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Antibacterial and phytochemical analysis *of Ceiba pentandra* (L.) Seed extracts

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Abstract
In the present study the aqueous, methanol, ethanol and acetone extract of *Ceiba pentandra* were screened for the presence of phytochemical components and tested for antibacterial activity against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aerogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Salmonella typhi, Staphylococcus epidermidis* and *Proteus vulgaris*. Results revealed the presence of anthraquinones, alkaloids, saponins, tannins, glycosides and phenolics. The acetone extracts had wide range of antibacterial activity against bacterial pathogens than the ethanol and methanol extract, where as aqueous extract were slightly higher antibacterial activity as ethanol extract. Antibacterial activity of various extract of seeds of *Ceiba pentandra* would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Further work is being carried out to isolate and identify the active constituents of the plants responsible for antibacterial activity.

Keywords: *Ceiba pentandra,* seed extracts, phytochemical screening, antibacterial activity

Introduction
Ceiba pentandra is a plant that is normally found in the wild, it belongs to the family of Bombacaceae. It is known as silk cotton tree or locally as dum is widely reputed in the African traditional medicine (Ueda *et al.,* 2002) [13]. Various morphological parts of the plant have been reported to be useful as effective remedies against diabetes, hypertension, headache, dizziness, constipation, mental diseases, fever, peptic ulcer and leprosy. It is also used as diuretics and to expel evil spirits. The bark contains a blackish mucilaginous gum, which swells in water and resembles tragacanth (Noumi *et al.,* 1999) [9]. Folk medicines in Nigeria use the bark for the treatment of infections. It is astringent and is used in India and Malaya for bowel complaints. The bark is believed to also contain tannin. In West Africa, it is generally used in the treatment of diarrhea (Kiritikar and Basu, 1987: Cowan, 1999) [6, 3].
Emergence of multidrug resistant pathogens has been reported to be one of the leading causes of death world (Reddy *et al.,* 2009) [10] wide with infectious diseases responsible for 68% of all deaths globally in 2012 (WHO, 2000) [14]. Many infectious microorganisms' are resistant to synthetic drugs and it has become the major concern for health institutions, pharmaceutical companies and governments all over the world; thus there is need for an alternative therapy (Tambekar and Dahikar, 2011) [11]. Medicinal plants contain numerous active constituents of great therapeutic value and have been used as an exemplary source for Centuries as an alternative remedy for treating human diseases Approximately 80% of the third world population depend on traditional medicines for maintaining general health and combating many diseases (Chindo *et al.,* 2002: Adebgoye *et al.,* 2008:) [2, 1].
Many researchers had studied antimicrobial activity of other parts of plant like bark, leaves and fruits of *Ceiba pentandra* which are used to cure many infectious diseases in traditional system of medicine but still very, less work has been done on antibacterial activities of seeds of *Ceiba pentandra*. To prove the validity of traditional medicine the present work has been undertaken to evaluate the antimicrobial screening of seeds of *Ceiba pentandra* against the human bacterial pathogens.

Materials and methods
Sample Collection
Ceiba pentandra seeds were collected from herbal garden maintained by Sanjivani Arts, C College, Kopargaon, India in the month of March and authenticated by Department of Botany, S.S.G.M College, Kopargaon (M.S), India.

Preparation of plant material
Seeds were collected and dried at room temperature. The dried samples were powdered separately. 100gm each of the sample was extracted separately with different solvents starting with polar to non polar solvents in the order of aqueous, ethanol, methanol and acetone. The crude residues were obtained by removing the solvents in rotary evaporator and each of the extracts were resuspended in the respective solvents for further study.

Preparation of extracts: Solvent extraction method Thirty grams of dried powder of *Ceiba pentandra* seeds were extracted with aqueous, ethanol, methanol and acetone using soxhlet apparatus for 48 hrs. The collected extracts were filtered with Whatman No.1 filter paper and used for estimation of phytochemicals and antibacterial activity.

Phytochemical screening: Preliminary qualitative phytochemical screening was carried out with the following methods (Khandelwal, 2001) [5].

Test for Tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added, observed for blue or green black coloration.

Test for Saponins: Two ml of distilled water was added to 2 ml of the test solution shaken well and observed for frothing.

Test for Flavonoids: A volume of 1.5 ml of 50% methanol was added to 4 ml of the extracts. The solution and magnesium metal was added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid was added to the solution and observed for red coloration.

Test for Steroids: (Salkwoski's test): Five drops of concentrated sulphuric acid (H2SO4) was added to 2 ml of each extract and observed for red coloration.

Test for Glycosides: To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of 2 layers and bluish green colour in upper layer.

Test for Alkaloids: To 4 ml of extract filtrate, a drop of Mayer‟s reagent was added along the sides of test tube. Creamy yellow or white precipitate indicates that the test is positive.

Test for Anthraquinones: One gram of powdered plant material was taken and extracted with 10 ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl4 then CCl4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red colour. One gram of second sample of the same plant material was extracted with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid then it was heated on water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

Test for phenolic compounds: Two ml of extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) [7] guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Proteus vulgaris	426
Staphylococcus epidermidis	435
Staphylococcus aureus	96
Escherichia coli	739
Pseudomonas aeruginosa	424
Klebsiella pneumoniae	109
Salmonella typhi	733
Enterobacter aerogenes	111
Salmonella typhimurium	98

Preparation of disc for antibacterial activities: The aqueous, ethanol, methanol and acetone extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc was 1mg, 2mg, 3mg, 4mg, 5mg of each seed extracts of *Ceiba pentandra*. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial activity using disc diffusion method: The modified paper disc diffusion method was employed to determine the antibacterial activity of aqueous, ethanol, methanol and acetone extracts. Turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002) [7]. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^0C. The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive.

Results and discussion
The present study was made in the seed of *Ceiba pentandra,* The Table 2 represents the presence or absence of secondary metabolites in the seed of *Ceiba pentandra*. The results showed that the seed of *Ceiba pentandra* possessed more abundant alkaloid, flavonoids, tannins and glycosides. The phenolic compounds were found to be normal in quantity and flavanoids were quite high. The Steroids and anthroquinones were totally absent in all the four extracts. Methanol and acetone extracts contains, Alkaloid, Flavanoids, Glycosides, Saponins, Tannins and Phenolic compounds, where as in

aqueous extract only Steroids and Tannins were found. Several plants which are rich in alkaloids, tannins and glycosides have been shown to possess antimicrobial activity against a number of microorganisms. Ethanol extracts contained a higher number of phytoconstiutuents than the aqueous extracts.

The seed extracts demonstrated antimicrobial activity against both Gram-negative bacteria used in this study with the methanol and acetone extracts demonstrating a higher activity. In this work, methanol extracted more of the phytoconstituents than water, thus demonstrating higher activity. The difference in concentration of phytoconstituents in the different solvents is responsible for the differences in antibacterial potency between the two solvents.

According to antibacterial profile (Table 3), maximum inhibitory effect of the aqueous extract observed only on *Staphylococcus epidermidis, Staphylococcus aureus,* and moderate antibacterial against *Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes,* but mild inhibitory effect on *Salmonella typhi, Salmonella typhimurium, Proteus vulgaris*. Methanol and ethanol extract showed strong antibacterial effect against *Staphylococcus epidermidis* and *Staphylococcus aureus* and moderate antibacterial against *Proteus vulgaris, Escherichia coli, Enterobacter aerogenes, Salmonella typhi* and *Salmonella typhimurium* but mild effect on *Pseudomonas aeruginosa.* Acetone extract showed maximum inhibitory effect on *Staphylococcus aureus, Proteus vulgaris, Staphylococcus epidermidis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhimurium,* but moderate inhibitory effect on *Escherichia coli, Enterobacter aerogenes.* Its been reported that different solvents have different ability of extracting phytoconstituents depending on their polarity an indication that the plant contains antibiotic substances that have broad spectrum of activity including antimicrobial activity. This is actually a very significant discovery giving hope for the possible development of a novel antibiotic from this plant that can be effective in controlling multidrug resistant bacteria and a variety of other microbial disease agents (Tella *et al.,* 2002) [12]. Several researchers have reported on the medicinal properties of plants derived compounds. These classes of compounds are known to show curative activity against several bacterial and it is not surprising that these plants extracts are used traditionally by herbalist to cure bacteria related ill-health. The biological activity of the acetone extract of *Ceiba petandra* can be attributed to the synergistic effect of the combination of flavonoids, steroids, terpinoids and saponins. The synergistic antibacterial effects of flavonoids, saponins, terpenes and steroids have been reported (Ghamba *et al*., 2012) [4].

Table 2: Phytochemical analysis of seed extract of *Ceiba pentandra*

Sr. No	Phytochemical Constitutes	Aqueous extract	Ethanol extract	Methanol extract	Acetone Extract
1	Alkaloid	-	+	+	+
2	Flavonoids	-	++	++	+++
3	Glycosides	+	+	+	+
4	Saponins	-	++	++	+
5	Steroids	-	-	-	-
6	Tannins	+	++	+++	+++
7	Anthroquinones	-	-	-	-
8	Phenolic compounds	-	+++	+++	+++

- : absent, +: present in low concentration, ++: present in moderate concentration, +++: present in high concentration

Bacterial Pathogens	Aqueous					Ethanol					Methanol					Acetone					Controls				Ampicillin (10mcg)
	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	Pet ether	Chloroform	Ethyl acetate	Methanol	
P. vulgaris	15	13	12	-	-	17	15	12	-	-	19	17	15	15	12	20	17	15	13	12	-	-	-	-	16
S. epidermidis	23	17	15	13	-	22	19	17	13	13	24	22	20	17	15	26	18	20	18	14	-	-	-	-	25
S. aureus	22	21	18	17	16	24	22	20	18	16	20	19	17	15	14	22	19	18	16	13	-	-	-	-	24
E.coli	17	15	13	-	-	17	15	14	12	-	22	19	16	14	-	17	15	14	12	-	-	-	-	-	11
P. aeruginosa	16	15	13	12	-	15	14	13	-	-	21	17	15	14	13	20	17	16	15	13	-	-	-	-	16
S. typhi	14	13	-	-	-	18	16	15	14	13	18	17	15	13	-	22	20	18	15	14	-	-	-	-	18
E. aerogenes	15	13	12	-	-	18	16	15	13	11	22	19	17	14	13	17	15	13	13	12	-	-	-	-	14
S. typhimurium	14	13	-	-	-	18	17	14	12	-	19	15	13	-	-	22	20	17	14	12	-	-	-	-	19

Conclusion: The results obtained in this study thus suggests that the identified phytochemicals may be the bioactive constituents responsible for the efficacy of seed extract of *Caiba petandra* against fever, syphilitic, ulcer, inflammatory disease wounds, conjunctivitis etc. Based on this, it suggested that the traditional medicinal use of *Cieba petrandra* be continued and scientific evaluation of its active constituents given serious attention. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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