Physico-chemical characterization and antimicrobial activity of *Ceiba pentandra* (Kapok) seed oil

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Abstract

Oil extracted from *Ceiba pentandra* seed was studied to explore its suitability for ethnomedical uses with a special emphasis on its physiochemical characterization, antimicrobial behavior and spectrophotometric parameters. Some of the physiochemical properties were examined and compared with those of standard oils and, in particular, any common characteristics with cotton seed oil were evaluated. Spectrophotometric analysis of oil was carried out to obtain information regarding the types, numbers and position of chromophores and auxochrome, and saturated and unsaturated compounds. Crude *Ceiba pentandra* oil was found to show good to moderate activity against bacteria, and in particular Gram +ve (*B. cereus, B. subtilis* and *S. aureus*) and Gram -Ve (*E. coli* and *P. aeurignosa*) and fungal stains, more specifically *Aspergillus flavans*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. Maximum activity was observed on bacterial strains compared with fungal strains. Among bacteria, *Bacillus subtilis* was highly sensitive; fungi were less susceptible to oil and *Saccharomyces cerivisiae* were the most susceptible. Minimum inhibitory concentrations and minimum bactericidal and fungicidal concentrations of the seed oil varied between 3 to 10 mg/50 µL against all bacterial and fungal strains used in this study. In conclusion, *Ceiba pentandra* oil is a natural antimicrobial agent and could have therapeutic potential.

Introduction

The progress of pathogenic microorganism resistance to currently existing antibiotics has led to a search for new antimicrobial agents.[1] Pathogens are extremely adjustable organisms because of the extremely short time required for generation and their tendency to allocate genetic information, even among diverse species of pathogens. An antibiotic may perhaps kill most of the pathogenic organisms in a certain environment but the resistant survivors can ultimately restore themselves and pass their resistance genes on to their offspring, or even, as often happens, to other species of bacteria. Both medical and veterinary use of antibiotics has led to the manifestation of resistant strains of pathogens. Resistant human pathogens may make it difficult to treat some diseases. However, if the resistant bacteria are not human pathogens, they may still put the patient at risk since they can relocate their antibiotic resistance genes to new pathogenic microorganisms.[2]

The overuse of antibiotics in dealing with infectious ailments, and the appearance of *multi-drug resistant* pathogenic strains, has stimulated research towards the study of antimicrobial agents from essential oils.[3,4] A few essential oils have antimicrobial activities and some oils are used in cancer treatment,[5,6] food preservation,[7] aromatherapy,[8] and in the perfume industry.[9] Essential oils are a rich source of biologically active compounds. Therefore, it is sensible to already have identified a variety of plant compounds in these oils, with precise as well as broad antimicrobial activities, for possible future therapeutic use.

Therefore, the first objective of this study was to identify some physiochemical characteristics and antimicrobial activities of *Ceiba pentandra* seed oil using spectrophotometric analysis. *Ceiba pentandra* is a tree found in the steamy emergent layer of rainforests. It belongs to the *Malvaceae* (*Malvalea*) family. It is found extensively all over the world, from tropical America to Asia through Africa, and on farms in Southeast Asia, Western South India, Sri Lanka and other parts of East Asia and Africa. It is being used as a conventional medicine for the treatment of several disorders, such as headache, dizziness, diabetes, diuretic, fever, hypertension, constipation, mental disorders, peptic ulcer, rheumatism and leprosy.[10]

Materials and Methods

Seed collection

Mature dried fruits of *Ceiba pentandra* were obtained from the area in and around Andhra University, Visakhapatnam, India, and were explored for physiochemical characterization, antimicrobial activities and for spectrophotometric analysis.

Soxhlet extraction

The seed oil was extracted using a soxhlet extraction method with analytical grade hexane as a refluxing solvent. On completion of the extraction process, the oil was recovered from the mixture by distillation and stored at 4^{0}C until use.[11]

The percentage of oil content can be calculated as follows:

$$\% \text{ of oil} = \frac{\text{Wt. oil obtained in grams}}{\text{Wt. seed taken in grams}} \times 100$$

Oil characterization

The crude oil sample obtained from the hexane extraction was characterized for acid value, saponification value, iodine value, peroxide value. Reichert-Meissl value (RMV) and Polenske value were based on official recommendations and Tentative Methods of the American Oil Chemist's Society.[12]

Spectrophotometric analysis

Ultraviolet (UV) and visible absorption spectra were carried out for the *Ceiba pentandra* extracted oil. Absorption was between 200-300 nm and 300-600 nm wave lengths in a quartz cell with 1 cm path length against a solvent blank in a matched cell using Shimadzu double beam UV using a visible spectrophotometer, model TCC240A with UV probe software.[13]

Antimicrobial activity

Microorganisms

The oil of *Ceiba pentandra* was individually tested on nine different pathogenic microorganisms. Five were bacteria: *Bacillus cereus* MTCC430, *Bacillus subtilis* MTCC441, *Escheri* -

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chia coli MTCC443, *Pseudomonas aeruginosa* MTCC424 and *Staphylococcus aureus* MTCC3160. Four were fungal strains: *Aspergillus flavans* MTCC3396, *Aspergillus niger* MTCC961, *Candida albicans* MTCC227 and *Saccharomyces cerevisiae* MTCC170. These were obtained from the microbial culture collection center in Chandigarh, India.

Inoculum preparation

Bacterial strains were maintained on nutrient agar. Overnight cultures of the bacterial strains were prepared in nutrient broth and they were incubated for 24 h at 37°C before use and standardized to 0.5 Mc Farland standards (10^6 cfumL^{-1}). The fungal isolates were grown on PDA at 25°C until they were sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water on the surface of the plate and the spores were later scraped with a sterile glass rod. The harvested fungal spores were standardized to a concentration of 10^6 spores/mL or to an OD_{600nm} of 0.1 before use.[14]

Agar well diffusion method

Antimicrobial assay was carried out by an agar well diffusion method.[15,16] Twenty milliliters (20 mL) of the molten nutrient agar were seeded with 100 µL inoculum of the test organism in sterile petri dishes rotated slowly to ensure a uniform distribution of the microorganisms, and allowed to solidify on the bench for 30 min. The seed oil of *Ceiba pentandra* was dissolved in hexane to a final concentration of 200 mg/ mL. The 6-mm wells were cut from the agar surface and each well was inoculated with 50 µL of the extract at a concentration of 10 mg well-1. After the incubation period (24 h at 37^{0}C for bacteria and 25°C for 96 h for fungi), wells were observed for zones of inhibition. The effect of extract on bacterial and fungal isolates was compared with those of rifampicin and fluconazole at a concentration of 1 mg/mL.

Minimum inhibitory concentration

Minimum inhibitory concentrations (MIC) of oil were prepared in test tubes using a broth dilution method.[17] Fifty microliters of *Ceiba pentandra* seed oil at concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mg/50 µL was added to 5 mL of nutrient broth for the bacterial strains and potato dextrose broth for the fungi containing cells as described above. A negative control tube was inoculated without extract. The test tubes were incubated for 24 h at 37°C for bacteria and 48 h at 25°C for fungi. During the incubation period, the tubes were submitted to a manual agitation every hour. After incubation, the MIC was recorded as the lowest concentration demonstrating no apparent growth compared to the negative control.[18]

Minimum bactericidal and fungicidal concentration

The bactericidal and fungicidal concentration of the oil was determined by a modification of the broth microdilution method according to the German DIN regulation 58940-7. Samples were taken from test tubes with no visible growth in the MIC assay, subcultured on freshly prepared nutrient agar plates and potato dextrose agar plates, and later incubated at 37°C for 48 h and 25°C for 72 h for bacteria and fungi, respectively. The minimum bactericidal and fungicidal concentrations were taken as the concentrations of oil that did not show any growth on a set of agar plates.

Figure 1. ***Ceiba Pentandra*** **seeds and oil.**

Table 1. Some physiochemical properties of *Ceiba pentandra* seed oil.

S/No	Parameter	Value
1	Color	Yellow
2	Odor	Pungent
3	Yield (%)	40%
4	PH	Acidic
5	Acid value (mg KOH/g)	15
6	Saponification value (mg KOH/g)	224
7	Iodine value	98
8	Peroxide value	12 mEqKg^{-1}
9	Reichert-Meissl value	0.36
	Polenske value	0.97

Table 2. Minimum inhibitory concentrations and minimum bactericidal and fungicidal concentrations.

Bacteria	MIC (mg/µL)	MBC (mg/µL)	Fungi	MIC (mg/µL)	MFC (mg/µL)
B. cereus (Gram+)	0.08	0.08	*A. flavus*	0.16	0.16
B. subtilis (Gram +)	0.06	0.06	*A. niger*	0.18	0.18
S. aureus (Gram+)	0.08	0.08	*C. albicans*	0.20	0.20
E. coli (Gram-)	0.08	0.08	*S. cerevisiae*	0.08	0.08
P. aeurignosa (Gram -)	0.08	0.08			

MIC, minimum inhibitory concentrations; MBC, minimum bactericidal concentrations; MFC, minimum bactericidal concentrations.

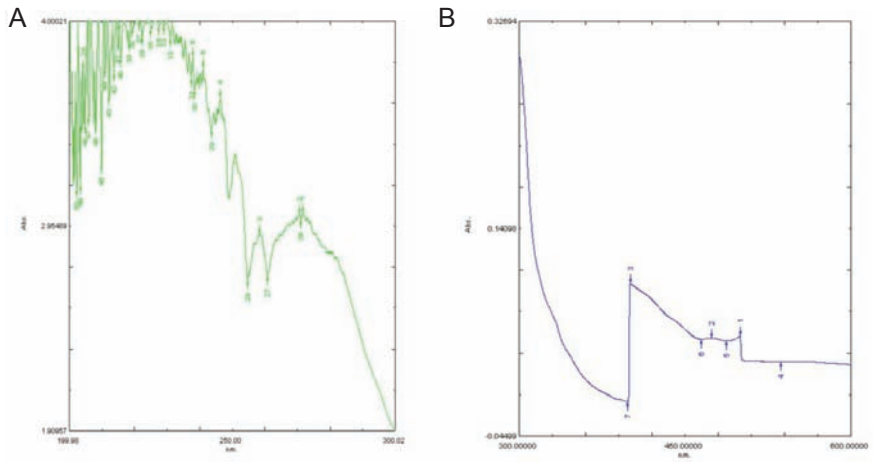

Figure 2. UV-Vis absorption spectrum of *Ceiba pentandra* seed oil. A) Absorption spectra from 200 nm to 300 nm; B) Absorption spectra from 300 nm to 600 nm.

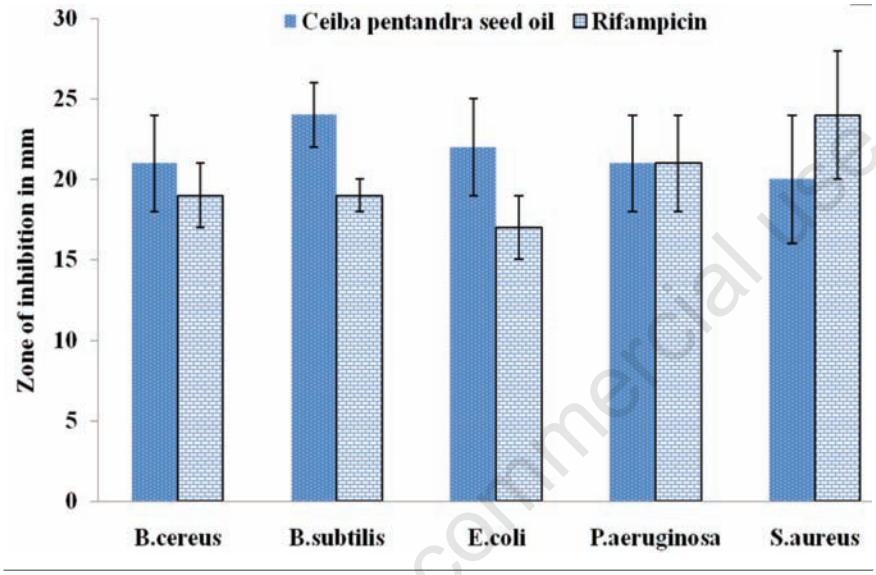

Figure 3. Antibacterial activity of *Ceiba pentandra* seed oil. Values represent average of triplicates and are expressed as mean ± standard deviation.

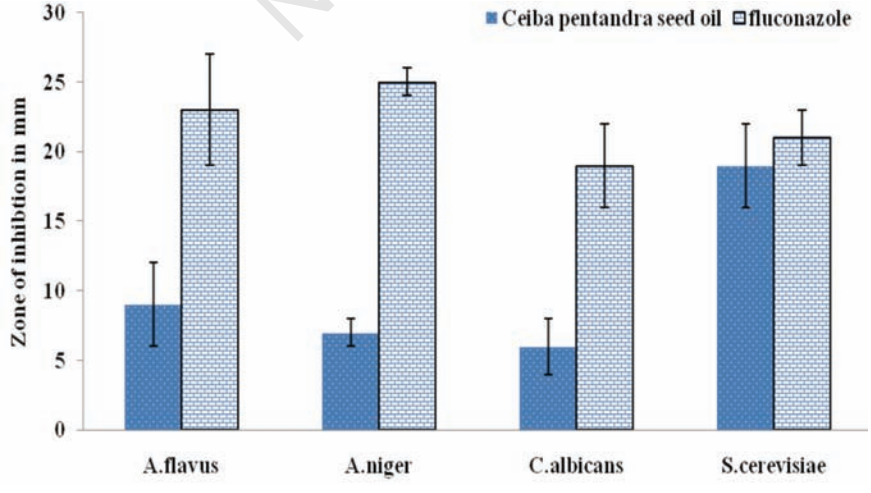

Figure 4. Antifungal activity of *Ceiba pentandra* seed oil. Values represent average of triplicates and expressed as mean ± standard deviation.

Results and Discussion

Physiochemical characterization

Oil extraction was carried out by a soxhlet extraction method according to Association of Official Analytical Chemists - AOAC - recommendations. Hexane was used as solvent to extract the oil from seeds and this was passed out for 10 h. The physiochemical characteristics were comparable to that of cotton seed oil (Table 1). The oil was thick and yellowish in color with a pungent odour and a 40% yield (Figure 1). The acid value of the oil was predicted to be 15, analogous to that of butter (0.46-35.0). The iodine value (degree of unsaturation) of the *Ceiba pentandra* seed oil was 98, comparable to that of cotton seed oil (103-111).The saponification value of the oil was 196, identical to that of cotton seed oil (194-196). Peroxide value, which indicates the extent of oil oxidation, was 12 $mEqKg^{-1}$. Fresh edible oils have a peroxide value of less than 10 $mEqKg^{-1}$, while rancid oils have values of more than 20 $mEqKg^{-1}$. The RMV of the oil was 0.36, the same as volatile water-soluble fatty acids present in the oil or fat. Relatively lower RMV of the oil was an indication of low content steam volatile fatty acids. The Polenske value of the oil was 0.97, which indicates low content of the volatile alcohol-soluble fatty acids in the oil.

Spectrophotometric analysis

UV-visible spectrum can be applied to identify the types, numbers and position of chromophores and auxochrome, and saturated and unsaturated compounds. UV-Vis absorption spectrums of *Ceiba pentandra* seed oil are shown in Figure 2. If no absorption peaks between 200~400 nm were detected, there is no conjugate double bond and C=O group, demonstrating that this is most probably a saturated compound. If there is a weak peak (=10~100) between 270~350 nm, and no other peaks detected over 200 nm it may contain >C=O, >C=C-O- or >C=C-N< etc. The weak peak was due to n - * transition. If there are many peaks in the UV region, some of them are even within the visible region, then the compounds may have long conjugation bonds. When λ_{max} is over 250 nm, and is between 1000~10000, the compound may contain aromatic structure. ε between 10000 ~ 20000 for the long wave absorption peak may be conjugated diene or carbonyl compounds.[19] If the peaks appear at the wavelengths 425, 455 and 480 nm in addition to 525, 570 and 590 nm chromophores, they may belong to carotenoids and flavonoids.[20] Given this, *Ceiba pentandra* crude oil may have unsaturated fatty acids with tandem conjugated double bonds, alkaloids, carotenoids, flavonoids, tannins and phenolic compounds, confirming its therapeutic potential.

Antimicrobial activity

In the present study, *Ceiba pentandra* seed oil in general showed antimicrobial activity against all the pathogenic bacterial and fungal strains studied. The bacteria and fungus used in this study were associated with various forms of diseases.[21] The antimicrobial and antifungal activity of the essential oil of L. *nobilis* has been demonstrated previously.[22] Simic *et al*.[22] reported low antifungal activity. In the present study, we found that the *Ceiba pentandra* seed oil showed significant action on bacterial strains compared with fungal strains (Figures 3 and 4). Results of antimicrobial susceptibility revealed that *Bascillus subtilis* was the most susceptible with an inhibition zone diameter (mm) of 24±2 followed by *Eschirichia coli* (22±3), *Pseudomonas aeruginosa* (21±3), *Bacillus cereus* (21±3), and *Staphylococcus aureus* (20±4). Among fungi, *Sacharomycetus cerevisiae* was the most susceptible with an inhibition zone diameter (mm) of 19±3 followed by *Aspergillus flavans* (9±3), *Aspergillus niger* (7±1) and *Candida albicans* (6±2). MICs and minimum bactericidal and fungicidal concentrations of the seed oil are shown in Table 2. These varied between 3-10 mg/50 µL against all bacterial and fungal strains used in this study. The varying degrees of susceptibilities of the bacterial and fungal strains may be due to the intrinsic tolerance of the microorganisms, and the nature and combinations of phyto compounds present in the oil. That fatty acids are potent antimicrobial agents with an inhibitory action has long been known.[23] Trace amounts of fatty acids have been shown to influence the growth of microorganisms in a very specific manner; some fatty acids, such as lauric acid, have been shown to have greater inhibitory action than others.[24] Kabara and coworkers examined several specific straight-chain saturated fatty acids and found lauric acid to be one of the most potent bacteriostatic fatty acids when tested on gram positive organisms.[25] In a study by Gudmundur *et al.,*[26] capric acid was found to inhibit the growth of *Candida albicans*, a fungal organism responsible for many infections. Medium chain free fatty acids and their corresponding 1-monoglyrides have been found to have a broad spectrum of microbicidal activity against enveloped viruses and various bacteria *in vitro*,[27,28] including pathogens such as herpes simplex virus,[29] Neisseria gonorrhoeae,[30] Chlamydia trachomatis, group A streptococci, group B streptococci, and Staphylococcus aureus.[31] An important characteristic of essential oils and their components is their hydrophobosity which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable.[32] Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death.[33] The activity of the essential oils would be expected to relate to the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components.[34] There were some reports about antimicrobial activity of terpinen-4ol, eugenol, and linalool components.[22,35] Indeed, the antibacterial activity of crude extracts has been attributed to the presence of some of the phytochemical components such as alkaloids, flavonoids, saponins and tannins which was in agreement with our results.[36,37]

Conclusions

The ethnomedicinal study of seed oils is important for modern day medicine but its usefulness cannot be fully appreciated until methods of comparison with other compounds are standardized and reproducible results are obtained. The present study has revealed the importance of seed oils in controlling resistant bacteria and fungi which are becoming a threat to human health. This can serve as a useful platform for the development of inexpensive, safe and effective natural medicines.

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