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Research Article

PHYTOCHEMICAL EVALUATION & ANTIMICROBIAL EFFICIENCY OF THREATENED MEDICINAL PLANT OF ANDHRA PRADESH *PTEROSPERMUM XYLOCARPUM* (THADA TREE)

K. Narendra, K. M. Sowjanya, J. Swathi, A. Krishna Satya*

Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur-522510. A.P. India

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*Email: akrishnasatya78@gmail.com

ABSTRACT

Pterospermum. Xylocarpum (Family: Sterculiaceae) is a wild medicinal plant distributed throughout India and south East Asia. It is used against treatment of joint diseases like gout, rheumatism, arthritis. In present study acetone, hexane, chloroform and methanol extracts were prepared from the leaf of *P.xylocarpum* and phytochemical analysis was done to screen alkaloids, steroids, saponins, tri terpinoidal saponins, carbohydrates, flavonoids, polyphenols, tannins and glycosides. Antimicrobial activity was tested against nine bacterial and nine fungal species. Among the four extracts methanol and acetone extracts showed significant anti bacterial activity and there is no significant activity against fungal strains and it was found to be effective against two tested bacterial species viz, *xanthomonas campestis* and *E.Coli*. Methanol crude extract was prepared by the cold maceration process and it was optimized for the yield, the crude extract was tested for anti bacterial activity at different concentrations viz, 250,500,1000mg/ml among them 500mg/ml concentration has shown significant antibacterial activity and in the methanol crude extract the phytochemical analysis showed the presence of alkaloids, steroids, phenols and cardiac glycosides.

KEY WORDS: antimicrobial assay, crude methanol extract, maceration, phytochemical assay, *Pterospermum xylocarpum*.

INTRODUCTION

From ancient times plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being[1, 2]. The vast chemical diversity present in the compounds from the natural resources shows the importance and demand of herbal medicine from the last two decades and these are felt for ensuring efficiency, quality and safety of herbal drugs. The most important plant produced; bioactive constituents are alkaloids, flavonoids, tannins and phenolic compounds[3]. These can show the significant physiological action on the human body[4].

The higher plants produce thousands of bioactive compounds which show diversified and different biological activities[5]. In indigenous system of Ayurveda, Unani, and Sidda more than 1500 plants have been used systematically. However, the Ethanopharmocologists, Microbiologists, Botanists and Natural Product Chemists over the world today are searching for the medical efficiency of plants and their phytochemicals. There is only few data available and comparatively it is very low before the vast number of plant population. In the present successive years drug safety became a global issue and drugs which we have used to treat infectious diseases show Adverse Drug Reaction (ADR) in 2.22 milion hospitalised patients and 1, 06,000 died in single year in USA. The herbal and natural products have been used in folk medicine for centuries throughout the world and there are only few incidences of adverse reaction and hence used as an alternative medicine to synthetic medicine[6].

Medicine plants are natural sources of compounds that can be used against many diseases today[7]. The screening of bioactive compounds from plants leads to discovery of new drugs that have efficient protection and treatment roles against various diseases[8].

Medicinal plants have therapeutic components against human diseases like epilepsy, malaria, diarrhoea, infantile convulsion, dysentery, fungal and bacterial infections[9]. These medicinal plants are the main sources of remedies used for the different disease conditions such as skin diseases, pains, hypertensions, and inflammation such as rheumatoid arthritis[10, 11, 12]. The phytochemical studies have drawn the attention of scientist due to development of new and sophisticated techniques. These played a significant role by adding additional resources of raw material for pharmaceutical industry. In medicinal plants the primary and secondary metabolites are of chemical origin. Finally the applications of chemical data to develop synthetic compounds have played a vital role and draw the attention of scientists[13].

Bacteria has developed resistance to most of the antibiotics existing[14], this lead to the search of new antimicrobial agents for the treatment of infections from resistant strain of bacteria[15]. The recent searches of antibiotics include various sources such as bioactive compounds from aquatic microorganisms, synthetic compounds and natural products from medicinal plants. More than 70% of people in Africa and other developing countries depend on the traditional healers and herbal practitioners for their health needs[16, 17]. The raising dominance of microorganisms to existing antibiotics, is alarming us to develop new easily affordable antimicrobial compounds[18]. Phytochemicals from medicinal plants shows better antimicrobial activities and have the potential to fill this need. The phytochemicals have different modes of action other than anti-microbial activity[19, 20].

Sterculiaceae family members have good ethnobotanical values practiced in all parts of the world. *Helictres isora* of *Sterculiaceae* is used in the traditional medicinal systems and is reported to be effective against HIV, diabetis, polio and asthma. The fruits are astringent, acrid, refrigerant, demulcent, constipating, stomachic, vermifuge vulnary, haemostatic and urinary astringent and has anti antispasmodic activity[21]. *Cola gigantea* A. Chev. belongs to the family *Sterculiaceae*. The nuts are often used to treat whooping cough, asthma, malaria, and fever. Other traditional uses include increasing the capacity for physical exertion and for enduring fatigue without food, stimulating a weak heart, and treating nervous debility, weakness, lack of emotion, nervous diarrhea, depression, despondency, brooding, anxiety, and sea sickness[22, 23]. *Sterculia setigera Del., (Sterculiaceae)* leaves are active against *M. tuberculosis*, *Pterospermum heyneanum*

wall. which is commonly known as barahakani is used for treating gout effected parts of the body[24].

Considering the medicinal history of the family the present study is focused on Threatened plant of Andhra Pradesh *Pterospermum xylocarpum* noted by[25] in Threatened Medicinal plants of Andhra pradesh 2002 and noted as Nearly Threatened by[26]. *Pterospermum xylocarpum* is a woody tree species belonging to the family *Sterculiaceae*. This plant is commonly known as "Tada Chettu" (in Telugu). It is widely distributed in India, south East Asia, Pakistan and North America. *P.xylocarpum* is common in deciduous forests and it is of 5-8mts tall tree. It is merely distributed in the tropical regions, found in the hills of Andhra Pradesh & Tamilnadu. There is no recorded evidence on its phytochemicals or its medicinal compounds; hence we preferred this massive tree species for its phytochemical & bioactive compound study.

MATERIALS AND METHODS

Collection of Sample

Kondapalli hills are located in Eastern Ghats nearby Krishna district, close to Vijayawada, the third largest city of Andhra Pradesh, India. The forest area in this hill range abounds in a type of lightwood known as 'ponuku' (*Gyrocapus jacquini*), which is used exclusively for the manufacture of the famous Kondpalli toys. The forest vegetation around Kondapalli and the nearby hills are also well known for medicinal plants and trees such as *Phyllanthus amarus* (Telugu local name "nela usiri"), *Phyllanthus*, *Andrographis paniculata* (local name: "adavi mirapa" or "nelavemu"), *Thedlapala* (*Wrightia tinctoria*), *Tephrosia purpurea*, *Albizia amara*, *Streulia urens* and *Chloroxylon swetenia*.

The medicinal plant (*Pterospermum xylocarpum*) leaves were collected from kondapalli hills. The plant was identified accordingly to various literatures, including other pertinent taxonomic literature.

Collection of Microorganisms

The micro organisms were collected from Department of Biotechnology, Acharya Nagarjuna University, Guntur and they were reconfirmed by gram staining and sub culturing on appropriate selective media.

Extraction Method: (Maceration)

The maceration is one of the well known methods of extraction process. In this process the plant material (10gms) mixed in the methanol solvent (100ml) and it is continuously shaken for few days[27].

Maceration Optimization

The five conical flasks of 10gms of plant material were taken and it was mixed in the 100ml methanol and it should be continuously shaken for three days. After that pre weighed empty boxes are taken and the components are filtered with the whatmann no1 filter paper. The filtrate should be air dried completely then the component was weighed to know the crude extract content. The method was followed for successive four days (i.e. 4^{th}, 5^{th}, 6^{th}, & 7^{th}). The yield was noted for five days and plotted optimization graph accordingly.

Phytochemical Screening

Preliminary phytochemical screening [28-31].

Standard screening tests of five extracts were carried out for various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones using standard procedures.

Crude Extract Phytochemical Screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Detection of Alkaloids: Extract was dissolved individually in dilute Hydrochloric acid and Solution was clarified by filtration.

Mayer's Test: Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of Phenols

Ferric Chloride Test: The filtered solution of extract was treated with three drops of freshly prepared 1% Ferric Chloride and potassium ferrocyanide. Formation of bluish-green colour was taken as positive.

The methanol extract was dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.

Detection of Flavonoids

Alkaline Reagent Test: The Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl acid, indicates the presence of flavonoids.

Lead Acetate Test: The Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Anthraquinones

Free Anthraquinones Test: (Borntrager's test) The extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered, and 5 ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red, or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.

Modified Borntrager's Test: The Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of Phytosterols

Salkowski's Test: The extract was dissolved in 2 ml chloroform in a test tube. Conc Sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown color at the interface indicated the presence of a steroid ring (i.e., the aglycone portion of the glycoside).

Libermann Burchard's Test: The Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Terpenoids: The extract was added to 2 ml of acetic anhydride and conc H_2SO_4. Formation of blue, green rings indicate the presence of terpenoids.

Detection of Fatty acids: The extract was mixed with 5 ml of ether. These extract was allowed for evaporation on filter paper and filter paper was dried. The appearance of

transparency on filter paper indicates the presence of fatty acids

Detection of Tannins

Ferric Chloride Test. The extract was dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in color to bluish black.

Lead Acetate Test. The extract was dissolved in water and to that 10% Lead acetate solution was added. The appearance of yellow precipitate confirms the tannins.

Pot. Dichromate Test. The extract was dissolved in water to that strong potassium dichromate solution was added, a yellow color precipitate indicates the presence of tannins and phenolic compounds.

Detection of Saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of "honey comb" froth indicates the presence of saponins.

Anthocyanins: The extract was added to 2 ml of 2N HCl and ammonia. Initial appearance of pink-red colour turning into blue-violet indicates the presence of anthocyanins

Leucoanthocyanins: The extract was added to 5 ml of isoamyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins

Coumarins: 3 ml of 10% NaOH was added to the extract, formation of yellow colour indicates the presence of coumarins

Emodins: 2 ml of NH_4 OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins

Detection of Reducing Sugars: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Keller - Kiliani test (for de-oxy sugars in cardiac glycosides): Methanol extract was obtained and the extract was dried. 50 mg of this was dissolved in 2 ml chloroform. H_2SO_4 was added to form a layer and the colour at interphase was recorded. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

Anti Microbial Activity

Agar Well Diffusion Method

Agar well-diffusion method was followed to determine the antimicrobial activity[32]. Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) plates were swabbed (sterile cotton swabs) with 24 hours old - broth culture of respective bacteria and fungi. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.5 ml of different concentrations (1000, 500, 250 mg/ml) of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37°C for 18-24 hours for bacterial pathogens and 28°C for fungal pathogens. Respective solvent control for leaf extracts was also maintained and the diameter of the zone of inhibition was recorded in mm and compared with standard values. It was maintained in triplicates and the experiment was repeated thrice, and the average values were recorded for antimicrobial activity.

Table 1: Preliminary Phytochemical Screening

S.No	Phytoconstituents	Water extract	Acetone extract	Hexane extract	Chloroform extract	Methanol extract
01.	Alkaloids	-	-	-	-	+
02.	Flavonoids	+	-	-	+	-
03.	Phenolics	-	+	+	-	+
04.	Anthraquinones	-	-	-	-	
05.	Steroids	+	-	-	-	+
06.	Fatty acids	-	-	-	-	-
07.	Tannins	-	-	-	-	-
08.	Saponins	-	-	-	-	-
09.	Anthocyanins	-	-	-	-	-
10.	Leucoanthocyanins	-	-	-	-	-
11.	Coumarins	-	-	-	-	-
12.	Emodins	-	-	-	-	-
13.	Reducing sugars	+	+	+	+	+

Table 2: Phytochemical Screening of Methanol Extract

S.No	Phytoconstituents	Methanol extract
01.	Alkaloids	+++
02.	Flavonoids	---
03.	Phenolics	+++
04.	Anthraquinones	---
05.	Steroids	+++
06.	Fatty acids	---
07.	Tannins	---
08.	Saponins	---
09.	Anthocyanins	---
10.	Leucoanthocyanins	---
11.	Coumarins	---
12.	Emodins	---
13.	Reducing sugars	+++

Table 3: Optimization of methanol extract

day	yield of compound
3rd day	0.91
4th day	1.16
5th day	0.78
6th day	0.7
7th day	0.66

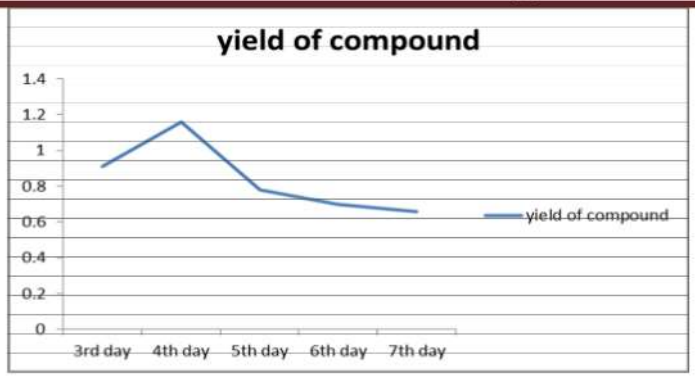


Table 4: Antimicrobial analysis of four extracts

	Zone of Inhibition in mm			
Microorganisms	acetone extract	hexane extract	chloroform extract	methanol extract
Staphylococcus aureus	14	-	11	15
Streptococcus mutans	13	-	10	13
Lactobacillus casei	15	-	10	11
Lactobacillus acidophilus	14	-	13	12
Enterococcus faecalis	16	-	9	10
Bacillus megaterium	15	-	12	11
Xanthomonas campestris	15	-	10	20
Escherichia coli	20	-	11	12
Pseudomonas aeruginosa	16	-	12	12
Candida albicans	15	-	9	11

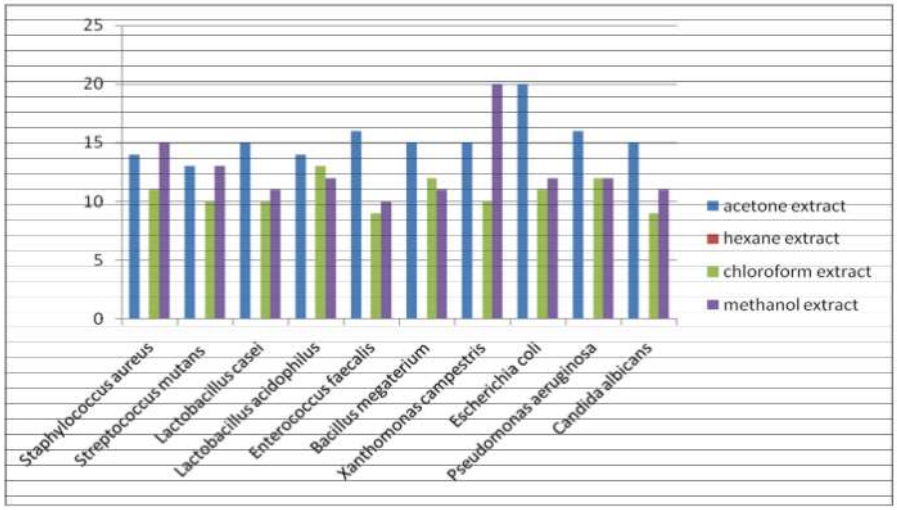
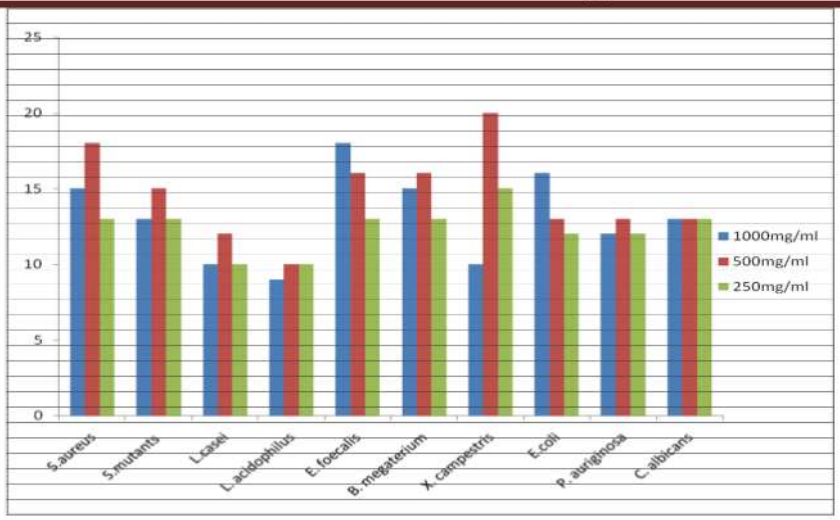


Table 5: Antimicrobial analysis of 7th day methanol extract concentrations

M.organism	1000mg/ml	500mg/ml	250mg/ml
Staphylococcus aureus	15	18	13
Streptococcus mutans	13	15	13
Lactobacillus casei	10	12	10
Lactobacillus acidophilus	9	10	10
Enterococcus faecalis	18	16	13
Bacillus megaterium	15	16	13
Xanthomonas campestris	10	20	15
Escherichia coli	16	13	12
Pseudomonas aeruginosa	12	13	12
Candida albicans	13	13	13



RESULTS

Preliminary Phytochemical Screening

The results of preliminary phytochemical screening were given in [Table 1]. It shows the presence of flavonoids, phenolic compounds, reducing sugars and steroids of *Pterospermum Xylocarpum.* Reducing sugars were present in all the extracts and alkaloids are present only in methanol extract. Phenolics are present in methanol, hexane and acetone extracts. Flavonoids are present in both water and chloroform extracts but absent in methanolic extract. Fatty acids, tannins, saponins, anthocyanins, leucoanthocyanins, coumarins and emodins were completely absent in all the five extracts.

The methanol extract yields high on 4th day maceration [Table 3]. The crude extract was screened for phytochemicals given in [Table 2] showed the presence of alkaloids, phenolics, reducing sugars and steroids.

Antimicrobial Activity

The results of antimicrobial activity of four extracts of acetone, hexane, chloroform and methanolic leaf extracts of *Pterospermum Xylocarpum* against various pathogens were tabulated in the [Table 4] . It was found that methanol leaf extract exhibited significant activity against *Xanthomonas campestris* and it has shown significant activity at 500mg/ml concentration among all the three concentrations [Table 5]. The methanol leaf extracts showed moderate activity against E.Coli, *Staphylococcus aureus*, *Enterococcus faecalis, Bacillus megaterium.* The methanol extract was tested against the nine fungal pathogens and none of them shows the antifungal activity. Antimicrobial potential of Acetone, Chloroform, Ethanol and Methanol leaf extracts were compared with standered DMSO to know and identify the potential antimicrobial activity of our desired plant extracts in respective solvents.

DISCUSSION & CONCLUSION

Plants are the major source for secondary metabolites they are meant for several biological activities in human and animals[9]. The plant secondary metabolite products are having several responsibilities. Phenolic compounds include Flavanoids and tannins that are the major group of compounds acting as primary antioxidants or free radical scavengers[33]. Tannins are known to possess general antimicrobial and antioxidant activities[34]. Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents[35]. Other compounds like saponins also have antifungal properties[36, 37]. Saponin is a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti inflammatory and weight loss, etc. It is also known to have anti-fungal properties[38]. Saponins have been implicated as bioactive antibacterial agents of plants[39, 40]. Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and anti- microbial properties. They are also used in nutrition, herbal medicine and cosmetics[41]. The efficiency of plant derived antimicrobials is needed to be determined completely. Generally plant extract shows more efficient microbial activity when compared to other extracts as they contain highly oxidized phenol which is highly toxic. In addition due to presence of steroids in leaf extract, it shows potential antimicrobial activity against all pathogens. The present study even deals with the antimicrobial property of different solvent extract of *Pterospermum Xylocarpum* by agar well diffusion method. With respect to agar well diffusion method the leaf extracts of ethanol extract is having potential antimicrobial property against *Xanthomonas campestris* at potential 500mg/ml concentrations. DMSO is taken as control for test organisms used for antimicrobial and antifungal activities. But desired plant extracts are not showing antifungal property by the conditions provided. Hence, by the conditions provided above, the plant extract is confirmed to have a potential antibacterial property. Further studies are to be conducted to purify the compounds showing antibacterial activity.

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