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Evaluation of Antimicrobial Activity and Phytochemical Analysis of Crude Extracts of Terminalia Paniculata Roth

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ABSTRACT

The present study was carried out with an objective to investigate the antibacterial and antifungal potentials of bark of Terminalia paniculata Roth. The aim was the study was to assess the antimicrobial activity and to determine the zone of inhibition of ethanolic (EtTP) and ethyl acetate (EATP) extracts of stem bark of Terminalia paniculata against medically important bacterial and fungal stains. The antimicrobial activity was assessed in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of extract (100, 200, 400 µg/ml) of Terminalia paniculata were tested against three gram negative Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhimurium human pathogenic bacteria and one fungal strain Candida albicans. Zone of inhibition of extracts were compared with that of standard drug Streptomycin. The results showed that the significant inhibition of bacterial growth against the tested organisms. Phytochemical analysis revealed the presence of alkaloids, carbohydrate, flavanoids, phenolics, steroids and alkaloids. The antimicrobial activity of Terminalia paniculata was due to the presence of various secondary metabolites. Therefore the bark of the plant can be used to isolate bioactive natural product which may serve as leads in the development of new pharmaceutical research activities.

KEYWORDS : Terminalia paniculata Roth, antibacterial, antifungal, Streptomycin, zone of inhibition, phytochemical analysis, secondary metabolites

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa and viruses. Antibiotics are those substances which are, produced by microorganisms, effective in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as antibiotics becoming less and less effective against certain illnesses, due to emergence of drug resistant bacteria and some toxic life threatening side effects. For this reason it is necessary to investigate newer drugs with lesser resistance and drugs derived from natural sources play a vital role in prevention and treatment of human disease. In many developing countries, traditional medicine occupies a place in the primary healthcare system (Farnsworth, 1993; Houghton, 1995) and curative potentials of these obtained from herbs are well documented (Dubey *et al*., 2004). Nearly 61% of new drugs developed between 1982 and 2002 based on natural products have been found to be successful in the areas of infectious disease and cancer (Cragg *et al*., 2005). Recent studies have shown that there is declining trend in rate of discovery of active novel chemical entities (Lam, 2007). Natural products of higher plants may provide a new source of antimicrobial agents with possible navel mechanisms of action (Runyoro *et al*., 2006; Shahidi, 2004). The antimicrobial effect of plant extracts have been studied by a very large number of researchers globally (Reddy et al., 2001) and extensive work has been carried out on ethno medicinal plants in India (Maheshwari *et al*., 1986). Plants serve as rich source of wide variety of secondary metabolites such as alkaloids, carbohydrate, flavanoids, phenolics, steroids and alkaloids which have been found in vitro to exhibit antimicrobial properties (Dahanukar *et al*., 2000; Cowan, 1999). Herbal medicines have been familiar to man for centuries and therapeutic efficacy of many indigenous plants for several health disorders have been described by practioners of traditional medicine (Ramaswamy & Charles, 2009). The WHO estimates that plant extract or their active constituents are used as folk medicines in traditional therapies of 80% of the world's population (Shaik *et al*., 1994). Antimicrobial properties of medicinal plants have been reported from different parts of the world. The harmful organisms can be controlled with drugs which results in the emergence of multiple drug resistant bacteria creating alarming clinical situations in the treatment of infections. Development of number of newer antibiotics resulted in increasing resistance to these drugs by microorganisms. Generally, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs used as therapeutic agents (Towers *et al*., 2001).

In an attempt to expand the spectrum of antibacterial agents, from natural resources, the present study is carried out to evaluate the antimicrobial potential of ethanolic (EtTP) and ethyl acetate (EATP) extract of stem bark of *Terminalia paniculata* Roth against bacterial (*Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhimurium*) and fungal (*Candida albicans*) pathogens and their phytochemical analysis which are responsible for antimicrobial activity.

Terminalia paniculata Roth (Family: Combretaceae) commonly known as Kindal or Kinjal is a tropical wild tree with large distribution in Western Ghats of India (Figure 1). Traditionally, flower juice and bark of *Terminalia paniculata* have been used as a remedy for cholera for the treatment of inflamed parotid glands and in menstrual disorders. It is used as a cardiotonic and diuretic. Timber is useful for shipbuilding and as a substitute for teak. Fruits are used for tanning and dying. However, till date, there has been no investigation supporting the pharmacological properties of this plant. In the present investigation carried out, a screening of ethanolic (EtTP) and ethyl acetate (EATP) extracts of bark of *Terminalia paniculata* against pathogenic bacteria and fungi is done in order to detect new sources of antimicrobial agents.

MATERIALS AND METHODS

Collection of plant material

The bark of Terminalia Paniculata Roth was collected from the Devarakadu (preserved forest) of Hosagunda Village of Sagar Taluk, Shivamogga District under the supervision of localites and forest officials. The plant material was identified and authenticated by Resident Botanist, Prof. R. Manjunatha of Botany Department, DVS College of Arts and Science, Shivamogga.

Preparation of plant extract

Freshly collected bark of *Terminalia paniculata* were dried in shade

and pulverized to get a coarse powder. A weighed quantity of powder (830g) was used for extraction. Dried powder of bark was exhaustively extracted successively using Ethyl acetate (EATP) and Ethanol (95%) (EtTP) respectively in Soxhlet apparatus. All the extracts were concentrated by rotary flash evaporator, under reduced pressure and controlled temperature, followed by freeze drying and stored at 40°C for further use. This crude extracts of ethyl acetate and ethanol were used for further investigation for antimicrobial potential.

Preliminary phytochemical screening

The ethyl acetate and ethanolic extracts were subject to preliminary phytochemical analysis to detect the presence of different chemical entities viz. glycosides, carbohydrate, flavanoids, phenolics, steroids, saponins, tannins and alkaloids (Khandelwal, 2009; Kokate, 2000; Kumar *et al*., 2009).

Test Microorganisms

Bacterial and fungal strains used in the present study were obtained from the laboratory of National College of Pharmacy, Shivamogga, Karnataka, India. Bacterial strains used were *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhimurium* and fungal strain used was *Candida albicans*.

Preparation of Media

It was prepared by dissolving nutrient agar (14g) in 500 cm^3 distilled water. pH of solution adjusted to 7.4 and then sterilized for 20 minutes at 15 lb pressure in an autoclave.

Preparation of subcultures

One day prior to the test, the microorganisms were inoculated in to sterilized nutrient broth tubes and incubated at 37°C for 24 hours. On the day of testing the organisms were subculture into sterile nutrient broth. After incubating the same for three hours, the growth thus obtained was used as inoculums for the test.

Preparation of solution of crude extract of *Terminalia paniculata* Roth

Ethyl acetate and ethanolic crude extracts (10mg) were dissolved in Dimethyl Sulfoxide (DMSO) (10cm^3) in serially labeled test tubes to get required concentration and stored at 4°C.

Method of testing-Determination of zone of inhibition

In vitro antibacterial and antifungal activities were investigated for ethyl acetate and ethanolic stem bark extracts of Terminalia paniculata Roth by the agar disc diffusion method (Alzoreky & Nakahara, 2003; Bauer *et al*., 1966; Rios *et al*., 1988). This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a petridish to an extent such that growth of added microorganisms is prevented entirely in a circular zone around the cavity containing a solution of antibiotic.

Nutrient agar plates were prepared for both the extracts, 50 µl inoculam of each selected microorganisms was uniformly spreaded on agar plants with the help of glass spreader. After 5 minutes wells of approximately 5 mm diameter was bored with the help of sterile borer. Using sterile pipettes, the standard and the sample solution (50µl) of known concentration were fed into the bored wells. The dishes were left standing for 2 hours at room temperature as a period of pre incubation diffusion to minimize to effect the variation in time among the application of different solutions. There were then incubated for 24 hours at 37°C. The sensitivities of microorganisms species to the plant extract were determined by measuring the size of inhibitory zones on the agar surfaces around the wells. Each zone of inhibition recorded was average of three measurements. Zone of inhibition for water was done separately.

RESULTS

Preliminary phytochemical analysis

It was found that the ethyl acetate and ethanolic extracts of bark of *Terminalia paniculata* contained alkaloids, carbohydrate, flavanoids, phenolics, amino acids, steroids, saponins, glycosides, tannins and alkaloids (Table 1).

Microbial activity

The antimicrobial activity of the ethyl acetate and ethanolic extracts of *Terminalia paniculata* Roth bark were studied at different concentrations (100, 200, 300, 400 µg/ml) against three pathogenic gram negative bacterial strains *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhimurium* and one fungal strain *Candida albicans*. Antimicrobial and antifungal activities of the extract were assessed in terms of zone of inhibition of microbial growth. The antibacterial and antifungal potential of the extract increased linearly with increase in the concentration of extracts (µg/ml). As compound with standard drug (Streptomycin), the results revealed that the extracts (EtTP and EATP) showed significant antimicrobial activity. However the efficacy of EATP was found to be better than EtTP. (Table 2 & 3 and Figure 2, 3 & 4).

DISCUSSION

Herbal medicines are valuable and readily available resources for primary health care and complimentary health care system. The plant kingdom, undoubtedly still holds many unexplored species of plants containing active principles of medicinal value. Though antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world, these plants may prove to be a rich source of constituents with possible antimicrobial activities, require further pharmacological investigations. The WHO estimates that plant extracts or their active constituents are used as folk medicines in traditional therapies of 80% of the world's population. In the present study, the ethyl acetate and ethanolic extracts obtained from *Terminalia paniculata* bark show significant activity against tested bacterial and fungal strains. The results were compared with standard antibiotic drug Streptomycin. In this screening work, ethanolic and ethyl acetate extracts of bark of *Terminalia paniculata* were found to be not inactive against any microorganisms.

The above results show that the bark extracts of *Terminalia paniculata* show significant antibacterial and antifungal activities. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemical analysis showed that the plant contains more or less components like carbohydrate, flavanoids, phenolics, amino acids, steroids, saponins, glycosides, tannins and alkaloids. It has been reported that the plant rich in tannins and phenolic compounds have been shown to possess antimicrobial activities against number of microorganisms.

CONCLUSION

The present study justified the claimed uses of bark of *Terminalia paniculata* Roth in the traditional system of medicine to treat various infections disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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Table 1: Qualitative phytochemical analysis of stem bark of *Terminalia paniculata*

Sl. No.	Constituents	Test	EATP	EtTP
01	Steroids	Salkowski's test Libermann test	+ +	+ +
02	Alkaloids	Maeyer's test Dragendorf's test	+ +	- +
03	Flavanoids	Neutral Ferric chloride test Conc. Sulphuric acid test Sodium hydroxide test Reduction test(Zn/HCl)	+ - + -	+ - + +
04	Carbohydrates	Molish's test	+	+
05	Amino acids	Millon's test Biuret test	+ +	+ +

06	Saponins	Foam test	+	+
07	Glycosides	Molish's test	+	+
08	Tannins	Keller-Killiani test Ferric chloride test	+	+

+ - Present, – - Absent

Table 2: In vitro antimicrobial activity of ethyl alcohol extract (EtTP) on pathogenic strains (Diameter of zone of inhibition in mm)

Concentration (µg/ml)	100	200	300	400
Standard (streptomycin)	16	19	27	33
Pseudomonas aeruginosa	3	4	5	6
Shigella flexneri	4	7	9	10
Salmonella typhimurium	3	4	7	11
Candida albicans	5	8	11	13

Table 3: In vitro antimicrobial activity of ethyl acetate extract (EATP) on pathogenic strains (Diameter of zone of inhibition in mm)

Concentration (µg/ml)	100	200	300	400
Standard (streptomycin)	15	18	25	32
Pseudomonas aeruginosa	3	5	7	10
Shigella flexneri	5	7	9	12
Salmonella typhimurium	4	9	12	15
Candida albicans	5	8	11	15

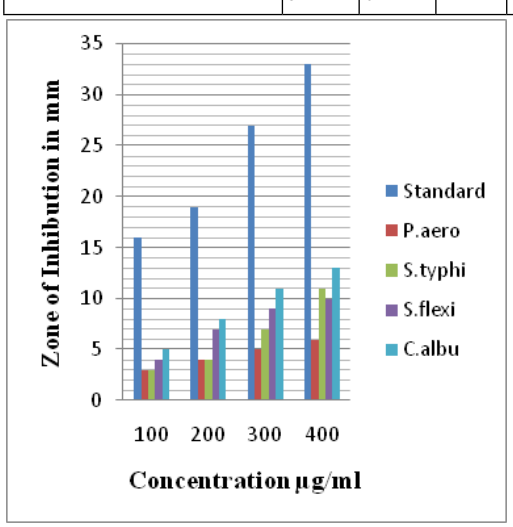


Figure 2: Antibiogram analysis of EtTP \

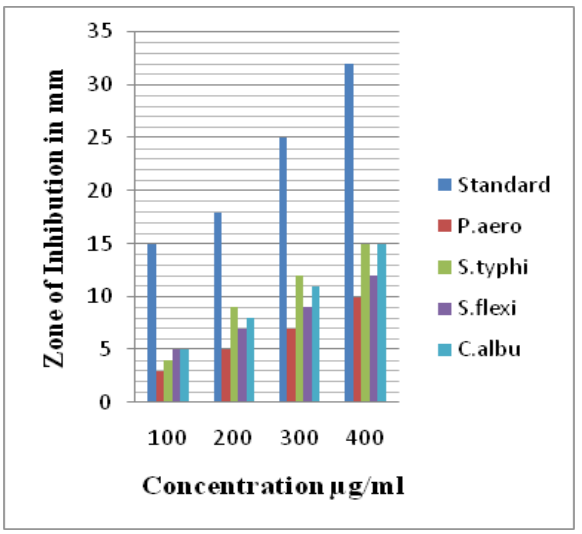


Figure 3: Antibiogram analysis of EATP

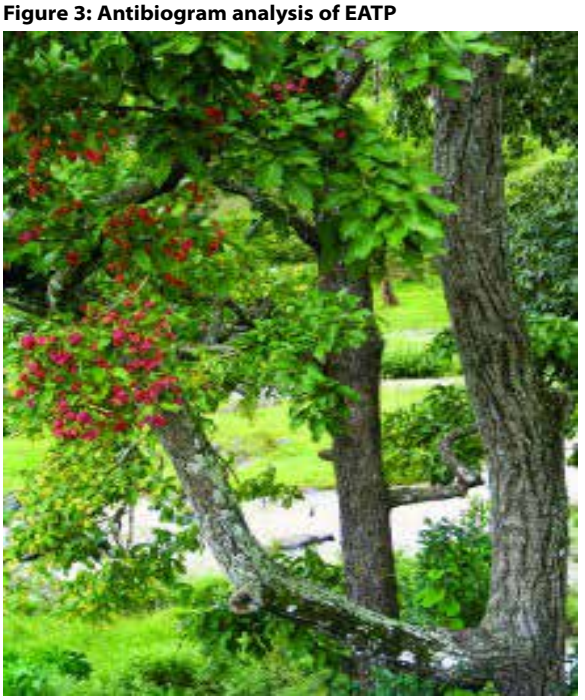
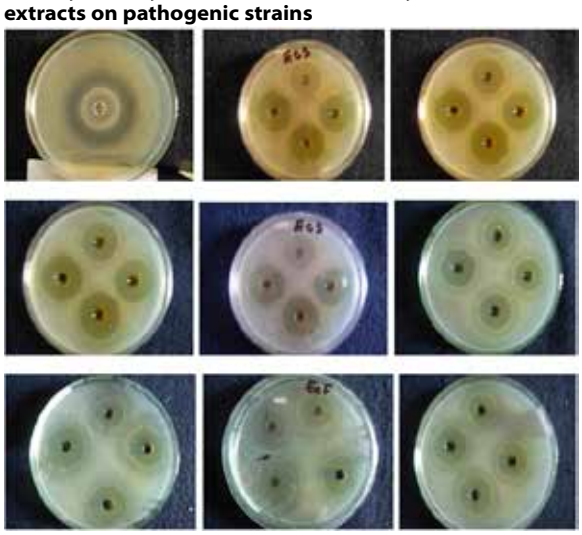


Figure 1: *Terminalia paniculata*

Figure 4. Antibiograms showing in vitro antimicrobial activity of ethyl alcohol (EtTP) and ethyl acetate (EATP) extracts on pathogenic strains



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