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Study on the Antibacterial Potential and Phytochemical Analysis of *Thespesia populnea* (L.) Sol. ex Corr. used in the Treatment of Diarrhoea and Dysentery.

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ABSTRACT

The current study was to investigate the hidden antibacterial potential of *Thespesia populnea* (L.) Sol. ex Corr. (Malvaceae) against some selected bacterial pathogenic strains responsible for diarrhoea and dysentery. The research work was done in the laboratory of P.G. Department of Botany, Utkal University, Odisha during the month of November 2012. Five bacterial strains were used in the study i.e. *Escherichia coli* (MTCC-614), *Shigella flexneri* (MTCC 9543), *Staphylococcus aureus* (MTCC-1430), *Salmonella paratyphi* (MTCC-3220) and *Salmonella enterica typhi* (MTCC-733). The study revealed that n-Hexane extract of *Thespesia populnea* exhibited the highest zone of inhibition against *S. aureus* at 6mg/ml and quite comparable with reference antibiotic Ciprofloxacin (0.5 mg/ml).The methanol extract was moderately effective against all above bacteria. From the phytochemical analysis it was found that the plant contained tannin, alkaloid, flavonoid and triterpenoid. The result indicated that the bark extracts of *Thespesia populnea* might be a source for a new drug against diarrhoea and dysentery which needs further investigation.

Keywords: Ciprofloxacin, *in-vitro* anti-bacterial activity, phytochemical analysis, *Thespesia populnea*.

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INTRODUCTION

Medicinal plants play an appreciable role in the development of modern herbal medicines. About 30% of worldwide drugs are based on natural products isolated from medicinal plants (Grabley and Thiericke, 1999)[1]. Since time immemorial various plants used in herbalism are thought to have medicinal properties. Since these are in common use by the local people are of great importance, that is why a lot of people are engaged in the trade of important medicinal herbs throughout the world (Elisa Betsy, 1990)[2]. Their dependence on plants around them made them to acquire knowledge of many useful as well as harmful plants which are accumulated and enriched through generations and passed on from one generation to other, without any written documentation (Heywood,1992)[3]. A number of traditional natural products have been found from selected ethno-medicinal plants for antibacterial activity against pathogenic strains of Gram negative and Gram positive bacteria. This antibacterial activity would support the folk therapy of infections whose symptoms might involve bacteria (Verpoorte *et al*., 1982)[4]. The development of antimicrobial agents for clinical use has bought unquestionable benefit to individuals and society. Many works have been done for antimicrobial and phytochemical constituents from medicinal plants to use in the treatment of microbial infections. Production of the secondary metabolites are the characteristic features of microorganisms and plants which can be used in control of Pathogens. (Nikapitiya, 2012)[5]. The search for the plants with antimicrobial activity has gained importance in recent years due to growing concern about rising in the rate of infection by antibiotic resistant microorganism. Phytochemical study and antibacterial activities are becoming popular as these are important potential sources for discovery of new drugs (Rojas *et al.*, 1992)[6]. The plant *Thespesia populnea* Soland. ex Correa of family Malvaceae is known as Indian tulip tree and considered to be used traditionally in the treatment of diarrhoea and cholera (Chopra *et al*., 1956,Warrier *et al.*, 1996)[7, 8]. It is also used in cutaneous infections, skin and liver diseases **(**Shirwaikar kumar *et al*., 1995)[9]. It is an important plant in traditional medicine system for the treatment of asthma, boil, gout, inflammations, rheumatism, tumour and ulcers, and as a tonic to regain vitality (Kirtikar & Basu, 1981[10], Watt & Breyer-Brandwijk, 1962[11]). In the plant *Thespesia populnea,* polyphenols have been reported to exhibit antibacterial activities (Haslam *et. al.,* 1996)[12]. Generally the optimal effectiveness is due to the combined action of different compounds originally in the plant (Gonzalez *et al.,* 1994)[13]. Diarrhoea and dysentery are two dreaded diseases causing heavy death toll in India. The causative organisms of these disease are *Escherichia coli, Shigella flexneri, Staphylococcus aureus, Salmonella paratyphi,* and

Salmonella enterica typhi. As the plant is claimed for treatment in diarrhoea and dysentery, the authentication is needed for further investigation to find out compounds which are responsible of bacterial inhibition. The interest in the scientific investigation of *Thespesia populnea* was based on the claims of its effective use in the treatment of diarrhoea and dysentery.

MATERIALS AND METHOD

Collection and identification of plants

The bark of the plants *Thespesia populnea* were collected from Chandaka reserve forest area during month of march and were identified following available literature (Saxena and Brahamam 1995)[14]. The voucher specimens of the plant species were deposited in the Herbarium of P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneswar. The collected barks were washed with tap water to remove all dust and foreign particles. Then the barks were dried for 10-15 days in shade. Then the dried bark materials were grinded to coarse-powder. This powdered material was used for preparation of extraction.

Method of preparation of plant extract

30 grams of air dried and coarsely powdered plant materials were kept in Soxhlet extraction unit and exhaustively extracted with n-hexane for 36 hours. To confirm whether the extraction is complete or not, the extract from the siphoning tube of Soxhlet apparatus was taken in a watch glass. If no residue remained in the watch glass the extraction was considered complete, if not, the extraction continued. The extracted plant material was then air-dried, repacked in Soxhlet apparatus and thoroughly extracted with methanol for such time hour and maintained again. The crude extract and the fraction obtained were filtered and distilled to evaporate the solvent from the extract. The liquid extracts were concentrated separately under vacuum rotary evaporator and the resulting dried extract was preserved in dessicator until further use. The dried extracts were weighed and their percentage in terms of dry weight of the plant material were estimated by the following formula:

Percentage of extract yield = (weight of dried extract/ weight of dried plant material) X 100

Microbial strain

Five pathogenic bacteria including three strains of gram-negative (*Shigella flexneri, Salmonella paratyphi, Salmonella enterica ser typhi, Escherichia coli)* and one strains of gram-positive *Staphylococcus aureus)* were used for bioassay study. The pure strains were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Microbial culture media

For antibacterial susceptibility of the extract was tested on solid (Agar-Agar) media on petridishes by applying agar well diffusion method. For bacterial assay nutrients agar (NA) (28 gm/L) [(HIMEDIA), REF- M001-500G, LOT-0000145979] was used for developing surface colony growth. The suspension culture for bacterial cells growth was done by preparing Nutrient Broth [(HIMEDIA), (REF-M088-500G, LOT- 0000154058)] was taken for evaluation. All the media prepared, were sterilized by autoclaving the media at 121°C for 20 minutes.

Preparation of fresh culture

The nutrient agar medium was prepared and dispersed in a number of clean test tubes to prepare slants (5 ml in each test tube). The test tubes were plugged with cotton and sterilized for 30 minutes. After sterilization, the test tubes were kept in an inclined position (45^{o}C) for solidification. The test organisms were transferred to the agar slants from the supplied pure cultures with the help of an inoculating loop in an aseptic condition. The inoculated slants were then incubated at 37^{o} C for 24 hours to assure the growth of test organisms. These fresh cultures were used for the sensitivity test.

Preparation of test plates

Nutrient agar media were transferred to the sterile petri dishes in sterile area. About 30 ml of the media was poured onto each petri dishes in such a way to keep a uniform depth of approximately 4 mm. The petri dishes were rotated several times, initially clockwise then anticlockwise. Then the plates were swabbed (sterile cotton swabs) with 8 hours old broth culture and kept preserved for applying samples.

Preparations of test samples

The stock solution of the two extracts (n-hexane and methanol) were prepared taking dimethyl sulphoxide (DMSO) at a concentration of 6mg/ml and further dilutions were made.

Agar well diffusion method

Agar well diffusion method (Atta Rahman, 1991)[15] was followed to determine the zone of inhibition. Wells (08mm diameter) were made in each of these swabbed plates using sterile cork borer. About 50 µl of this two solvent extracts were added with sterile syringe into the wells and allowed to diffuse at room temperature for 2 hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°c for 24 hours for bacterial pathogens. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.The zone of inhibition obtained by methanolic and n-hexane extracts of *Thespesia*

populnea were compared to that exhibited by reference antibiotic Ciprofloxacin (0.5mg/ml, equivalent to 25µg/well of Ciprofloxacin).

Measurement of the zone of inhibition

The diameter of the inhibition zone was measured with Himedia antibiotic zone scale and the inhibition zone was recorded. The antibacterial activity of n-hexane and methanol extract of 6mg/ml. was tested against five bacteria. Ciprofloxacin was used for comparing the bioassay.

Phytochemical Screening

Phytochemical screening was carried out to determine the presence of Saponins, Tannins, Flavonoids, Glycosides, Triterpenoids, Phytosterols. (Khandelwal & Harbourne, 2000)[16, 17] The solvents used were methanol and distilled water in different tests.

RESULTS AND DISCUSSION

The percentage yield of n-hexane and methanol extracts of *T. populnea* bark was found to be 6.5% and 12% respectively.

Phytochemical Studies

The phytochemical studies showed the presence of triterpenoids, alkaloids and flavonoids. Tannin was present only in n-hexane extract of *T. populnea*. Whereas phytosterol was present in methanolic extract of *T. populnea*. Other phytochemicals were found in both solvent extracts (Table 2). This study indicated that some bioactive molecules were present in both extracts and could be used in developing of new drugs for different diseases.

Anti-microbial activity studies

The n-hexane and methanolic extracts of the plant *Thespesia populnea* were subjected to antibacterial activity and the results were expressed in terms of zone of inhibition. Among the two extracts of the plant, the n-hexane extract showed maximum activity against four strains of bacteria *Shigella flexneri* (10mm), *Escherichia coli* (27mm), *S. aureus* (23mm), *S. paratyphi* (24mm) at 6mg/ml concentration of extract, but it was ineffective against *S. enterica typhi*. The methanolic extract was quite effective against *Escherichia coli* (15mm), *Shigella flexneri* (16mm), *S. aureus* (16mm), and moderately effective against *S. paratyphi* (14mm) and *S. enterica typhi* (11mm) at 6mg/ml concentration of extract. Ciprofloxacin which was taken as standard antibiotic showed *Escherichia coli* (30mm), *Shigella flexneri* (29mm), *S. aureus* (20mm), *S. paratyphi* (28mm) and *S. enterica typhi* (32mm) at the concentration (10mg/ml). The comparison between standard and extracts revealed that n-hexane extract was quite effective against all five strains whereas methanolic extract was moderately effective.

Table-1: Percentage of yield of *Thespesia populnea* bark.

Solvent for extraction	Weight of dry material (gm)	Weight of extract (gm)	% yield
n- Hexane	30	1.95	6.5%
Methanol	30	3.6	12%

Table 2. Phytochemical analysis of bark of *Thespesia populnea*

Name of the plant	Extracts	Saponins	Tannins	Alkaloids	Flavonoids	Phytosterol	Triterpenoids
Thespesia populnea	n-Hexane	_	+	+	+	_	+
	Methanol	_	_	+	+	+	+

Table 3: Extracts showing zones of inhibition (in mm) against bacterial species

Name of the Plant	Test Agent	Concentration	*Escherichia coli*	*S. enterica typhi*	*S. paratyphi*	*S. aureus*	*Shigella flexneri*
Thespesia populnea	Ciprofloxacin(RA)	0.5mg/ml	30±0.816	32±1.69	28 ±0.81	20±0.816	29±1.69
	n-Hexane	6 mg/ml	27.23±0.25	--	24±0.3	23.466±0.15	10.2±0.2
	Extract	3 mg/ml	22±0.264	--	19.2±0.173	19 .1±0.173	--
		1.5 mg/ml	18.2±0.2	--	11.2±0.1	13.2±0.173	--
		0.75mg/ml	14.533±0.2	--	10.56±0.25	11.06±0.115	--
	Methanol	6mg/ml	15.3±0.264	11.06±0.35	14.2±0.1	16.33±0.251	16.56±0.208
	Extract	3 mg/ml	12.36±0.05	10.13±0.12	12.23±0.208	12.56±0.057	
		1.5mg/ml	10.06±0.11	--	--	--	10.133±0.230
		0.75mg/ml	--	--	--	--	--

RA- Reference Antibiotic

CONCLUSION

It was found that the n-Hexane extract of *T. populnea* shows more potent antibacterial activity than methanolic extract. The inhibitory effect of the plant extract against bacteria *E. coli*, *S. paratyphii* and *S. aureus* was very promising which indicated the capability of the plant as an effective source of medicine against pathogens of diarrhea and dysentery. The plant can further be studied for the isolation of important chemical constituents which are specific for the antimicrobial activity. The phytochemical analysis showed the presence of tannin, alkaloid, flavonoid, phytosterol and triterpenoids which might be responsible for its potent antibacterial activity. From the above study it can be concluded that the plant is potent for the pharmaceutical industry and needs further investigation and study.

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