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Bioprospecting of *Callistemon citrinus* explored for present chemical species using FTIR

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Abstract
Despite a great breakthrough of scientific advancements and knowledge about diversity, biochemical composition of a vast array of plant species still remains unestablished or unquantified. FTIR spectroscopy was used in this study to evaluate the phytochemical potential of *Callistemon citrinus.* Leaves, stems and bark tissues of the mentioned species were sampled, and the ATR-FTIR spectra recorded in the spectral range 485 - 4000 cm^{-1} at a resolution of 4 cm^{-1}. The data indicated presence of the following significant functional groups; β-carophyllene, tristerpenes betulin and α-pinene in the leaf samples, phemol, Linalool, β-Pinene (bicyclic monoterpenes), phloroglucinol derivatives, alkaloids and terpenen-4-ol in the bark samples, pectin,1-8 cineol, alkynes and γ-terpineole in the stem samples. These results demonstrates a great potential of application of *C. citrinus* plant species in pharmaceutical industry recommends further scrutiny on quantities of each of the compound present.

Keywords: *Callistemon citrinus*, FTIR, Biodiversity, Functional group, *Callistemon citrinus*

1. Introduction
Vegetation biodiversity is one of the greatest wealth that nature has endowed every nation with. Even with such great natural wealth, man has not been able to get the most out of the plants that grow around him [1, 2]. The main reason for such incapacity is only attributable to lack of sufficient and accurate knowledge of what each vegetation species contain in terms of biochemical [3, 4]. In other occasions, lack of knowledge about an alternative plant species containing similar biochemical to those whose utility is already in place, has led to over exploitation [5, 6]. Such incidences of overexploitation of a narrow range of vegetation species by man in the previous decades has led to declaration of some vegetation species as threatened, endangered and even some have become extinct and are no longer replaceable on the planet. Overexploitation of specific species being one of the major drivers to loss of biodiversity can be arrested by diversifying the sources of such biochemical that leads to the utility of the species in question [6, 7, 8, 9, 10, 11]

This diversification of sources can be achieved through a through screening of plants to establish their biochemical content similarities. Man's utility for vegetation is as old as man himself but his knowledge and information about the economically important biochemical in plants still remains limited even in the modern times [12]. Many economically potential plants have remained unutilized to date due lack of knowledge about their biochemical composition [13]. This limitation and inadequacy of information could be attributed to lack of proper and accurate biochemical testing tools and techniques. In the instances, where the economically important biochemical have been suspected in certain plants, quantification of such biochemical to ensure economic viability of harnessing them on the other hand has also represented a real challenge [14, 15,16]

The time-honored techniques of plant biochemical detection are faulty and based on trial and error methods. In some instances, for example in the fields like herbal medicine and Ayurveda, cases of human poisoning have been reported due to consumption of poisonous concoctions or overdose to such mixtures of dubious contents. For instance, between 1978 and 2008, "more than 80 cases of lead poisoning associated with Ayurveda medicine use were reported worldwide". In 2012, the U.S. Centers for Disease Control and Prevention (CDC) linked Ayurveda drugs to lead poisoning, based on cases where toxic materials were found in the blood of pregnant women who had taken Ayurveda drugs (17). Therefore the aim of this study was to determine the biochemical content of the various plant tissues (Leaf, stem and bark) of *Callistemon citrinus.*

1.2. *Callistemon citrinus*

Callistemon citrinus is one of the main species of the myrtaceae family of plants which is closely related to *melaleuca.* The genus callistemon comprise of about 30 species that are Australian native and endemic in areas swampy areas of Queensland, New South Wales Victoria. The plant is now widely distributed in the wet tropics all over the world [18]. Physiognomically, C. *citrinus* is either a shrub or a tree since it grows to a height of between 0.5-7 M with white papery bark and narrow attractive lanceolate of (ca. 3-6 mm wide and 40-70 mm long) as its foliage. The flowers are spikes with conspicuous red stamens [19]. *C.citrinus* is widely cultivated in homesteads and in city streets as ornamental plant due to its decorative flowers. It may also be used for forestry as a windbreaker, reclamation of degraded lands and as a bio indicator for environmental management among other applications [19, 20]. Studies have shown that plants of this family are also an important source of essential oils that can be used in the manufacture of various compounds for use as antibacterial, insecticides and antifungals [21, 22, 23].

1.2.1. Studies on leaves of callistemon species

Many authors [24, 25, 26] have studied the biochemical composition of *Callistemon* species leaves. However, to the best of our knowledge only limited number of these studies have been carried out on *Callistemon citrinus*. Mostly, they concentrated on other species like *C. lanceolatus, C. rigidus* and *C. viminalis.* [27, 28]. Furthermore the methodologies and equipment used were basically Thin Layer Chromatography and Gas Chromatography which involve drying, heat-induced hemolysis and hydrolysis of the samples [29] and does not give the composition of the leaves before metabolism, breakdown and transformation of some components within the fresh samples [30, 31]. Evaluation of pharmaceutical importance of leaves *Callistemon* genus has also been widely done revealing its ability to work as an anti-bacterial substance [31, 32, 33].

1.2.2. Stem and stem bark

Although several studies have been carried out to analyze the chemical composition of *callistemon* family, many of these studies have been focusing on leaves and not study of various parts of the plant [30, 33, 34]. This paper therefore for the first time is reporting findings of chemical constituents of *Callistemon citrinus* bark from the northern India. Nevertheless, studies of the *Callistemon* stems have revealed presence of constituents like; steroids, saponins, terpinoids and flavonoids [35].

2.1. Materials and Methods

2.1.1. Study Site

The study was carried out between the month of January and March, 2015, in the University of Allahabad, India. The University is located in the Allahabad city which lies on the upper northern quarter of the Indian sub-continent at a geographical position of (81.49^0 E, 25.26^0N) and an elevation of 104M or 341 ft Above Sea Level (A.S.L). The city is located in the Uttar Pradesh (U.P) state of India, about 180 Kms from Lucknow city, which is the Headquarters of UP and 579 Kms south-east of Delhi, the capital city of India. Allahabad has three seasons: a hot, dry summer, a cool, dry winter and a warm, humid monsoon. Summer lasts from April to June with temperatures in the low 30 °C (86.0 °F); during dry spells, maximum temperatures often exceed 40 °C (104 °F) in May and June. The monsoon begins in early July, and lasts till September. Winter runs from December to February, with temperatures rarely dropping to the freezing point (36). The daily average maximum temperature is about 22 °C (72 °F) and the minimum about 9 °C (48 °F). Although Allahabad experiences dense fog in January, resulting in traffic and travel delays, the city does not receive snow. Its highest recorded temperature is 48 °C (118.4 °F), and its lowest is −2 °C (28 °F).

2.1.2. Sample Collection and Preparation

The Plant tissues (stems, barks and leaves) which are main biochemical storage areas [37] of 30 individual plants (*Callistemon citrinus*) used in this experiment were harvested from the branches of mature trees (approx. 15-20yrs of age) growing within and around the University of Allahabad. In order to rule out the deviation in biochemical composition among individuals in each species due to the influence of soil (minerals) and other similar surrounding environmental factors, a distance of at least 800 square meters from each sampled individual to the next was maintained. Health of the plant individuals sampled was also put into consideration.

The samples were analyzed in Saha's laboratory, Physics department in University of Allahabad, where they were cleaned and air dried until their surfaces were completely free from the cleaning reagent (water), see figure 1. The air drying process was done carefully taking only approximately thirty [30] minutes to prevent any dehydration of the plant tissues which could lead to inaccuracy in determining the amount of water present in the samples. This would be reflected in the Intensity (I) of the portion of the spectra corresponding to the water. This would also have occurred to other biochemical components of the respective sample in case of delayed recording due to various transformations and conversions of the biochemical resulting from detachment of the tissues from their normal functioning systems.

Fig 1: Photos of plant tissues arranged for Air drying in the laboratory

2.1.3. Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR)

ATR-FTIR was used to obtain spectra. After calibration of the FTIR machine (MB 3000) to the wavelength between 485 cm^{-1} - 4000cm^{-1} and a resolution of 4cm^{-1}. The laboratory room background condition was recorded. The machine automatically saves the background reference information and

also automatically subtracts it from the recorded information for every sample tested within one hour. Upon elapsing of one hour a new room background reference should be obtained. Then the interferograms for samples were then recorded by placing each sample at a time on the crystal of the FTIR machine, then clipping it gently with a sample holder, and finally scanning by issuing a record command from the HORIZON MB® software present in the computer. The interferogram data for each sample was the saved and plotted into a graph using ORIGIN 6.1® LAB to facilitate further informative manipulations.

3.1. Results and Discussion

The observed spectrum (figure 2) illustrates important chemical compounds present in the leave samples. The wave peaks numbers obtained are 572 cm^{-1}, 663cm^{-1}, 1031cm^{-1}, 1174cm^{-1}, 1369cm^{-1}, 1456cm^{-1}, 1685cm^{-1}, 2358cm^{-1}, 2854cm^{-1}, 2923cm^{-1}, 3379cm^{-1} and 3728cm^{-1}. These corresponds to terpinen-4-ol, Cellulose, polysaccharides, β-carophyllene, proteins, triterpenes botulin, lipids and α-pinene as shown in table 1 below.

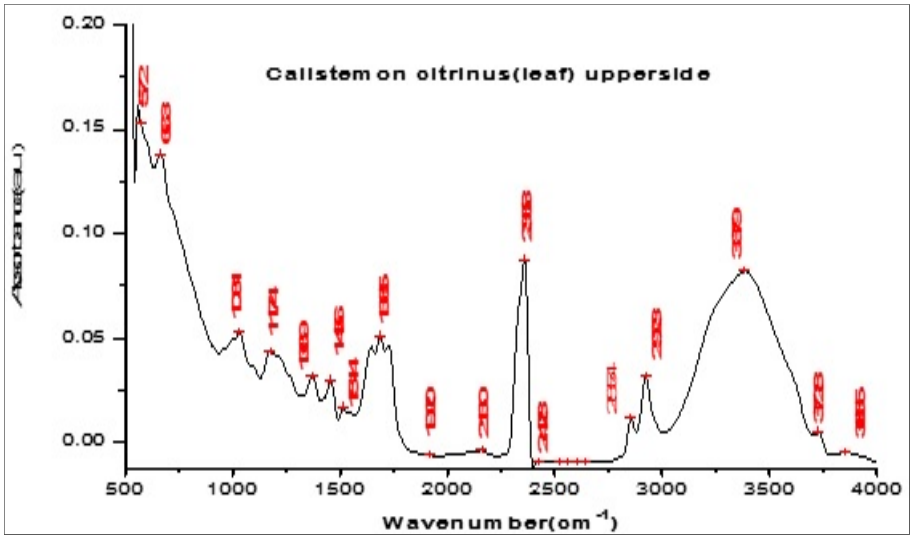

Fig 2: Recorded ATR-FTIR spectrum of the leaf of *Callistemon citrinus* at resolution of 4cm^{-1}

Table 1: Observed & reported IR-bands, Identified functional group and detected compounds of the *Callistemon citrinus* (leaf) upper surface (Abs mode)

Observed IR-band (cm^{-1})	Observed Absorbance	Reported IR-bands (cm^{-1})	Identified Functional group	Remarks(strong, weak, sharp, diffused)	Identified Probable compound
572	0.1528	690-515	C-Br-stretch(alkyl halides)	Weak	Terpinen-4-ol
663	0.1383	600-900	CH out-of- plane bending vibrations	Weak	
1031	0.0532	1031	v(CC), v(CCO) v(CO)	Medium	Cellulose (polysaccharides)
1174	0.0439	1173	Non- hydrogen- bonded stretching mode of C-OH groups	Weak	Polysaccharides
1369	0.0317	1369/70	□ sym(CH_3)	Weak	β-carophyllene (sequesterpenes)
1456	0.03	1456	CH_3 bending vibration (lipids and proteins)	Weak	
1514	0.0167	1514	Amide II □(N-H)$^+$ V(C-N)	Weak	Protein
1685	0.0511	1685	Amide I (disordered structure –non-hydrogen bonded)	Medium	Triterpenes betulin
2854	0.0118	2853	lipids Asymmetric CH2 stretching mode of methylene chains in membrane lipids	Weak	Lipid
2923	0.0315	2923/5	Stretching C-H	Medium	α-pinene
3379	0.0821	3400-3250	N-H stretch(1^0,2^0 amines, amide	Strong	1-8 cineol(bicyclic monoterpenes

Figure 3 shows the recorded spectrum illustrating the important chemical compounds present in the specimen of *Callistemon citrinus* bark. The major bands observed in this spectrum lie at wave numbers; 536cm^{-1}, 1031cm^{-1}, 1220cm^{-1}, 1367cm^{-1}, 1450cm^{-1}, 1639cm^{-1}, 1730cm^{-1}, 2358cm^{-1}, 2856cm^{-1}, 2927cm^{-1}, 3365cm^{-1} and 3730 cm^{-1}. These peaks corresponds to cellulose, phenol, terpinen-4-ol, linalool, proteins, α-pinene, phloroglucinol derivatives and alkaloids among others (see table 2 below).

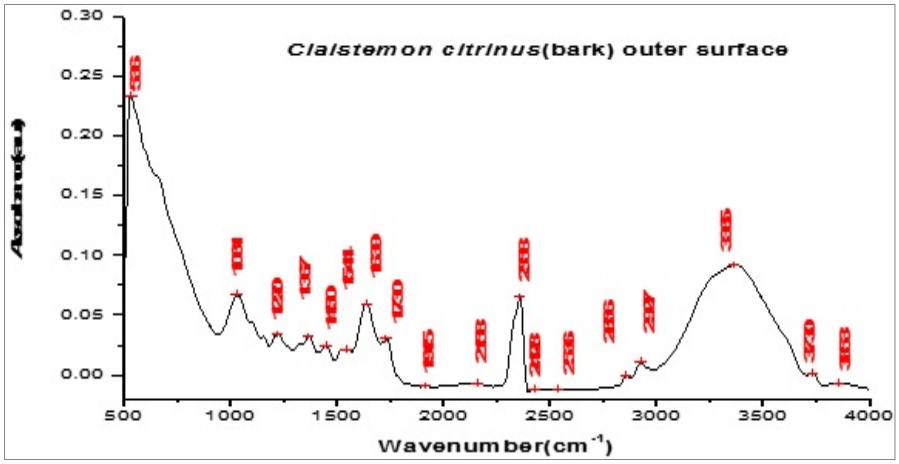

Fig 3: Recorded ATR-FTIR spectrum of the bark of *Callistemon citrinus* at resolution of 4cm^{-1}

Table 2: Observed & reported IR-band, Identified functional group and detected compounds in *Callistemon citrinus* (bark) outer surface (Abs mode)

Observed IR-band (cm^{-1})	Observed Absorbance	Reported IR-bands (cm^{-1})	Identified Functional group	Remarks (Strong, weak, sharp, diffused)	Detected Compound
536	0.2348				
1031	0.0678	1031	*V*(CC),*v(CC)*, v(CO)	Medium, sharp	Cellulose (polysaccharides)
1220	0.0341	1220	PO_2^- Asymmetric vibrations of nucleic acids when it is highly hydrogen bonded. Asymmetric hydrogen bonded phosphate stretching mode	Weak	Phenol
1367	0.0324	1367	sym CH_3 (C=O)	Medium	Terpinen-4-ol
1450	0.0245	1450	Methylene deformation in biomolecules	Weak	Linalool
1544	0.021	1544	Amide II bands	Weak	Protein
1639	0.0601	1639	*v(C=C)*	Medium, sharp	α-pinene(bicyclic monoterpene)
1730	0.0301	1730	Fatty acids ester band	Weak	Phloroglucinol derivatives
2358	0.0653				
2856	0.0012	2853	Vs CH2 of Lipids, Asymmetric CH2stretching mode of the methylene chains in membrane lipids	Weak	Lipid
2927	0.0108	2928	Stretching C-H	Weak	Terpinen-4-ol
3365	0.0925	3362	O-H,N-H,C-H	Strong	Alkanoids

The prominent wavenumber peaks observed from the stem sample of *Callistemon citrinus* include;1031cm^{-1}, 1218 cm^{-1}, 1319 cm^{-1}, 1446 cm^{-1}, 1620 cm^{-1}, 2358 cm^{-1}, 2929 cm^{-1}, 3353 cm^{-1}, 3728 cm^{-1} as shown in Fig 4. They correspond to various chemical compounds as illustrated in table 3 below.

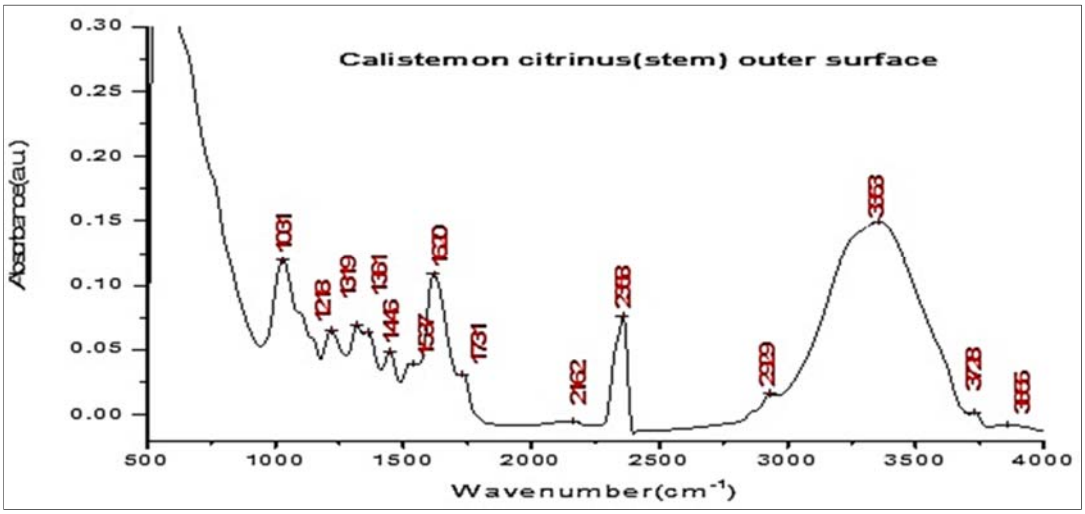

Fig 4: Recorded ATR-FTIR spectrum of the stem sample of *Callistemon citrinus* at a resolution of 4cm^{-1}

Table1 3: Observed IR-band & Identified functional group in FTIR spectrum of *Citrinus callistemon* (stem) outer surface (Abs mode)

Observed IR-band (cm^{-1})	Observed Absorbance	Reported IR-bands (cm^{-1})	Identified Functional group	Remarks(strong, weak, sharp, diffused)	Identified Probable compound
484	1.2242				
1031	0.1196	1031	ν(CCO), ν(CC)	Medium &sharp	Cellulose (polysaccharides)
1095	0.0788	1095	Asymetrical C-O-C vibrational stretching	Diffuse	
1143	0.0591	1145	C-O stretching	v. weak	Pectin
1218	0.0647	1217	υ_{as} (C-O-C)	Weak	1-8 cineol(bicyclic monoterpenes
1319	0.0689	1317	Amide III band component s of proteins collagen	Weak	Alcohols, carboxylic acids, esters
1361	0.0635	1358	Stretching C-O, deformation C-H, deformation N-H	Weak	
1446	0.0491	1444	$\delta(CH_2)$	Weak	Terinen-4-ol
1620	0.1088	1620	Peak of nucleic acids due to the base carbonyl stretching and ring breathing mode	Medium, sharp	Phloroglucinol derivatives
1731	0.031	1730	Absorption band of fatty acid esters. Fatty acids esters	Weak	Aldehydes, saturated aliphatic
2160	-0.005	2100-2260	-C≡C- stretch	v.weak	Alkynes
2358	0.076			Medium, sharp	
2929	0.016	2928	Stretching C-H	Weak	γ- terpineole
3353	0.1496	3353	Stretching N-H asymmetric	Strong	$1^0,2^0$, amines, amides

4. Conclusion

Although this study was not focused on quantification the various constituents occurring in each of the specimen samples used, it on the other hand strongly agrees with findings of studies by various authors about the chemical species present in *Callistemon* genus. For instance, while using Thin Layer Chromatography and Gas chromatography (38) established that the major components in the leaves of *Callistemon citrinus* and *Callistemon viminalis* from south Africa include; 1.8-cineole (61.2% and 83.2%), and α-pinene (13.4% and 6.4%) of the total essential oils respectively and other component such as, β-pinene and linalool in lower quantities. (30) further established presence of 1.8-cineole (66.3%) and α-pinene (18.7%) in leaves of *Callisemon citrinus* growing in the lower Himalayan plain which he claims differ greatly in percentage with similar components of the same plants growing in the northern India. However, this study established compounds like phenol, phloroglucinol derivatives, γ- terpineole, alkynes and alkaloids among others were detected in other parts (bark and leaves) of *C. citrinus* by application of ATR-FTIR spectroscopy. Preliminary testing of identified compounds for anti-microbial activity has shown positive results as indicated by the following authors (30, 31, 38). Additionally, several compounds in mixtures can be measured and identified simultaneously with the ATR-FTIR instrument. Therefore, ATR-FTIR methodology represents a significant advancement in measurement technology which provides a high level performance and adaptation for broad range of application that requires minimal sample preparation. Finally, this study has revealed the high potential of *Callistemon citrinus* for production of essential oils for pharmaceutical application.

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