifungal, anti-viral, immunomodulatary	APE 15
Phyllanthus flaccidus	Anti-edematic, anti-dysentry antimicrobial	APE 16
Phyllanthus Polyphyllus	Anti-inflammatory, sedative, anti-bacterial	APE 17
Phyllanthus piscatorum	Antibacterial, anti-dysentery, immunomodulatory.	APE 18

Table 2: Results of phytochemical tests

Plants	AL	EA	IR	LI	MDC	ST	TA	TT	pH
B.ramnoids	++	+	-	+	+	+	+	-	++
B.montana	++	-	+	-	+	+	++	-	++
B.ferruginea	++	++	++	+	+	+	+	++	++
M.phillipensis	++	-	-	++	-	+	+	+	++
P.Emblica	+++	+	+	+	-	-	++	+	++
P.flaccidus	++	+	+	-	+	++	+++	++	+++
P.Polyphyllus	++	+++	-	-	-	+	++	-	+
P.piscatorum	+++	+	+	+++	+	+	+++	+	++

AL – Alkaloids, EA – Ellagic acids, IR – Iridoids, LI – Lignans, MDC – Methelene dioxy compounds, ST – Steroids, TA – Tannins, TT – Triterpenoids, PH – Phenols,
+++ = High Amount; ++ = Moderate Amount; + = Low Amount; - Absent

Table 3: Antibacterial activity of the crude plant extracts by well diffusion method

Plants	B.st	S.A	B.S	M.l	S.t	E.a	P.m	P.v
B. ramnoids	--	--	5 ± 0.2	-	-	4 ± 0.2	-	-
B. montana	-	-	-	-	-	-	-	-
B. ferruginea	-	-	8 ± 0.2	8 ± 0.1	-	8 ± 0.2	8 ± 0.1	10 ± 0.1
M. phillipensis	-	3 ± 0.1	3± 0.1	3± 0.2	-	3 ± 0.2	3 ± 0.2	-
P. Emblica	-	9± 0.1	10 ± 0.1	10	8 ± 0.1	13 ± 0.2	11± 0.1	13 ± 0.1
P. flaccidus	-	-	-	-	-	-	-	-
P. Polyphyllus	-	-	-	-	-	-	-	-
P. piscatorum	-	6± 0.1	10± 0.2	9 ± 0.2	-	9 ± 0.1	10 ± 0.1	9 ± 0.2
Ampicillin	-	16±0.1	14±0.1	12±0.1	10±0.1	12±0.2	16±0.1	15±0.1

Key: B.st – *Bacillus stearothermophilus*, S.a – *Staphylococcus aureus*, B.s – *Bacillus subtilis*, M.l– *Micrococcus leuteus*, S.t – Salmonella typhi, E.a – *Enterobacter aerogens*, P.m – *Proteus mirabilis*, P.v. – *Proteus vulgaris*,
Figures indicate average zone of inhibition (in mm), (-) = No inhibition, Ampicillin = Commercial antibiotic.

Table 4: Efficacies of crude extracts as compared to standard ampicillin

Plant species	B.st	S.A	B.S	M.l	S.t	E.a	P.m	P.v
B.ramnoids	-	-	36%	-	-	33%	-	-
B.montana	-	-	-	-	-	-	-	-
B.ferruginea	-	-	57%	65%	-	65%	50%	66%
M.phillipensis	-	19%	22%	25%	-	25%	19%	-
P.Emblica	-	57%	72%	81%	80%	109%	67%	86%
P.flaccidus	-	-	-	-	-	-	-	-
P.Polyphyllus	-	-	-	-	-	-	-	-
P.piscatorum	-	38%	72%	73%	-	73%	61%	57%

Key: B.st – *Bacillus stearothermophilus*, S.a – *Staphylococcus aureus*,
B.s – *Bacillus subtilis*, M.l– *Micrococcus leuteus*, S.t – Salmonella typhi,
E.a – *Enterobacter aerogens*, P.m – *Proteus mirabilis*, P.v. – *Proteus vulgaris*,
% - Efficacy as compared to ampicillin in inhibiting the bacteria.

Table 5: Minimum inhibitory and bactericidal concentrations of methanol extracts (mcg/ml)

Plants	B.st		S.a		B.s		M.l		S.t		E.a		P.m		P.v	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B.ramnoids	135	150	90	105	85	105	75	90	115	130	90	115	130	160	85	130
B.montana	110	130	135	60	70	95	95	115	90	110	110	125	80	105	70	105
B.ferruginea	70	85	95	125	60	85	65	90	85	110	50	75	40	65	75	85
M.phillipensis	95	105	85	100	85	110	70	105	95	120	110	135	90	120	80	95
P.Emblica	95	105	50	65	40	60	50	80	35	45	45	50	30	40	60	80
P.flaccidus	130	160	110	135	90	115	95	135	85	95	85	110	110	125	95	105
P.Polyphyllus	140	155	85	115	95	130	85	105	125	155	80	105	95	120	80	105
P.piscatorum	90	120	75	105	30	55	50	65	80	110	45	65	40	50	70	85

Key: B.st – *Bacillus stearothermophilus*, S.a – *Staphylococcus aureus*, B.s – *Bacillus subtilis*, M.l– *Micrococcus leuteus*, S.t – Salmonella typhi, E.a – *Enterobacter aerogens*, P.m – *Proteus mirabilis*, P.v. – *Proteus vulgaris*,
MIC – Minimum inhibitory concentration, MBC – Minimum bactericidal concentration,;mcg/ml – Microgram per milliliter

2005). However, *B. montana* revealed the presence of phenols, tannins, alkaloids, ellagic acid and lignans in contrast to the report of Irobi, where he reported the presence of only phenols and tannins (Irobi et al., 1994). The difference in the findings might be due to the nature of solvent used for extraction which determines the presence or absence of a metabolite in the extract.

Eight whole plant methanol extracts were tested against 4 gram +ve and 4 gram -ve bacteria. The results of antibacterial activity of the methanol extracts and their efficacies as compared to standard ampicillin are depicted in Tables 3 and 4 respectively. In agar well diffusion assay, the diameter of inhibition zones ranged from 3-13 mm (Table 3). *P. emblica* showed maximum antibacterial activity against *E. aerogens* (13 mm), *Proteus vulgaris* (13 mm) and *Proteus mirabilis* (11 mm) with an efficiency of 109%, 86% and 67% to those of Ampicillin. Maximum zones of clearance by *P. emblica* were observed in gram –ve bacteria. Similar results were obtained by Mazumder,

where the extract showed significant concentration dependent antibacterial activity particularly against gram –ve microbes (Mazumder et al., 2006). *P. emblica* showed inhibitory activity against 7 organisms including 4 gram –ve bacteria. Phytochemicals tests revealed the presence of high amounts of alkaloids and also phenols in the extract of *P. emblica*. Alkaloids (Kabir et al., 2005) and phenols (Houghton et al., 1999) have been reported to possess antimicrobial activity.

None of the extracts or Ampicillin was active on *B. stearothermophilus*. So this bacteria is considered as most resistant towards all the extracts tested. *B. montana, P. flaccidus* and *P. polyphyllus* showed no inhibition. Irobi reported that *Bridelia* species produced antimicrobial activities ranging from 4-20 mm (Irobi et al., 1994). This is due to high concentration of 5 mg/ml of extracts tested as compared to 100 µg/ml of extract tested in present study. This other wise can be stated as significant concentration dependent antibacterial activity (Mazumder et al., 2006).

Lowest antibacterial activity was exhibited by *M. phillipensis* (3 mm) against 3 gram +ve and 2 gram –ve bacteria. This confirms the earlier report of Chattopadhyay, where the crude methanol extract was found to be active against *S. aureus*, *B. subtilis* and *P. mirabilis* (Chattopadhyay et al., 2002) . Taylor reported that bacteria causing diarrhea and dysentery were effectively inhibited by extract of *M. phillipensis* (Taylor et al, 1996). *P. piscatorum* showed maximum activity of 10 mm against *B. subtilis* and *P. mirabilis* with an efficacy of 72% and 61% respectively as that of standard Ampicillin (Table 4). Gertsch reported that *P. piscatorum* had no inhibitory activity against gram +ve bacterial strains (Gertsch et al., 2004) . In this study the extract showed inhibitory activity against 3 gram +ve and 3 gram –ve among the 8 tested bacterial strains. The reason for the difference in activities in both of the findings is supposed to be dependent on plant habitat (Rajakaruna et al., 2002). The antibacterial activity of *P .piscatorum* might be due to the presence of high amounts of lignans as revealed in phytochemical tests. Lin reported the antimicrobial activity of lignans isolated from *Phyllanthus sps* (Lin et al., 1995). The inhibitory activity of *B. ferruginea* against two gram +ve and three gram -ve bacteria was consistent with the earlier report of Akinpelu where he reported inhibitory activity on seven out of 8 organisms tested (Akinpelu, 2000). Maximum inhibitory zone of 10 mm was noticed as compared to Kabir et al., (2005) with 4.7 and 4.6 mm zones of activity by *B. ferruginea*.

The MICs and MBCs of the eight extracts is 30-140 mcg/ml and 40-160 mcg/ml, respectively (Table 5) *P. piscatorum* and *P.emblica* showed lowest MIC (30 mcg/ml) against *B.subtilis* and *P.mirabilis* respectively. *P .emblica* showed the lowest MBC (40 mcg/ml) against *P .mirabilis*. This result is similar with that of Onoch, where *Phyllanthus* species were active against *P.mirabilis* (Onoch et al, 2003). According to Panthi, *P. emblica* was more effective in fresh rather than in dry condition (Panthi and Chaudhary, 2006). So >30 mcg/ml (MIC) and >40 mcg/ml (MBC) can be expected of the same. This is interesting and such low concentrations could be used in combination with other plant extracts. *P. emblica* is one of the best constituents of a well known drug 'Triphala' i.e combination of *P. emblica, T. chebula* and *T. bellirica* in equal proportion is used to manage stomach disorder. *P. polyphyllus* with MIC of 140 mcg/ml and *P.flaccidus* with MBC of 160 mcg/ml showed highest concentrations on *B. stearothermophilus*, which when compared to MIC of 2 mg/ml and MBC of 6 mg/ml (Ngemenya et al., 2006) is very low in concentration. The antimicrobial activity of these plant species can be attributed by the presence of alkaloids phenols and tannins (Table 2). It has been reported that Alkaloids, phenols and tannins are plant metabolites well known for antimicrobial activity (Tschesche, 1970).

P .piscatorum, B .ferruginea and *P. emblica* showed MIC of <100 mcg/ml on all the bacteria tested , so these 3 extracts can be considered to have broad spectrum antibiotic values. This is in parallel with the findings of Kabir et al (2005) at MIC of > 100 mcg/ml for *B.ferruginea*. The antimicrobial activity of *P. piscatorum* might be due to arylnaphthalide lignan Justicidin B (Gertsch, 2004). *B.subtilis, M.leuteus* and *P.vulgaris* were inhibited at <100 mcg/ml (MIC) by all the extracts tested. These 3 bacteria can be treated as sensitive towards all the extracts used. From Table 5 it is clear that extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations.

Conclusion

It is evident from the present study that some of the extracts inhibited the tested microorganisms. The extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations.

Acknowledgements

Authors are thankful to S.Girisham, Head, Department of Microbiology for providing the necessary

facilities and Mr.Chaeraalu for technical assistance.

References

1. Ahmed, I., Mehmood, Z and Mohammad, F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol **62:** 183-193.
2. Akinpelu, D.A. and Olorunmola, F..O. (2000). Antimicrobial activity of *Bridelia ferruginea* fruit. Fitoterapia **71:** 75-76.
3. Chattopadhyay D, Arunachalam G, Mandal A.B, Sur T.K, Mandal S.C and Bhattacharya S.K (2002). Antimicrobial and anti-inflammatory activity of folkfore: *Mallotus Peltatus* leaf extract. J Ethnopharmacol **82:** 229-37.
4. Davis, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. Science **264:** 375-382.
5. Desta, B (2005). Ethiopian traditional herbal drugs part 11.Antimicrobial activity of 63 medicinal plants. J Ethnopharmacol **100:** 168-175.
6. Gibbs R.D (1974). chemotaxonomy of flowering plants. McGill-Queen's university press, Nontreal and London vol I: 523-619.
7. Gertsch J, Niomawe, Gertsch-Roost K. and Sticher O (2004). *Phyllanthus Piscatorum*, Ethnopharmacological studies on a women's medicinal plant of the Yanomami Amerindians. J Ethnopharmacol **91:** 181-188.
8. Houghton P.J, Woldemariam T.Z, O'Shea S, Mullen J.E, Rostron T, Bantvala J.E,Walker E, Thyagarajan S.P, Soloman S and Yeap Foo L (1999). The anti-HIV activity of an aqueous extract and polyphenolic compounds of *Phyllanthus amarus* J Pharm Pharmacol **51:**100.
9. Irobi O.N, Moon-Yong M & Anderson W.A. and Daramola S.A. (1994). Antimicrobial activity of bark extracts of *Bridelia ferruginea* (Euphorbiaceae), J. Ethnopharmacol. **43:** 185-90.
10. Kabir O. A, Olukayode O, chidi E O, Christopher C and fasure K.A (2005). Screening of crude extracts of six medicinal plants used in south-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus activity.* Comp Alt Med **5:** 6.
11. Karaman I, Sahin P, Gulluce M, Oguten H, Songul M and Adiguzed, A (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus L.* J Ethanopharmacol **2837:** 1-5.
12. Lin M.J, Lec S.S and Liu K.C.S.C (1995) Phyllamyricins A-C, 3 Novel Lignans from *Phyllanthus myrtifolius* J Nat Prod **58:** 244-249.
13. Mazumder A, Mahato A. and Mazumder R (2006). Antimicrobial potentiality of *Phyllanthus amarus* against drug resistant pathogens. Nat Prod Res. **20:** 323-326.
14. Moerman D.E (1996) An analysis of the food plants and drug plants of Native North America. J Ethnopharmacol **52:** 1-22.
15. Ngemenya, Moses N.,James A, Mbah, Pierre Jane and Vincent P.K. Titanji (2006) Antibacterial effects of some Cameroonian medicinal plants against common pathogenic bacteria. Afr J Tra Comp and Alt Med **2:** 84-93.
16. Onoch P.A, Opegbemi A.O, Kadri O.A, Ajayi K.M and Okorie D.A (2003) Antimicrobial properties of Nigerian Euphorbiaceae. Nigerian J Natural Products Med **7:** 9-12.
17. Panthi M.P and Chaudhary R.P (2006) Antibacterial activity of some selected folklore medicinal plants from west Nepal. Scientific World **4:** 205-210.
18. Rajasekharan P.E and Ganeshan S (2002) Conservation of medicinal plant biodiversity in Indian perspective. J Med Aromatic Pl Sci **24:** 132-147.
19. Rajakaruna N, Harris C and Towers G.H.N (2002) Antimicrobial activity of plants collected from serpentine outcrops in Srilanka. Pharm biol **40:** 235-244.
20. Sashi Kumar J, Remya M and Janardhan K (2003) Antimicrobial activity of ethnomedicinal plants of Nilgiri Biosphere reserve and Western ghats. Asian J Microbial Biotech Env Sci **5:** 183-185.
21. Schultes R.E (1978) The Kingdom of plants In: W.A.R. Thomson (ed.), Medicines from the earth, McGraw-Hill Book Co., New York, pp 208.
22. Stock Well C (1988) Natures pharmacy. Century Hutchinson Ltd., London, United Kingdom.
23. Taylor R.S.L, Edel F, Manandhar N.P and Towers G.H.N (1996) Antiviral activities of Nepalese medicinal plants. J Ethnopharmacol **52:** 157-163.
24. Trease,G.E and Evans W.C (1989) A text-book of pharmacognosy. Bailliere Tinall Ltd., London. p53.
25. Tschesche R (1970) Advances in the chemistry of antibiotic substances from higher plants pharmacognosy and phytochemistry. In: Proceedings of the 1st international congress, Murich, Edited by Wagner H, Horharmmer L. Springer – verlag, Berlin Heidelberg, New York 171: 274-289.
26. Vongvanich N, Kittakoop P, Kramyu j, Tanticharoen M and Thebtaranonth Y (2000) Phyllanthusols A and B, Cotytoxic norbisabolane glcosides from *Phyllanthus acidus* skeels. J Org Chem **65:** 5420-3.