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Research Article

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Comparative account of allelopathic potential of different parts of *Cassia occidentalis* and its correlation with bio-molecular profile through FTIR

Komal Arora

Department of Botany, DAV College, Jalandhar, Punjab, India

ABSTRACT

Present study deals with the biochemical analysis of different parts of Cassia occidentalis L. *and its correlation with their allopathic potential. Growth studies on Echinochloa crus-galli, a common agricultural weed of rice ecosystems showed significant reduction with flower and leaf extracts as compared to root and stem extracts of C. occidentalis. Metabolic fingerprinting was achieved by using Fourier Transform Technique (FTIR) to understand differential distribution of different phytochemicals in root, stem, flower and leaf tissues. Presence of different functional groups C=O, C–H, C=C, C–O, C–C and N–H were identified which indicated the presence of alkyl groups, methyl groups, alcohols, ethers, esters, carboxylic acid and anhydrides in different tissues. The results showed that flower and leaf tissues were rich in lipids, proteins, amides (aromatic compounds) corresponding tomany sharp bands in the region of 1700-1500 cm^{-1}. Thus, FTIR studies not only provided the bio-molecular profile of C. occidentalis but also established a correlation of allopathic potential of different tissues with relative distribution of different phytochemicals.*

Keywords: FTIR, *Cassia occidentalis*, Biomolecules, Allelopathy, Phytochemicals

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INTRODUCTION

Cassia occidentalis L. is non-nitrogen fixing leguminous plant belonging to subfamily Caesalpinioideae of family Fabaceae. It is a smooth, semi-woody, 0.8 to 1.5 m tall herb. Tap root is hard, stout; stem reddish purple; leaves alternate, paripinnate, with 4 – 6 pairs of leaflets; inflorescence composed of axillary and terminal racemes, flowers perfect, yellow; fruit dehiscent, laterally compressed, sickle shaped legume containing 25 to 50 smooth, brown coloured seeds.

C. occidentalis is medicinally important as it finds many applications in traditional system of medicine involving skin infections, stomach disorders, snake bites etc. [1]. Plant is significant ecologically as it has been reported major weed of 23 crops in 66 countries worldwide [2]. Many studies have established theallopathic inhibition of other plants by aqueous extracts of *C. occidentalis* [3,4,5] and its antioxidant, antimicrobial and phytochemical properties [6,7] but the biomolecular analysis through Fourier transform infrared spectroscopy (FTIR) has not been determined so far. Initially, the use of infrared spectroscopic method was restricted only for structural elucidation of isolated compounds, but now this technique is being used as a fingerprinting device in phytochemical studies.

The aim of present study is to explore the bio-molecular profile of different parts of *C. occidentalis* correlating it with their varying levels of allopathic potential against *Echinochloa crus-galli,* a common weed of rice agricultural ecosystems.

EXPERIMENTAL SECTION

2.1 Collection and Identification of Plant Material

Plant material of *C. occidentalis* was collected from different sites in Jalandhar, Punjab, India. It was identified by various resources available online and the herbarium of Panjab University, Chandigarh, India. It was shade dried; various parts were ground to fine powder and stored in air tight jars till used for growth and FTIR study. Seeds of *E. crus-galli* were purchased from Punjab Agricultural University, Ludhiana, Punjab, India.

2.2 Growth Studies

Aqueous extracts of the flower, leaf, root and stem tissues were prepared by dipping 5 gm of air dried material in 100 ml distilled water for 24 h at 25 ± 5°C. It was filtered through Whatman filter paper no.1 and the volume of filtrate made to 100 ml [8]. Different dilutions of the extracts *i.e.* 2.5%, 1% and 0.5% were prepared from stock solution (5%, w/v). Healthy and viable seeds of *E. crus-galli* were soaked in distilled water for 24 h. Each Petri dish (9 cm diameter) was lined with Whatman filter paper No. 1 and made wet by 6 ml of respective extract concentration or distilled water in case of control. Ten imbibed seeds of *E. crus-galli* were placed equidistantly on filter paper and the Petri dishes were covered with glass covering. They were incubated for seven days at standard conditions of light and dark (16/8 h) and relative humidity 85% at 30°C. On eighth day, observations on germination, seedling length and dry weight accumulation were taken. The whole set of experiment was repeated thrice.All parameters were expressed as mean values and analysed statistically through one-way Analysis of Variance (ANOVA) followed by separation of treatment means from the control at $p < 0.05$.

2.3 Sample Preparation & Spectroscopic Analysis

FTIR analysis was performed using Perkin Elmer spectrophotometer system which was used to detect the characteristic peaks and their functional groups. The powdered samples of leaf, flower, stem and root tissues of *C. occidentalis* were mixed with dried KBr and prepared as pellets, scanned at room temperature 25±2ºC at 4000 – 400 cm^{-1} spectral range. Special care was taken to prepare pellets of same thickness applying same pressure using same amount of sample. Therefore, in present study it was possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups [9].

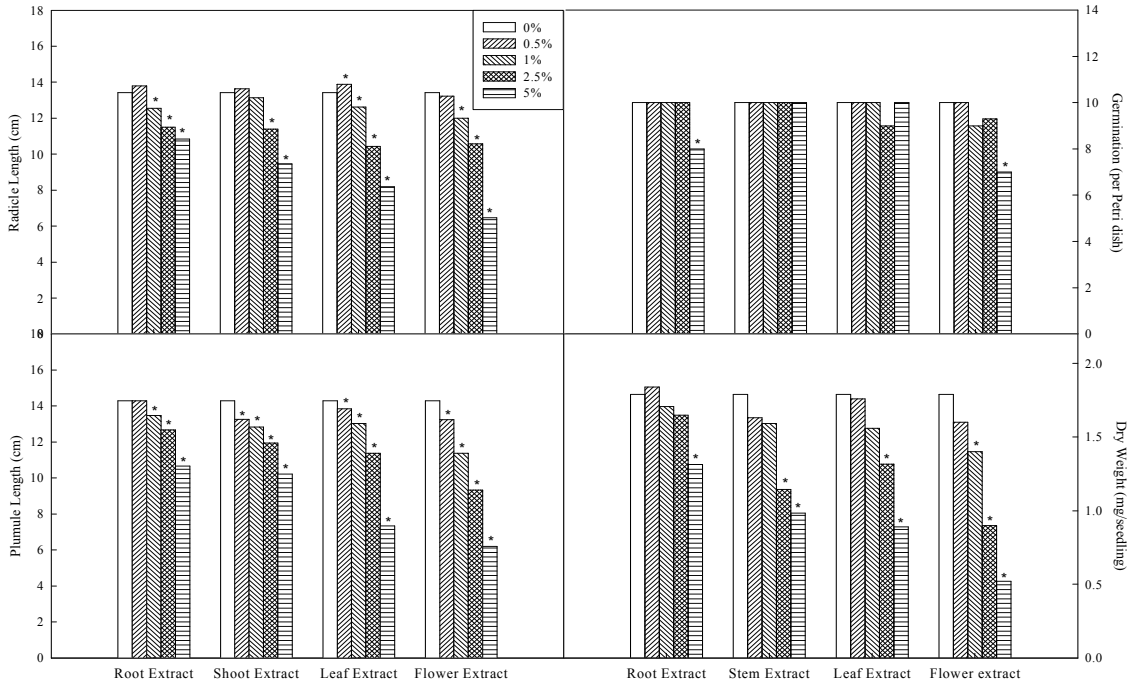
RESULTS AND DISCUSSION

All tissue extracts inhibited the growth of *E. crus-galli* under lab conditions. However, the reduction was maximum with flower extract followed by leaf extract (Fig. 1). Germination of seeds was not affected much, but radicle length and plumule lengths decreased to 6.47 ± 0.31 and 6.2 ± 0.25 cm at 5% (w/v) concentration of flower extract as compared to 13.423 ± 0.45 and 14.29 ± 0.53 cm in control, respectively. Dry weight reduction was quite significant with both flower and leaf extracts with least reduction in case of root extracts (Fig. 1). Allopathic influence of *C. occidentalis* on other plants has been reported in previous studies also [4, 5].

The FTIR spectra of different tissues of *C. occidentalis* revealed presence of various characteristic functional groups (Fig. 2). The absorption bands and major peaks in the spectra have been listed in Table 1. Peaks in the region of 500-600 cm^{-1} were attributed to C–Br stretching in acyclic and aromatic compounds [10]. Weak absorption bands in region 600-900 cm^{-1} were attributed to CH out of plane bending vibrations [m]. Sharp peak at 892 cm^{-1} in all tissues corresponded to C–C, C–O stretching in deoxyribose. Medium and sharp bands in the region of 900-1300 cm^{-1} were attributed to phosphodiester region. In flower, stem and leaf tissues, weak band at 940 cm^{-1} indicated presence of carotenoids in these tissues.

Root and stem tissue FTIR spectra indicated strong peaks at wavelength range 1000-50 cm^{-1} corresponding to ring stretching vibrations mixed strongly with CH in-plane bending. The bands at 1000-200 cm^{-1} corresponding to C–OH bonds in all samples indicated presence of oligosaccharides such as mannose and galactose. Medium band at 1035 cm^{-1} in root tissue can be attributed to skeletal *trans* conformation of DNA. Medium sharp peaks at 1052 cm^{-1} in flower tissue may be attributed to phosphate I band in DNA. A sharp peak at 1077.7 cm^{-1} in leaf tissues corresponded to C–OH stretching band of oligosaccharide residues. Weak band in the region of 1180-300 cm^{-1} were attributed to amide III band region in all tissues indicating presence of abundant proteins in all. Weak bands at 1237.2 cm^{-1} in flower tissue, 1245.9 cm^{-1} in stem tissue, 1248.1 cm^{-1} in leaf tissue and 1262.7 cm^{-1} in root tissue may be due to asymmetric PO_2^-stretching of phosphate I. Sharp peak at 1419 cm^{-1} in all tissues may be attributed to presence of abundant polysaccharides and pectin in all plant tissues. Similarly common band at 1456 cm^{-1} can be due to CH_3 bending vibrations indicating presence of lipids and proteins. A sharp peak at 1637 cm^{-1} appeared due to C=C uracyl and C=O stretching vibrations in all tissues. Sharp absorption peaks in the region of 1700-800 cm^{-1} in flower, stem and root regions were attributed to fatty acid esters. Two medium peaks in the region of 2250-700 cm^{-1} corresponded to N-H stretching in amine salts [11].

Fig. 1: Effect of aqueous extracts of different parts of *C. occidentalis* on growth of *E. crus-galli*

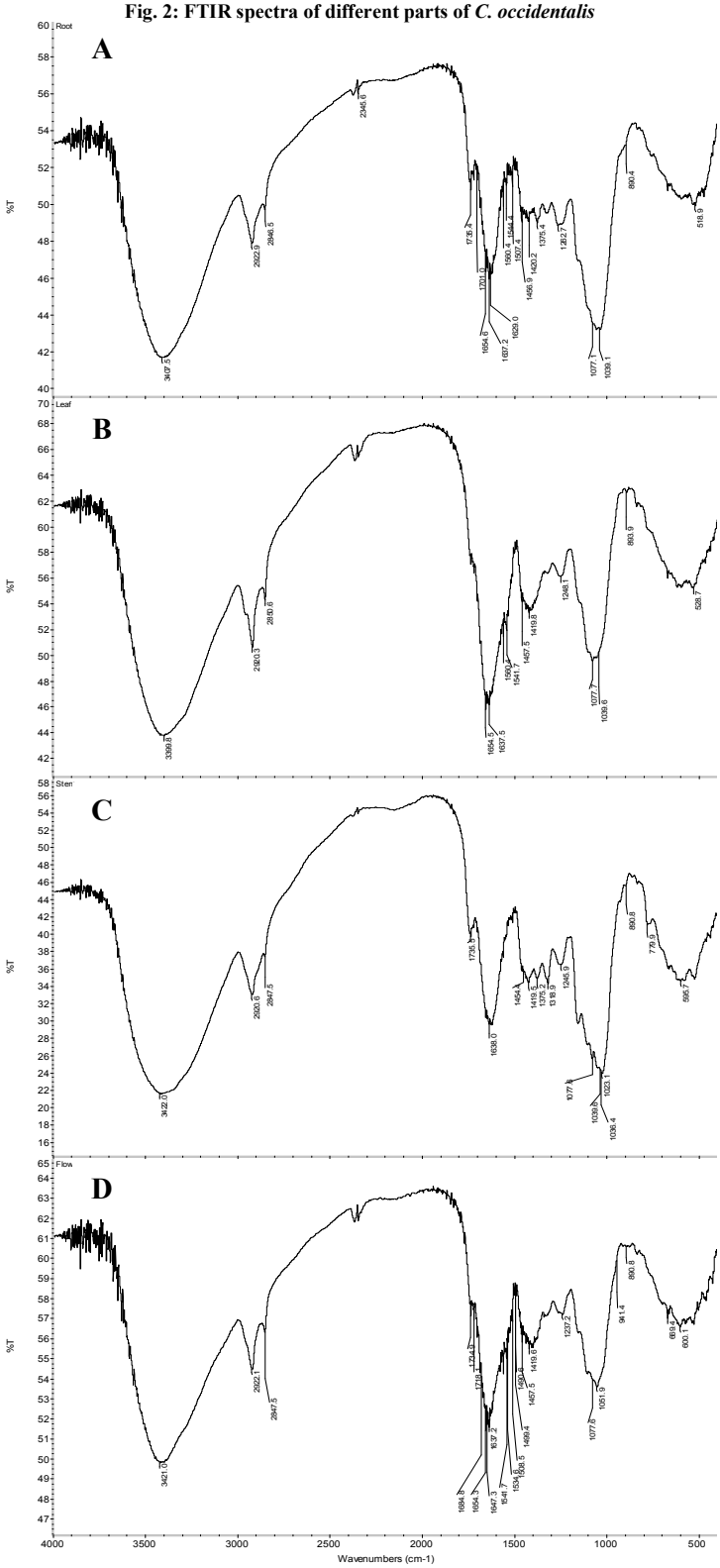


Concentrations of different extracts (0, 0.5, 1, 2.5 & 5%) are expressed as w/v; Values are means;
* indicate significant difference from controls at P < 0.05, applying Dunnett's test

Table 1.FTIR peak values of different tissues of *Cassia occidentalis*

SN.	Peak	Assignment
1	600-900 cm^{-1}	CH out-of-plane bending vibrations
2	892 cm^{-1}	C–C, C–O deoxyribose
3	900-1300 cm^{-1}	Phosphodiester region
4	940 cm^{-1}	Carotenoids
5	1000-140 cm^{-1}	Protein amide I absorption
6	1000-1050 cm^{-1}	Ring stretching vibrations mixed strongly with CH in-plane bending
7	1000-200 cm^{-1}	C–OH bonds in oligosaccharides such as mannose and galactose
8	1035 cm^{-1}	Skeletal trans conformation (CC) of DNA
9	1039/40 cm^{-1}	Stretching C–O Ribose
10	1052 cm^{-1}	Phosphate I band for two different C–O vibrations of deoxyribose in DNA
11	1078 cm^{-1}	Phosphate I in RNA, Symmetric phosphate, C–OH stretching band of oligosaccharide residues
12	1237/38, 1245-1248, 1262 cm^{-1}	Stretching PO_2^- asymmetric (phosphate I)
14	1220-350 cm^{-1}	Amide III
15	1419 cm^{-1}	ν_s (COO^-) polysaccharides, pectin
16	1456 cm^{-1}	CH_3 bending vibration (lipids & proteins)
17	1480-600 cm^{-1}	Amide II band in tissue proteins
18	1600-720 cm^{-1}	Amide I band of tissues protein
19	1637 cm^{-1}	C=C uracyl, C=O
20	1700-15 cm^{-1}	The region of the bases
21	1700-800 cm^{-1}	Fatty acid esters
22	2250-2700 cm^{-1}	N–H stretching in amine salts
23	2800-3000 cm^{-1}	C–H lipid region
24	2922 cm^{-1}	Asymmetric stretching vibration of CH_2 of acyl chains (lipids)
25	3200-550 cm^{-1}	Symmetric asymmetric vibrations attributed to water

Fig. 2: FTIR spectra of different parts of *C. occidentalis*



A: Root; B: Stem; C: Leaf & D: Flower tissues

Two medium peaks in the region of 2800-3000 cm^{-1} in all samples corresponded to C–H stretching vibrations in lipid and proteins.

Asymmetric stretching vibrations of CH_2 of acyl chains at 2922.9, 2922.1, 2920.6 and 2920.3 cm^{-1} in root, flower, stem and leaf tissues, respectively, indicated presence of lipids. Broad absorption peaks at 3422.0, 3399.8, 3407.5 and 3421.0 cm^{-1} in stem, leaf, root and flower tissues, respectively were stretching O–H vibrations attributed to water [12]. The FTIR spectra of flower and leaf samples showed similar variation with more bands in the region of 1500-1700 cm^{-1} corresponding to aromatic C=C bending.

CONCLUSION

The coherence of phytochemical distribution in FTIR spectra of different *C. occidentalis* tissues and allopathic study conducted on *E. crus-galli* do indicate richest phytochemistry of flower tissues among all. All samples showed presence of carbohydrates, lipids, proteins, carotenoids and nucleic acids. However, the biomolecular concentration was different in different parts of *C. occidentalis*. The results of growth study were further corroborated by FTIR data.

This study confirmed that FTIR spectroscopy can be used for reliable discrimination between different parts of a plant species based on biomolecular profiling.

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