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Phytochemical Screening of Leaves, Bark and Stem of *cassia siamea* by FTIR Spectroscopy

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Abstract: ***The global wealth of plant species range between 400,000-500,000. However, only a small percentage of species have been phytochemically investigated. The fraction that has so far been subjected to biological or pharmacological screening is even smaller. This scenario presents a huge knowledge gap and underutilization of modern technologies for bioprospection leading to overexploitation of a narrow range of plant species to provide mankind with secondary metabolites for pharmaceutical, cosmetic, food additives and many other modern applications. The ATR- FTIR spectra of various tissues (leaf, bark and stem) of Cassia siamea used in this experiment have revealed presence of a wide range of biochemical compounds with pharmaceutical importance. Apart from presence of main primary metabolites such as protein, carbohydrate and lignin, there was abundance of Anthraquinone, saponins, and cassiarins in all the plant tissues sampled. Differences in other components present in the sampled tissues was noted as follows; limone and bicyclic monoterpenes were detected in the leaf while tetraterpenes, monoterpenes and pectin were conspicuously present in the bark and stem samples. In conclusion this studies therefore, reveal that Cassia siamea is a plant with great potential of some active biocomponents with therapeutic capacity.***

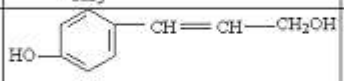
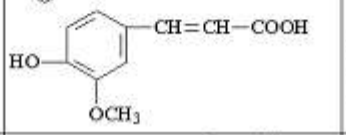
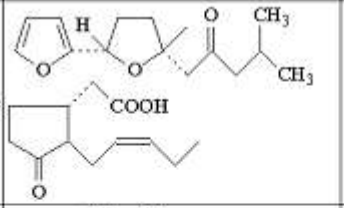
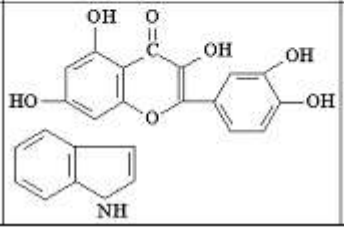
Key words: Cassia siamea, bioprospection, FTIR, biochemical, tissue

1. Introduction

Since time immemorial, plants have played very crucial role in the support of other life forms-animals. (Y.R. Alli Smith, 2009). The life of plants themselves is supported by various chemical compounds which they contain or else synthesize (Kretovich, 2005). Such chemical compounds by which plants drive their physiological processes are collectively referred to as biochemical and may be categorized either as primary or secondary metabolites (Schafer et al., 2009). They also enormously contribute to defense or immunity of plants to and/or against disease causing pathogens and parasites-especially epiphytes (Bennett, R. N. and Wallsgrove, R. M., 1994; Brooks and Watson, 1985). The Biochemical composition of plants vary greatly between and among individuals of the same species as well as among the different tissues within the same individual plant. For instance, the biochemical composition of the roots of a given plant may be different from those found in its stem, bark, fruits, flowers and leaves (Ajaiyeoba et al., 2008; Y.R. Alli Smith, 2009). However, some biochemical appears to be conspicuously present in all individuals sharing a common genus or family. In other instances, the similarity or variability of biochemical composition in the individual plant species with a common origin may also be affected by environmental factors such as; climate, soils and pollutant loadings among others (Prozumenshchikova, 1981). Based on abundance and bioactivity, plant biochemical are utilized by man to meet various needs. For instance, presence and abundance of artemisinin in the *Artemisia annua* makes it suitable for production of anti-maralia drugs while *Azadrachta indica* has been extensively used in herbal medicine to treat a variety of health issues due to its wealth of biochemical with pharmaceutical importance (Mohammad A. Hossain et. *al*, 2013; Schwikkard and van Heerden, 2002).

In the effort of biochemical prospection, only about 100,000 secondary metabolites have been described from various plant species. Most uniquely, they have been found to be chemical substances with representatives of all main classes of organic compounds (Aliphatic, Aromatic, hydro-aromatic and heterocyclic) with inimitable carbon skeletons occurring along with multiplicity of functional groups (Hadacek, 2002)

Table 1: Major secondary metabolites and their chemical structures. *Source*: Edreva et al

Chemical types	Formulae	Representatives
Aliphatic	$NH_2—CH_2—CH_2—CH_2—CH_2—NH_2$	Polyamines
	$CH_2=CH_2$	Ethylene
	$CH_2=C(CH_3)—CH=CH_2$	Isoprene
Aromatic		Phenolic alcohols
		Phenolic acids
		Unsaturated aromatic carbonic acids
Hydroaromatic		Terpenoids Jasmonic acid
Heterocyclic		Flavonoids Indole derivatives

The global wealth of plant species range between 400,000-500,000. However, only a small percentage of species have

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been phytochemically investigated and the fraction that has so far been subjected to biological or pharmacological screening is even smaller. According to Fransworth, (1990), about 119 of the characterized drugs are still being commercially obtained from higher plants and out of these 74% have been found from ethnobotanical information. This scenario presents a huge knowledge gap and underutilization of modern technologies for bioprospection leading to overexploitation of a narrow range of plant species to provide mankind with secondary metabolites for pharmaceutical, cosmetic, food additives and many other modern applications.

In this study, Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy was used to evaluate the biochemical composition of *Cassia siamea* with an aim of demonstrating *Cassia sp*. as an alternative source of various chemical compounds of economic importance to human being. Further, the study also established that ATR-FTIR is a superior, rapid, environmentally friendly, reproducible and accurate technique of detecting biochemical contents of any vegetation species.

1.1 Cassia siamea

Cassia siamea is a medium size evergreen tree that grows to an optimum height of 18m and a trunk diameter of 30cm. The tree is adapted to a wide range of climatic conditions within the world's tropics. It is a native species in most of the Asian countries such as; India, Brunei, Thailand, Philippines and China among others. *Cassia siamea* has a smooth light brown or grey bark that becomes slightly fissured with age. The leaves are about 23-33 cm long, alternate and pinnately compound with tinged axis and 6-12 pairs of leaflets. The flowers are bright yellow pentamerous clusters with imbricate sepals and obtuse apex. Since *Cassia* is a leguminous plant, it produces numerous dehiscent pods of 5-25 cm long and 12-20mm wide (Orwa *et al.*, 2009).

1.2 Uses of *Cassia Siamea*

Among the traditional folklore, *C. siamea* has a wide range of applications. For instance, in the traditional Chineese medicine, *C. siamea* is used for treatment of diarrhea, gastritis, ringworm, and fungal skin infections (S. Rajan, 2001; J. Ma, 2004). Additionally, it provides a wide range of goods such as; timber, fuel, fodder, tannin, human vegetables and services such as; soil amendment, shade, ornamental and land reclamation among others (Kiepe P., 1995; Orwa *et al.*, 2009).

1.3 Studies on Cassia plant

Recent studies (Ajaiyeoba *et al.*, 2003; 2005) have demonstrated that apart from the ethnobatanical use of *Cassia siamea* as a laxative, for insomnia, diabetes and hypertension, new findings have shown usefulness of its bark extract as an antimaralial remedy. (Yan-Qing Ye, *et al.*, 2014), revealed extraction of two new anthraquinones, siameaquinones A (1) and B (2) from the air dried stems of *C. siamea* using column chromatography on silica gel, Sephadex LH-20, and RP-18 and preparative HPLC. Due to cytotoxicity potential of anthraquinone, (D. Jeevitha and K. Amarnath, 2013; M. Endale, 2013), testing of these compounds, further revealed cytotoxicity against five human tumor cell lines.

2. Study Site and Methodology

2.1 Study Site

The study was carried out between the month of January and May, 2015, in the University of Allahabad, India and its environs. 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 U.P 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 (Allahabad Climate, 2012). 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).

2.2 Materials and Method

2.2.1 Sample Collection and Preparation

The Plant tissues (stems, barks and leaves) of 30 individual plants *Cassia siamea* were obtained /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 consistency in biochemical composition among individuals in samples due to the influence of soil (minerals) and other similar surrounding environmental factors, a distance of at least 800 square metres from each sampled individual to the next was maintained. Health of the plant individuals sampled was also put into consideration.

The samples were taken to 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). 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 recording/determining the amount of water present in the samples.

2.3 General Experimental procedure

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

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recorded. 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. Relevant and authentic literature was then used to ascertain the various functional groups and chemical compounds that corresponds to various wave peaks observed in the interferograms of each sample.

3. Results and Discussion

The spectrum (Fig.1) shows highest concentration of chemical compounds in the leaf of *C. siamea* at band waves numbers; 552 cm^{-1}, 1029cm^{-1}, 1377cm^{-1}, 1463cm^{-1}, 1641cm^{-1} , 2848cm^{-1}, 2916cm^{-1} and 3371 cm^{-1}. These wave peaks corresponds to functional groups and chemical compounds contained in the leaf sample as shown in the table 1 below.

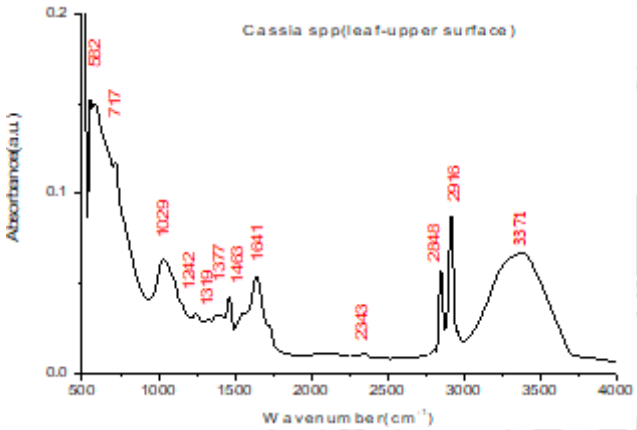

Figure 1: Recorded ATR-FTIR spectrum of the leaf of *Cassia siamea* at a resolution of 4cm^{-1}

Table 1: Observed IR-band & Identified functional group in FTIR spectrum of leaf of *Cassia siamea* (upper -lower surface) (Abs mode)

Observed IR-band (cm^{-1})	Observed Absorbance	Reported IR-bands (cm^{-1})	Identified Functional group	Identified Probable compound
554	0.3748	553		Saponins
1010	0.1955	1010	υ(C-O), υ (CC) ring	Cellulose
1149	0.0741	1150	υ(C-O-C)	Pectin
1242	0.0397	1242	Amide III	Protein
1411	0.046	1411/4	Stretching C-N, deformation N-H, deformation C-H	Lignin
1456	0.0462	1456	CH3 bending vibration (lipids and proteins)	Tetraterpenes (β-carotene)
1548	0.059	1549	Amide II Amide II of proteins	Protein
1643	0.1111	1643	Amide I band (arises from C-O stretching vibrations)	Cassarin
2927	0.0292	2727/731	Stretching NH (NH3+)	Anthraqui-none
3344	0.1664	3330/5/7/ 9/43	Stretching N-H asymmetric	Alkaloid

The spectra obtained from C. siamea bark (fig.2) shows major bands at wavenumbers 554cm^{-1}, 1010cm^{-1}, 1149cm^{-1}, 1548cm^{-1}, 1643cm^{-1}, 2358cm^{-1}, 2927cm^{-1}, 3344cm^{-1}, and 3739cm^{-1}. These bands corresponds to various chemical compounds and functional groups illustrated in table 3 below.

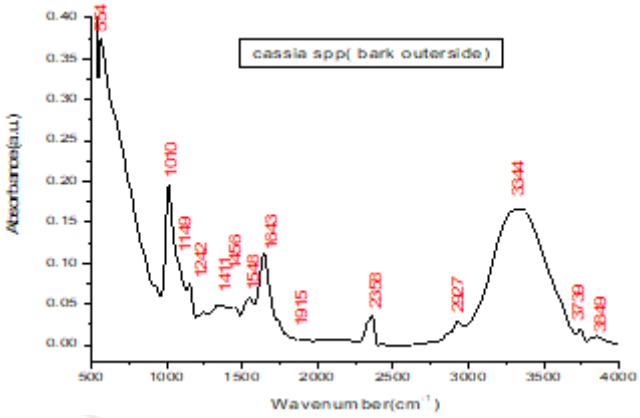

Figure 2: Recorded ATR-FTIR spectrum of the bark of *Cassia siamea* at a resolution of 4cm^{-1}

Table 3: Observed IR-band & Identified functional group in FTIR spectrum of *Cassia siamea* (bark-outer surface)(Abs mode)

Observed IR-band (cm^{-1})	Observed Absorbance	Reported IR-bands (cm^{-1})	Identified Functional group	Identified Probable compound
552	0.1176	552-3		Saponins
1029	0.0633	1029/30	C-O stretch	Carbohydrates
1242	0.0329	1242	Amide III	Proteins
1377	0.0323	1380	Δsym CH_3(C-O)	1-8 cineol(bicyclic monoterpenes)
1463	0.0424	1464	C-H deformation	Lignin
1641	0.0542	1640	υ ethylene C=C	Limonene
2848	0.0571	2848	CH_3 symmetric stretch	Lipid
2916	0.0876	2916	CH_2 asymmetric stretching	Lipid
3371	0.0671	3370	O-H, N-H	Cassiarins

The most prominent bands in the shown spectrogram (fig. 3) are indicated by wavenumbers; 553cm^{-1}, 1027cm^{-1}, 1514cm^{-1}, 1548cm^{-1}, 1639cm^{-1}, 2358cm^{-1}, 3350cm^{-1}, 3737cm^{-1} and 3858cm^{-1}. These wave numbers are described by the functional groups and chemical compounds shown in table 4 below.

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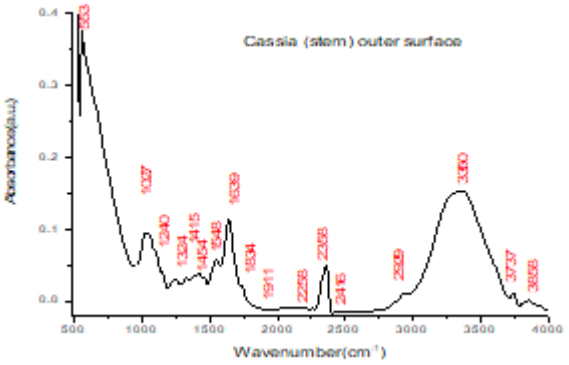

Figure 3: Recorded ATR-FTIR spectrum of the stem of Cassia siamea at a resolution of 4cm^{-1}

Table 3: Observed IR-band & Identified functional group in FTIR spectrum of *Cassia siamea*(stem –outer surface) (Abs mode)

Observed IR-band (cm^{-1})	Observed Absorbance	Reported IR-bands (cm^{-1})	Identified Functional group	Identified Probable compound
553	0.3756	552-3		Saponins
1027	0.095	1028	C-O and C-C stretching and C-O-H deformation motions	carbohydrate
1240	0.0308	1240	Vas PO2- Collagen Asymmetric non-hydrogenated bonded phosphate stretching mode	Saponins
1324	0.0321	1327/8	C-C & C-O skeletal Stretch	Anthraquinone
1415	0.0391	1416	υs (COO)	Pectin
1454	0.0346	1454	Asymmetical methyl deformation	Alkaloids
1548	0.0575	1549	Amide II δ(N-H)+ υ(C-N)	Proteins
1639	0.1135	1639	υ(C=C)	Bicyclic Monoterpenes (β-pinine)
2358	0.0496			Polyphenols
3350	0.1544	3350	O-H,N-H,C-H	

4. Conclusion

The ATR- FTIR spectra of various tissues (leaf, bark and stem) of *Cassia siamea* used in this experiment have revealed presence of a wide range of biochemical compounds with pharmaceutical importance. Apart from presence of main primary metabolites such as protein, carbohydrate and lignin, there was abundance of Anthraquinone, saponins, and cassiarins in all the plant tissues sampled. However, there was variations in the other components found in the sampled tissues. For example, limone and bicyclic monoterpenes were detected in the leaf while tetraterpenes, monoterpenes and pectin were conspicuously present in the bark and stem samples.

These findings concur with information revealed by other authors who have previously studied *Cassia siamea* using other techniques. For example, Jun Deguchi *et al.*, 2012, using HPLC and ^{13}C NMR extracted alkaloids and cassiarins (G, H, J and K) from the leaves of *C. siamea* which shown moderate antiplasmodial activity against *plasmodium falciparum.* Besides this study, (Yan-Qing Ye, et al., 2014) also showed presence of Anthraquinone and siameaquinone A (1) and B (2) in *Cassia siamea* plant. However, some chemical compounds like; limone, terpenes (Mono and ttetratepenes) as well as pectins were detected in the current study have not been recorded in many of the previous studies of *Cassia* plant. In conclusion this studies therefore, reveal that *Cassia siamea* is a plant with great potential of some active biocomponents with therapeutic capacity.

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