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A study on antimicrobial activity and phyto chemical constituents of some phyllanthus plants in South Indian region

Ananthagiri Komuraiah[1]* and A. Ragan[2]

[1-2] *Department of Botany, Kakatiya University, Warangal, Telangana state India.*

*Email: akbotany@gmail.com

Abstract

Phytochemical tests and Antibacterial activity of the methanol extracts of four *Phyllanthus* species (*P. tenellus*, *P.emblica, P. simplex and P. acidus)* are evaluated. The diameter of inhibition zones ranged from 5 - 23 mm is found in agar well diffusion assay. *Phyllanthus tenellus* showed maximum activity of 23 mm. The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) observed for *Bacillus stearothermophilus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Salmonella typhi*, *Enterobacter aerogenes*, *Proteus mirabilis*, and *Proteus vulgaris. P. tenellus* and *P.emblica* showed the lowest MIC (30 µg/ml) as well as MBC (40 µg/ml) and thus an effective inhibitor of the tested bacteria. Alkaloids, Lignans, Triterpenoids,Tannins and Phenols were detected in all the four tested plants.

Keywords: Plant extracts, phytochemical tests, antibacterial activity

INTRODUCTION

The *Phyllanthus* is one of the important genus belongs to the *Euphorbiaceae* family. *Phyllanthus* species widely distributed throughout subtropical and tropical region and these are used in folk remedies. Therefore these Phyllanthus species have extensive history in traditional medicine for the treatment of diabetes, jaundice, gall, flu, liver diseases (Unander *et al*, 1995, Calixto *et al* 1998, Dhiman and Chawla 2005, Komuraiah *et al* 2009). It is very large genus having approximately 750 to 800 species.

India is one of the rich knowledge based countries in medicinal plants and that led to keen interest by pharmaceutical and Health care companies. These companies use the source of the knowledge for their Research and Development programmes in the pursuit of discovering novel drugs (Rajashekaran and Ganeshan 2002). The number of *Phyllanthus* species has been reported to have extensive history in medicinal system (Unander *et al* 1990, 1991, Komuraiah *et al* 2009). In India, more than 500 species of plants are used for medicinal applications. Among them, 90% of plants provide raw materials for the herbal pharmaceutical which are collected from wild habitat.

Microorganisms have created immense clinical problems in the treatment of infectious diseases and these have developed resistance to many antibiotics (Davis 1994). So the scientists started searching for new antimicrobial substance from various sources including traditional medicinal plants (Karaman et al 2003). Another factor for the renewed interest in the fast two decades has been the rapid rate of plant species extension. The secondary metabolites of antimicrobial importance has been isolated in around 12000 plants, these compounds fall in one of the major groups like phenols, flavonoids, tannins, terpenoids, alkaloids and other mixtures (Schultes 1978). *Phyllanthus* genus is an important source for phytochemicals. *Phyllanthus* extracts has secondary metabolites such as alkaloids, flavonoids, lignin, phenols, terpenes and tannins which are found in leaf stem and root of the plants (sheshi *et al* 2003).

Research and review on indigenous *Phyllanthus* species in some countries are known for its numerous antimicrobial activities. Phyllanthin antimicrobial compound was isolated from *Phyllanthus species* (Sayyada *et al* 2006 ,Iqbal *et al* 2001,Sayyada *et al* 2005). However in India, several plants are used in the form of crude extracts, without scientific evidence (Ahmed *et al* 1998). So there is a need of interest to

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determine the scientific bases for traditional use of medicinal plants. In the present study, we reveal the antimicrobial properties and phytochemicals through *in vitro* investigation for four medicinal plants.

MATERIALS AND METHODS

Plant Materials

Four 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 Herbarium of Botany Department, Kakatiya University. The whole plants were oven dried at 60^{o}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.

Table-1. Four species of *Phyllanthus* used.

Plant species	Activity	Voucher number
Phyllanthus acidus	Cytotoxic.	APE 14
Phyllanthus emblica	Antiaging, Anticancer, Anti-inflammatory, antimicrobial, Immunomodulatary	APE15
Phyllanthus Simplex	Antiviral Antithrombotic, Anti-cancer , Anti Hepatitis-B	APE16
Phyllanthus tenellus	Antibacterial, Parasitic, Immunomodulator	APE15

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, methylene 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 luteus*, and four gram negative bacteria, *Salmonella typhi, Enterobacter aerogenes, 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^{o}C 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 × 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^{o}C. 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 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 × 10^{7} cfu/ml) was added to each tube. Control tubes were maintained simultaneously. The tubes were incubated aerobically at 37^{0}C 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 four medicinal plants used in this study is shown in Table-1. Tests are conducted for the presences of phytochemicals in all of these methanolic extracts are present in Table-2. Alkaloids, Lignans, tannins, triterpenoids and phenols are detected in all the 4 tested plants. These results are in similar 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), which determines the presence or absence of a metabolite in the extract.

Table.2. Analysis of phenolic acids and secondary metabolities in 4 species of Phyllanthus

Plant species	AL	EA	IR	LI	MDC	ST	TA	TT	PH
Phyllanthus acidus	+++	-	-	+++	-	-	+++	++	++
Phyllanthus emblica	++	+	+	+	-	-	++	+	++
Phyllanthus simplex	++	-	-	+	-	-	++	++	++
Phyllanthus tenellus	+++	++	++	++	+	+	+++	++	+

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 acidus	-	5	13	8	12	14	12	18
Phyllanthus emblica	-	9	10	10	8	13	11	13
Phyllanthus simplex	-	7	-	10	-	12	18	15
Phyllanthus tenellus	-	8	5	14	16	16	23	21
Ampicillin	-	23	16	15	23	18	24	20

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

Four plant methanol extracts are 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 5- 23 mm (Table-3). *P. tenellus showed* maximum antibacterial activity against *Proteus mirabilis* (23 mm) and *Proteus vulgaris* (21 mm) with an efficiency of 92.6 and 100% compared to ampicillin. Maximum zones of clearance by *P. tenellus* are observed in gram –ve bacteria. These results similar to the results were obtained by Mazumder *et al*. (2006), where the extract showed significant concentration dependent antibacterial activity particularly against gram –ve microbes. *P.tenellus* shows 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.tenellus.* Alkaloids (Kabir *et al*., 2005) and phenols (Houghton *et al*., 1999) have been reported to own antimicrobial activity. None of the extracts or ampicillin is active on *Bacillus stearothermophilus*. So this bacterium is considered as most resistant towards all the extracts tested. The lower antimicrobial activity is exhibited by *P. tenellus* and *P.acidus* (5 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 four extracts are 30 - 205 µg/ml and 40 – 230 µg/ml, respectively (Table-5). *P. tenellus, P.acidus and P.emblica showed the lowest MIC (30*µg/ml) as well as MBC (40 µg/ml) against *Proteus mirabilis*. 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. acidus* with MIC of 205 µg/ml and MBC of 230 µg/ml showed highest concentrations on *B. stearothermophilus*, 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. tenullus* 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. tenullus* may be due to phyllanthin (Mazumder *et al*., 2006). *P. vulgaris* and *M. leuteus* are inhibited at lower MIC concentrations by all the extracts tested. These two bacteria can be treated as sensitive towards all the extracts used. From Table-5, it is clear that extracts were

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 acidus	-	38%	72%	73%	-	73%	61%	57%
Phyllanthus emblica	-	57%	72%	81%	80%	109%	67%	86%
Phyllanthus simplex	-	80%	-	-	-	75.5%	60%	45%
Phyllanthus tenellus	-	33.3%	35%	87.5%	66.6%	88.8%	92.6%	100%

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-5. Minimum inhibitory and bactericidal concentrations of methanol extract (µg/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 tenellus	160	180	130	150	90	105	50	60	35	50	100	130	30	40	50	70
Phyllanthus emblica	95	105	50	65	40	60	50	80	35	45	45	50	30	40	60	80
Phyllanthus simplex	150	120	160	125	130	140	55	90	20	55	135	155	110	80	65	95
Phyllanthus acidus	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, Figures indicate average zone of inhibition (in mm), (-) = No inhibition, Ampicillin = Commercial antibiotic

bacteriostatic at lower concentrations and bactericidal at higher concentrations.

Competing interests

The authors have declared that no competing interests exist.

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