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Screening of Antibacterial Activity of the Medicinal Plant *Phyllanthus amarus* Against Urinary Tract Infection Causing Bacterial Pathogens

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Abstract: Urinary tract infections (UTI) are one of the most commonly occurring infections in hospitals. But micro organisms causing UTI vary in their susceptibility from place to place and from time to time. In this present study, the herbal plant *Phyllanthus amarus* was tested for its antibacterial activity against urinary tract infection causing bacterial isolates *viz*., *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli*, *Enterobacter* sp., *Streptococcus fecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The *Phyllanthus amarus* was shade dried and extracted with methanol, acetone, chloroform, petroleum ether and hexane. The antibacterial activity of *Phyllanthus amarus* was determined by agar well diffusion method. It was found that methanol extract of *Phyllanthus amarus* showed highest inhibitory activity against UTI causing bacterial pathogens when compared to other solvent extracts. From this it was concluded that the solvent methanol is able to leach out antimicrobial principles very effectively from the plant than the other solvents. Phytochemical analysis showed the presence of alkaloids, flavonoids, phenols and triterpenes.

Key words: Urinary Tract Infections · *Phyllanthus amarus* · Organic Solvents · Antibacterial Activity · Disc Diffusion Method · Phytochemical Analysis

INTRODUCTION

Urinary tract infection (UTI) is the second most common infection. Worldwide about 150 million people are diagnosed with UTI each year, costing more than 6 billion US dollars. Among both out patients and inpatients, *Escherichia coli* is the primary clinical isolate accounting for 75 to 95 % of uncomplicated UTI isolates. *Klebsiella, Proteus, Pseudomonas, Enterococcus* and *Enterobacter* sp. are less commonly isolated from outpatients [1].

Urinary tract infections are a serious health problem affecting millions of people each year. Urinary tract infections account for about 8.3 million doctor visits each year. Women are especially prone to UTIs for reasons that are not yet well understood. One woman in five develops a UTI during her lifetime. UTIs in men are not as common as in women but can be very serious when they do occur. Although a variety of etiologies is involved with UTI, *Escherichia coli* and other coliforms account for large majority of naturally acquired urinary tract infections. They are the frequent cause of nosocomial infections in many hospitals. Bacteriological investigations of UTI are not complete without an antibiotic sensitivity test of the isolate [2].

Developing a medicinal plants sector, across the various states of India has become an important issue. Different stakeholders in the medicinal plants sector have projected Tamil Nadu, one of the southern states, as an "Herbal State". The significant contribution to the society, traditional medicine has experienced very little attention in modern research and development and less effort has been done to upgrade the practice [3]. The antimicrobial properties of plants have been investigated by number of researchers' worldwide. Since past few decades antibiotics from microbial origin and other chemotherapeutic agents have been used for control of bacterial disease. However due to indiscriminate use of these drugs, various pathogenic bacteria have developed resistance to many of the currently available antibiotics [4]. Other drawbacks are their high cost and undesirable side effects [5].

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There are over 300 genera with over 5000 species in the Euphorbiaceae worldwide. The *Phyllanthus* is one of the genus that falls under this enormous family. *Phyllanthus* has about 750-800 species, found in tropical and subtropical regions worldwide. *Phyllanthus amarus* is an erect annual herb of not more than one and half feet tall and has small leaves and yellow flowers [7].

Several bioactive compounds were isolated from this plant and some of them interact with most key enzymes. In traditional medicine, it is used for its hepatoprotective, anti-diabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties. *Phyllanthus amarus* leaf extract as a hepatoprotective agent (block DNA polymerase during Hepatitis B virus reproduction). The plant is also used in the treatment of stomach disorders, skin diseases and cold. It has anti-diarrhea, anti-carcinogenic and antimutagenic activities. It also has anti-nociceptive and antilipidemic potentials [8, 9].

Where's the aim of the study??

MATERIALS AND METHODS

Collection and Drying of Plant Materials: Mature leaves of *Phyllanthus amarus* were collected from different areas of Chidambaram, Cuddalore district, Tamil Nadu. The leaves of *Phyllanthus amarus* were washed thoroughly three times with water and once with distilled water. The plant materials were shade dried and powdered. The powdered samples were sealed in separate polythene bags until the time of extraction.

Preparation of Plant Extract: Forty g of powdered leaves were extracted successively with 200 ml of methanol (56-60°C), acetone (60-62°C), chloroform (60-62°C), petroleum ether (40-60°C) and hexane (62-66°C) in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use [10].

Test Microorganisms: Eight pathogenic urinary tract infection causing bacterial isolates, *viz*., *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli*, *Enterobacter* sp., *Streptococcus fecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* were used during the present study and were obtained from RJMC Hospital, Annamalai University, Chidambaram. The cultures were sub-cultured and maintained on nutrient agar slants and stored at 4°C.

Determination of Antibacterial Activity (Agar Well Diffusion Method): Bacterial inoculums were prepared by inoculating a loopful of test organisms in 5 ml of nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc McFarland standards.

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculums on the surface of the media. Wells (6 mm in diameter) were punched in the agar. Ethanol and ethyl acetate extracts with different concentrations (25, 50, 75 and 100 mg/ml) were mixed with 1 ml of dimethyl sulfoxide (DMSO) and added into the well. Well containing DMSO alone act as a negative control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

Phytochemical Analysis: Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloids [11, 12], glycosides [11], terpenoids and steroids [11], flavonoids [11], reducing sugars, triterpenes, phenolic compounds and tannins [13].

RESULTS AND DISCUSSIONS

Herbal medicine is still the main stay of about 75-80% of the whole population, in India and the major part of traditional therapy involves the use of plant extract and their active constituents [14]. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines [15].

In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [16].

The antibacterial activity of various plant extracts determined by agar well diffusion method was showed in Tables 1-5. Among the various solvents tested, the

Table 1: Antibacterial activity of methanol extract of *Phyllanthus amarus*

	Concentration of extract (Zone in mm)							
	25mg		50mg		75mg		100mg	
Organism	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
S. aureus	-	R	19	S	23	S	26	S
S. marcescens	22	S	32	S	35	S	37	S
E. coli	27	S	32	S	32	S	35	S
Enterobacter sp.	20	S	28	S	33	S	36	S
S. fecalis	-	R	20	S	24	S	31	S
K. pneumoniae	-	R	25	S	29	S	33	S
P. mirabilis	-	R	23	S	27	S	30	S
P. aeruginosa	-	R	27	S	32	S	35	S

R - Resistant; S - Sensitive

Table 2: Antibacterial activity of acetone extract of *Phyllanthus amarus*

	Concentration of extract (Zone in mm)							
	25mg		50mg		75mg		100mg	
Organisms	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
S. aureus	-	R	18	S	20	S	24	S
S. marcescens	15	S	22	S	26	S	30	S
E. coli	20	S	25	S	30	S	33	S
Enterobacter sp.	12	S	16	S	20	S	24	S
S. fecalis	-	R	23	S	25	S	28	S
K. pneumoniae	-	R	20	S	24	S	28	S
P. mirabilis	-	R	22	S	25	S	29	S
P. aeruginosa	10	S	29	S	32	S	30	S

R - Resistant; S - Sensitive

Table 3: Antibacterial activity of chloroform extract of *Phyllanthus amarus*

	Concentration of extract (Zone in mm)							
	25mg		50mg		75mg		100mg	
Organisms	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
S. aureus	-	R	12	S	15	S	18	S
S. marcescens	9	S	20	S	23	S	27	S
E. coli	12	S	17	S	22	S	28	S
Enterobacter sp.	8	S	14	S	20	S	25	S
S. fecalis	-	R	12	S	18	S	24	S
K. pneumoniae	-	R	10	S	20	S	22	S
P. mirabilis	-	R	20	S	23	S	25	S
P. aeruginosa	-	R	21	S	24	S	30	S

R - Resistant; S - Sensitive (How could be zone of inhibition 8 or 9 sensitive, what about the diameter of the well???

methanol extract showed highest inhibitory activity when compared to other solvent extracts. Next to methanol extract, acetone extract showed good inhibitory activity followed by chloroform extract and petroleum ether extract. The inhibitory activity of hexane extract was relatively low when compared to the other tested solvent extracts.

Earlier research work on the medicinal plants *Phyllanthus amarus* grown in the same research location have shown that extracts from some plants posses antimicrobial properties [17].

Saranraj *et al*. [21] investigated the antibacterial potentiality of ethanol and ethyl acetate extracts of *Acalypha indica* leaves against human pathogenic

Table 4: Antibacterial activity of petroleum ether extract of *Phyllanthus amarus*

	Concentration of extract (Zone in mm)							
	25mg		50mg		75mg		100mg	
Organisms	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
S. aureus	-	R	-	R	-	R	-	R
S. marcescens	-	R	10	S	15	S	20	S
E. coli	-	R	9	S	14	S	20	S
Enterobacter sp.	-	R	-	R	11	S	17	S
S. fecalis	-	R	8	S	12	S	19	S
K. pneumoniae	-	R	-	R	10	S	15	S
P. mirabilis	-	R	-	R	12	S	17	S
P. aeruginosa	-	R	11	S	15	S	20	S

R - Resistant; S - Sensitive

Table 5: Antibacterial activity of hexane extract of *Phyllanthus amarus*

	Concentration of extract (Zone in mm)							
	25mg		50mg		75mg		100mg	
Organisms	Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
S. aureus	-	R	-	R	-	R	-	R
S. marcescens	-	R	-	R	12	S	15	S
E. coli	-	R	-	R	13	S	15	S
Enterobacter sp.	-	R	-	R	-	R	13	S
S. fecalis	-	R	-	R	10	S	14	S
K. pneumoniae	-	R	-	R	-	R	-	R
P. mirabilis	-	R	-	R	8	S	12	S
P. aeruginosa	-	R	-	R	11	S	16	S

R - Resistant; S - Sensitive

Table 6: Phytochemical analysis of *Phyllanthus amarus* extracts

S. No.	Test	Result
1	Alkaloids	+
2	Glycosides	-
3	Tripenoid and steroids	-
4	Flavonoids	+
5	Reducing sugars	-
6	Triterpenes	+
7	Phenolic compounds	+
8	Tannins	-

bacteria and concluded that the ethanol extract shows more inhibitory activity against human pathogenic bacteria when compared to ethyl acetate extract. In this study, methanol was best solution for extracting the effective antimicrobial substances from the medicinal plant *Phyllanthus amarus* than other solvents.

Siva Sakthi *et al*. [22] evaluated the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Datura metel* against nine pathogenic bacteria isolates *viz*., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The ethanol extract of *Datura metel* showed maximum zone of inhibition against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition. The ethyl acetate extract of *Datura metel* showed maximum zone of inhibition against *Escherichia coli*. There was no zone of inhibition against *Pseudomonas aeruginosa*. In this study, methanol was best solution for extracting the effective antibacterial substances from the medicinal plant *Phyllanthus amarus* than other solvents used. This could be related to those bioactive metabolites present in *Phyllanthus amarus* which are not soluble in other solvents but they can be soluble in methanol.

Recently, Murugan and Saranraj [23] investigated the antibacterial activity of *Acalypha indica* by agar well diffusion method. It was found that 50mg/ml of methanol extract of the plant are able to inhibit the growth of nosocomial infection causing bacteria when compared to other solvent extracts. From this it was concluded that the solvent methanol is able to leach out antimicrobial principles very effectively from the plant than the other solvents.

Saranraj *et al*. [24] determined the antimicrobial activity of *Mangifera indica* ethanol extract by disc diffusion method. The zone of inhibition of *Mangifera indica* ethanol extract against bacteria was maximumal against *Vibrio cholerae* followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The least zone of inhibition was recorded against *Salmonella typhi*.

The phytochemical compounds present in the *Phyllanthus amarus* extracts was analyzed in the present study and the results were shown in Table 6. The *Phyllanthus amarus* showed the presence of alkaloids, flavonoids, phenols and triterpenes. Some studies concerning the effectiveness of extraction methods highlighted that methanol extract yields higher antibacterial activity than n-hexane and ethyl acetate [25]. Whereas other report concluded that chloroform is better than methanol and benzene [26]. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method [27].

From the present study, it can be concluded that the methanol extract of *Phyllanthus amarus* have more inhibitory activity against urinary tract infection causing bacterial isolates when compared to other solvent extracts and the antibacterial potentiality was due to the presence of phytochemical compounds like alkaloids, flavonoids, phenols and triterpenes. Further chemical and pharmacological investigations can be done to isolate and identify minor chemical constituents in the seeds and to screen other potential bioactivities.

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