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Full Length Research Paper

Antibacterial studies and phytochemical constituents of South Indian *Phyllanthus* species

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Antibacterial activity and phytochemical tests of the methanol extracts of six *Phyllanthus* species were evaluated. In agar well diffusion assay the diameter of inhibition zones ranged from 3 - 22 mm. *Phyllanthus amarus* showed maximum activity of 22 mm. The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) observed for *Bacillus stearothermophilus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus leuteus*, *Salmonella typhi*, *Enterobacter aerogens*, *Proteus mirabilis*, and *Proteus vulgaris* were 30 - 205 µg/ml and 40 - 230 µg/ml, respectively. *P. amarus*, *P. hookeri* and *P. maderaspatensis* showed the lowest MIC (30 µg/ml) as well as MBC (40 µg/ml) and thus an effective inhibitor of the tested bacteria. Lignans, triterpenoids and phenols were detected in all the 6 tested plants.

Key words: Plant extracts, antibacterial activity, phytochemical tests.

INTRODUCTION

The *Phyllanthus* genus belongs to the Euphorbiaceae family. There are over 300 genera with over 5000 species in the Euphorbiaceae worldwide. *Phyllanthus* has about 750 - 800 species, found in tropical and subtropical regions. A number of the *Phyllanthus* species have been reported to have extensive history in medicine systems. Substantial amount of secondary metabolites present in the genus are used widely in traditional medicine for the treatment of flu, dropsy, diabetes, jaundice, gall and bladder calculus, liver disease (Unander et al., 1995; Calixto et al., 1998; Dhiman and Chawla, 2005).

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). The *Phyllanthus* genus is a source of plant chemicals. Extracts of *Phyllanthus* have secondary compounds like alkaloid, flavonoid, lignin, phenol, tannin and terpene. Many of the “active“ constituents are attributed to biologically active lignin, glycosides, flavonoids, lkaloids, ellagilannins and phenyl propanoids that are found in the leaf, stem and roots of the plant. mmon lipids such as sterols and flavonols also occur in the plant. Infectious diseases account for high proportion of health problems in the developing countries (Sashi et al., 2003). In India, about 500 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

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Table 1. Profile of Six medicinal plants used.

Plant species	Activity	Voucher number
Phyllanthus amarus	Antibacterial, antifertility, Immunomodulatory, bodyache, Abscess, Boils	APE 19
Phyllanthus debilis	Antimicrobial, swelling, intestinal worms, cold, sedative, cuts, inflammation, rheumatism	APE 20
Phyllanthus hookeri	Antidiabetic, spasmatic, wound, fever, inflammation, antibacterial and snake bite	APE 21
Phyllanthus kozhikodianus	Anticonvulascent, Antidysentry, jaundice, ulcer, itching, anti microbial	APE 22
Phyllanthus maderaspatensis	Anti – edematic, anti dysentery, Immunomodulatory, fever, ulcer, burn, jaundice, cold, anti microbial.	APE 23
Phyllanthus nozeranii	Anti viral, spasmatic, piles, anti bacterial, headache, boils, indigestion	APE 24

for research and development programmes in the pursuit of discovering novel drugs (Rajasekharan and Ganeshan, 2002).

A number of the *Phyllanthus* species have been reported to have extensive history in medicine systems (Unander et al., 1990, 1991). Researches and review on *Phyllanthus* species indigenous to some countries are known for its numerous antimicrobial and antiviral activities. An antimicrobial compound phyllanthin was isolated from *Phyllanthus amarus* (Sayyada et al., 2006). Antimicrobial activities have been reported from *Phyllanthus debilis* (Iqbal et al., 2001) and *Phyllanthus maderaspatensis* (Sayyada et al., 2005). However, several plants are used in India in the form of crude extracts without scientific evidence of efficacy (Ahmed et al., 1998). 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 6 selected medicinal plants through *in vitro* investigation.

MATERIALS AND METHODS

Plant materials

Six 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°C 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°C and sub cultured for 24 h before use.

Bacterial susceptibility testing

The agar plate well–diffusion method was used as described by Desta (2005). A standardized inoculum 1 - 2 × 107 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 methanol extract at a concentration of 50 µg/ml was introduced into each well. The agar plates were incubated aerobically at 37°C. After 24 h, the inhibition zones were measured with a ruler and compared with the control well containing only methanol. 30 µg/ml of ampicillin served as control. The work was done in triplicates.

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 µg/ml. Each volume of each extract and nutrient broth were mixed in a test tube and 0.1 ml of standard inoculum (1 - 2 × 107 cfu/ml) was added to each tube. Control tubes were maintained simultaneously. The tubes were incubated aerobically at 37°C for 24 h. The lowest concentration of extract that produced no visible bacterial growth (no turbidity) when compared with control tube was regarded as

Table 2. Results of phytochemical tests.

Plant species	AL	EA	IR	LI	MDC	ST	TA	TT	PH
Phyllanthus amarus	+++	++	+++	++	+	+	+++	++	+
Phyllanthus debilis	–	–	–	++	+	–	+++	–	++
Phyllanthus hookeri	++	++	+++	+++	+	+++	+++	+	+++
Phyllanthus kozhikodianus	+++	++	–	+	+	–	+++	++	+++
Phyllanthus maderaspatensis	–	++	+	+++	–	–	+++	+	++
Phyllanthus nozeranii	+++	–	–	+++	–	–	+++	++	++

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

Plant species	*B.st*	*S.a*	*B.s*	*M.l*	*S.t*	*E.a*	*P.m*	*P.v*
Phyllanthus amarus	–	8	7	14	16	16	22	20
Phyllanthus debilis	–	–	–	–	–	–	–	–
Phyllanthus hookeri	–	3	–	15	20	–	–	5
Phyllanthus kozhikodianus	–	–	12	8	–	6	10	13
Phyllanthus maderaspatensis	–	–	–	–	3	14	18	10
Phyllanthus nozeranii	–	5	13	8	12	14	12	18
Ampicillin	–	24	20	16	24	18	24	20

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.

MIC. MBC was determined by sub-culturing the test dilution onto a fresh agar plate (without extract) and incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as MBC.

RESULTS AND DISCUSSION

The profile of six 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). Lignans, triterpenoids and phenols were detected in all the 6 tested plants. These results are in parallel to the earlier studies conducted on terpenes, alkaloids, lignans, flavonoids and tannins in *Phyllanthus* species (Vongvanich et al., 2000; Houghton et al., 1999; Lin et al., 1995). 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. Six 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 - 22 mm (Table 3). *P. amarus* showed maximum antibacterial activity against *P. mirabilis* (22 mm) and *Proteus vulgaris* (20 mm) with an efficiency of 91.6 and 100% compared to ampicillin. Maximum zones of clearance by *P. amarus* were observed in gram –ve bacteria. Similar results were obtained by Mazumder et al. (2006), where the extract showed significant concentration dependent antibacterial activity particularly against gram –ve microbes. *P. amarus* showed inhibitory activity against 7 organisms including 4 gram –ve bacteria. Phytochemicals tests revealed the presence of high amounts of alkaloids and phenols in the extract of *P. amarus.* 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. *P. debilis* showed no inhibition. Lowest antibacterial activity was exhibited by *P. hookeri* and *P. maderaspatensis* (3 mm). Mazumder et al. (2006) reported that bacteria causing diarrhea and dysentery were effectively inhibited by extract of *P. amarus*. 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 results obtained in antimicrobial activity are similar to those of Lin et al. (1995). The MICs and MBCs of the six extracts are 30 - 205 µg/ml and 40 – 230 µg/ml, respectively (Table 5). *P. amarus*, *P. maderas-*

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*
Phyllanthus amarus	–	33.3%	35%	87.5%	66.6%	88.8%	91.6%	100%
Phyllanthus debilis	–	–	–	–	–	–	–	–
Phyllanthus hookeri	–	12.5%	–	93.7%	83.3%	–	–	25%
Phyllanthus kozhikodianus	–	–	58.3%	50%	–	33.3%	41.6%	65%
Phyllanthus maderaspatensis	–	–	–	–	12.5%	77.7%	75%	50%
Phyllanthus nozeranii	–	20.8%	53.8%	50%	50%	77.7%	50%	90%

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 extract (mcg/ml)

Plant species	*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
Phyllanthus amarus	160	180	130	150	90	105	50	60	35	50	100	130	30	40	50	70
Phyllanthus debilis	140	160	135	150	165	175	135	150	120	140	135	145	140	160	120	135
Phyllanthus hookeri	125	140	110	125	135	150	80	100	30	40	140	160	120	140	95	105
Phyllanthus kozhikodianus	160	200	135	150	115	130	105	120	120	140	110	120	75	90	60	70
Phyllanthus maderaspatensis	120	150	140	160	120	140	135	150	100	115	50	60	30	40	70	80
Phyllanthus nozeranii	205	230	150	170	85	95	70	85	80	100	65	75	90	110	50	70

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

patensis and *P. hookeri* showed the lowest MIC (30 µg/ml) as well as MBC (40 µg/ml) against *P. mirabilis* and *S. typhi*, respectively. This result is similar with that of Onoch et al. (2003), where *Phyllanthus* species were active against *P. mirabilis*. According to Panthi and Chaudhary (2006) such low concentrations could be used in combination with other plant extracts. *P. nozeranii* with MIC of 205 µg/ml and MBC of 230 µg/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. amarus* showed the least MIC on all the bacteria tested, so this extract can be considered to have broad-spectrum antibiotic values. The antimicrobial activity of *P. amarus* might be due to phyllanthin (Mazumder et al., 2006). *P. vulgaris* and *M. leuteus* were inhibited at lower MIC concentrations by all the extracts tested. These 2 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.

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REFERENCES

Ahmed I, Mehmood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62: 183-193.

Calixto JB, Santos AR, Cechinel FV, Yunes RA (1998). A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. Med. Res. Rev. 18: 225-258

Davis J (1994). Inactivation of antibiotics and the dissemination of resistance genes. Science 264: 375-382.

Desta B (2005). Ethiopian traditional herbal drugs part 11.Antimicrobial activity of 63 medicinal plants. J. Ethnopharmacol. 100: 168-175.

Dhiman RK, Chawla YK (2005). Herbal Medicine for Liver Diseases. Dig. Dis. Sci. 50: 1807-1812.

Gibbs RD (1974). Chemotaxonomy of flowering plants. µgill-Queen's university press, Nontreal and London I: 523- 619.

Houghton PJ, Woldemariam TZ, O'Shea S, Mullen JE, Rostron T, Bantvala JE, Walker E, Thyagarajan SP, Soloman S , Yeap Foo L (1999). The anti-HIV activity of an aqueous extract and polyphenolic compounds of *Phyllanthus amarus* J. Pharm. Pharmacol. 51:100.

Iqbal MCM, Meiyalaghan S, Wijesekara KB, Abeyratne KP (2001). Antifungal activity from water extracts of some common weeds. Pakistan J. Bio. Sci. 4 (7): 843 – 845.

Kabir OA, Olukayode O, chidi EO, Christopher C, Fasure KA (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.

Karaman I, Sahin P, Gulluce M, Oguten H, Songul M, Adiguzed A (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L.. J. Ethanopharmacol. 2837: 1-5.

Lin MJ, Lec SS, Liu KCSC (1995). Phyllamyricins A-C, 3 Novel Lignans from *Phyllanthus myrtifolius* J. Nat. Prod. 58: 244-249.

Mazumder A, Mahato A. Mazumder R (2006). Antimicrobial potentiality of *Phyllanthus amarus* against drug *resistant pathogens.* Nat. Prod. Res. 20: 323-326.

Ngemenya MJN, Mbah P, Jane A, Vincent TPK (2006) Antibacterial effects of some Cameroonian medicinal plants against common pathogenic bacteria. Afr. J. Tra. Comp. Alt. Med. 2: 84-93.

Onoch PA, Opegbemi AO, Kadri OA, Ajayi KM, Okorie DA (2003) Antimicrobial properties of Nigerian Euphorbiaceae. Nigerian J. Natural Products Med. 7: 9-12.

Panthi MP, Chaudhary RP (2006) Antibacterial activity of some selected folklore medicinal plants from *west Nepal.* Scientific World 4: 205-210.

Rajasekharan PE Ganeshan S (2002). Conservation of medicinal plant biodiversity in Indian perspective. J. Med. Aromatic Pl. Sci. 24: 132-147.

Rajakaruna N, Harris C, Towers GHN (2002) Antimicrobial activity of plants collected from serpentine outcrops in Srilanka. Pharm. Boil. 40: 235-244.

Sashi Kumar J, Remya M, Janardhan K (2003) Antimicrobial activity of ethnomedicinal plants of Nilgiri Biosphere reserve and Western ghats. Asian J. Microbial. Biotech. Env. Sci. 5: 183-185.

Sayyada K, Vartika R, Ajay KSR, Shanta M (2005). Comparative pharmacognostic studies of three *Phyllanthus* species. J. Ethnopharmacol. 104: 79-86.

Sayyada K, Vartika R, Ajay K, Singh R, Shanta M (2006) Comparative pharmacognostic studies of three *Phyllanthus* species. J. Ethnopharmacol. 104: 79–86

Schultes RE (1978) The Kingdom of plants In: W.A.R. Thomson (ed.), Medicines from the earth, µgraw-Hill Book Co., New York, p. 208.

Trease GE, Evans WC (1989) A text-book of pharmacognosy. Bailliere Tinall Ltd., London. p. 53.

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.

Unander DW, Webster GL, Blumberg BS (1995). Usage and bioassays in *Phyllanthus* (Euphorbiaceae): IV. Clustering of antiviral uses and other effect. J. Ethnopharmacol. 45: 1-18.

Vongvanich N, Kittakoop P, Kramyu J, Tanticharoen M, Thebtaranonth Y (2000) Phyllanthusols A and B, Cotytoxic norbisabolane glcosides from *Phyllanthus acidus* skeels. J. Org. Chem. 65: 5420-3.