Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Suppl 2, 2013 ISSN - 0974-2441

Research Article

STUDIES ON SOME PRIMARY METABOLITE'S EXTRACTION AND QUANTIFICATION IN DIFFERENT PLANT PARTS OF SELECTED *CASSIA* SPECIES

B. L. SHARMA[1],DAULAT SINGH[1],SANTOSH K. SHARMA[2*],AFZAL HASHMI[3],ARJUN SINGH[4],ANIL BANSAL[2]

[1]Department of Life Sciences, Biyani Girls College, Jaipur Rajasthan,[2*]Corresponding Author:Department of Botany, University of Rajasthan, Jaipur.[3]Central Council for Research in Unani Medicine, New Delhi-58,[4]Central Council for Research in Ayurvedic Sciences, New Delhi-58. ***Received: 28 March 2013, Revised and Accepted: 11 April 2013***

ABSTRACT

During the present investigation , comparative study of primary metabolites i.e. Carbohyrates, Protein, Ascorbic acid, Starch, Lipids, Nucleic acids, Chlorophylls and Caretenoids, of genus Cassia were determined. The three species *Cassia pumila*, *C. nodosa* and *C .renigera* were undertaken for study and all the parts of respective plants were analyzed for their chemical composition. It was found that the higher level of starch, lipids, chlorophylls and nucleic acid was observed in *Cassia nodosa* leaves and phenols contents was higher in leaves of *C. nodosa* as well as in pods of *C. pumila.* The protein and lipid were found to be higher in pods of *C. renigera.*

Keywords: Cassia pumila, C.nodosa, C.renigera, primary metabolites.

INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial.The plants provided food, clothing, shelter and medicine. Recent estimates suggest that over 9,000 plants have known medicinal applications in all cultures of various countries and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth, 1998).The potential of these plants depends upon the presence of phytochemicals inside those may be primary metabolites or secondary metabolites those are secreted by plants during life.

In the present study, the primary metabolites Carbohyrates, Protein, Ascorbic acid, Starch, Lipids, Nucleic acids,Chlorophylls and Caretenoids, of genus Cassia were studied. Primary metabolite are those organic substances which are synthesis during photosynthesis and these organic compound is essential for plant life, growth and development. Out of the known species of the genus Cassia, any systematic study on the primary metabolites viz. starch, ascorbic acid, proteins, lipids, nucleic acids, chlorophylls, carotenoids and phenols was not carried out in details so far (Sayeed et al., 1999). The genus Cassia has 540 species of trees, shrubs, vines and herbs with numerous species growing in the tropical and sub tropical part of world. Many species have been used medicinally and these tropical plant have a rich history in natural medicine. Indigenous to Brazil, it is also found in warmer climates and tropical area of South and North America (Pari and Latha, 2002). It is in the same genus as Senna and in same times called "Coffee Senna' since its seeds, found in long seed pods are sometimes roasted and made into a coffee-like beverage (Soukup, 1970). The three species Cassia pumila, C.nodosa and C.renigera were undertaken for study. As their name implies carbohydrates are composed of the elements of water and carbon so their formula approximates to a multiple of CH_2O. Most of the dry weight of plants is carbohydrate of one kind or another. All carbohydrates are polar and the low molecular forms are what we commonly know as sugars(Sambaiah and Lokesh 1998). Starch is a substance that plants use to store energy. It is the end product of photosynthesis and can be stored for later use in seeds, tubers, and roots. Chemically, starch is a polysaccharide comprised of glucose molecules linked together in long chains (Barthakur et al., 1995).Ascorbic acid is a major metabolite in plants. It is an antioxidant and in association with other components of the antioxidant system, protects plants against oxidative damage resulting from aerobic metabolism, photosynthesis and a range of pollutants(Lange, 2002).Lipids are fatty substances with long hydrocarbon chains and often ester linkages somewhere in the molecule. There are three classes of lipids in plants. The simplest are the triglycerides or fats in which three fatty acids are attached to a glycerol molecule by ester bonds. These are the most energy-rich form of food reserve (for plants and for us). Plants tend to accumulate fats only when it is important to pack a lot of energy into a small space, such as a seed (Govaerts, 2001).Proteins are also derived partly from carbohydrates through the formation of amino acids. These latter simple compounds are then combined with nitrates from the soil and other substances to form the highly complex protein molecule. The main characteristic of proteins is their high nitrogen content (Fairbairn and Shrestha, 1967). Nucleic acid are another group of nitrogen containing compounds with special functions in the cell. They are mostly based on five organic bases. Two of these, thymine and uracil (U) differ only in the presence or absence of one methyl group. These are often combined with ribose (a pentose sugar) and one or more phosphate groups(Kashiwada et. al., 1996).Chlorophylls are greenish pigments which contain a porphyrin ring. This is a stable ring-shaped molecule around which electrons are free to migrate. There are several kinds of chlorophyll, the most important being chlorophyll "a" i.e. universal is occurrence. This is the molecule which makes photosynthesis possible. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in other higher plants except fungi. A third form of chlorophyll which is common is (not surprisingly) called chlorophyll "c", d – red and e – yellow green and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. Carotenoids are usually red, orange, or yellow pigments and include the familiar compound carotene(Kumar et al., 1966).

MATERIAL METHOD

Extraction Procedure

The plant material has been collected from Jaygragh fort of Ajmer,and Garganesh temple of Jaipur, Rajasthan, India. The plant was identified at Herbarium, Department of Botany, University of Rajasthan, Jaipur. 50 gm each of the experimental materials were soxhlet extracted successively with petroleum ether (60°-80°), benzine, chloroform, acetone, alcohol and water 24-36 h. Each of the resultant extract was filtered , dried in vacuo and weighed to calculate the extractive value on dry weight basis. Latter, following the established protocols (Paech and Tracey, 1955) each of the test sample was processed further to used to evaluate the presence of carbohydrates, proteins, tannins and flavonoids. Before doing so, each test sample was reconstituted in the respective solvent .

CARBOHYDRATES

Extraction Procedure

A. Total soluble sugars

Each of the dried and powdered test sample (50 gm) was macerated in a mortar and pestal with 20 ml of 80% ethanol and left overnight. Each of the homogenates was centrifuged (1200 rpm, 15 min), the supernatants were removed and concentrated on a water bath. Later each resultant concentrate was raised to 50 ml with distilled water (Ext. I) and processed further following the method of Loomis and Shull (1937) for total soluble sugars.

B. Starch

For starch, the residual pellet obtained out of the above process in each case was suspended in 5 ml of 52% perchloric acid and 6.5 ml of distilled water, shaken vigorously (5 min) and then centrifuged (2500 rpm; McCready et al., 1950). This step was repeated thrice and the supernatant of each sample were pooled together and the volume was raised to 100 ml with distilled water (Ext. II). Out of this, 1 ml aliquot was measured separately to estimate starch quantitatively.

Quantification

Aliquots (1 ml) of each test simple were used to estimate quantitatively the total levels of carbohydrates following the protocol of Dubois et. al., (1951) using phenol-sulphuric acid reagent, which included the preparation of a regression curve for the standard glucose.

A stock solution of glucose (100 mg/ml) was prepared in distilled water, out of which 0.1 to 0.8 ml were separately pipetted into the test tubes and the volume of each was raised to 1 ml with distilled water. Each of these were kept in an ice-chest, 1 ml of 5% aqueous phenol was added and shaken gently. Later, 5 ml of conc. H_2SO_4 was added rapidly, accompanied with gentle agitation during the addition of the acid. These were allowed to stand in a water bath at 26-30°C for 20 min before taking the optical densities (ODs) of the yellow-orange colours thus developed at 490 nm in a spectrophotometer after setting for 100% transmission against the blank (which was prepared by substituting distilled water for the sugar solution). Five replicates in each were run and their mean values were calculated. A regression curve was computed between its known concentration and the respective OD, which followed the Beer's Law.

The concentration values of the total soluble sugar in the test samples was directly worked out from the regression curve of the standard glucose. Five replicates of each experimental sample were taken and their mean values were recorded. The sugar contents in terms of glucose equivalent and the use of conversion factor 0.9 to convert the values of glucose to starch was made in each case.

ASCORBIC ACID

Extraction procedure

Each of the fresh experiment materials (400 mg) was homogenize thoroughly with 10 ml of acetate buffer (pH 4.8) and centrifuged (1200 rpm, 20 min.). The supernatants were separately collected, out of which 1 ml was measured to other test tube, 4 ml of 4% trichloroacetic acid (TCA) was added, left overnight and later, centrifuged (Roe and Kuenthar, 1943). To the supernatant of each sample, 1 ml of the colour reagent (prepared by mixing 90 ml of 2.2%, 2,4-dinitrophenylhydrazine in 10N H_2SO_4, 5 ml of 5% thiourea and 5 ml of 0.6% CuSO4 solution), was added and incubated at 57% for 45 min. Later, on cooling 7 ml of 65% H_2SO_4 was added to each mixture and cooled again.

Quantification

From the stock solution of ascorbic acid (10 mg/100 ml in 4% TCA), varied concentrations (0.01 to 0.09 mg/ml) were prepared in different test tube. The volume of each was raised to 5 ml by adding 4% TCA solution and left overnight at the room temperature. To these, 1 ml of the colour. Later, to each of these, 7 ml of 65% H2SO4 was added, brought to the room temperature and the ODs were measured at 540 nm in a spectrophotometer against a blank. A regression curve was computed between the main optical density and the concentration of standard ascorbic acid, which followed Beer's Law.

LIPIDS

Extraction and Quantification

Each of the dried and powdered test samples (1 g) was homogenized using a mortar and pestle with 10 ml distilled water (Jayaraman, 1958). The resulted pulp was transferred to a conical flask (250 ml), 30 ml of chloroform-.methanol (2: 1, v /v) mixture was added and later, mixed thoroughly. The above mixture was kept overnight at room temperature; and each 20 ml of chloroform and distilled water was added. On centrifugation, three layers- a clear lower of coloured layer chloroform containing all the lipids, a coloured aqueous layer of methanol with all soluble materials and a thick pasty interphase were observed. The methanol layer was discarded and the lower layer was collected. This organic layer in case was taken in pre-weighed beakers and concentrated carefully. On complete evaporation, the weight was determined again which was taken as the weight of total lipids/g of the dried plant material (s).

NUCLEIC ACIDS

Extraction Procedure

Each of the dried and powdered experimental materials (100 mg) was suspended in 10 m1 of 95% ethanol and left for' over-night. The mixture was centrifuged at 4°C and the supernatant (s) was removed (Ogur and Rosen, 1950). To each of the pellet, a mixture of ethanol and ether (1: 3, v/v) was added, heated to 60°C (10 min) cooled and centrifuged. The resulting supernatants were again and this extraction step was repeated in the same order thrice so as to ensure complete extraction of interfering compounds was chilled to 0°C for 2h, 5 ml of 0.2 m perchloric acid was drained off at once. To this, 5ml of 1 M perchloric acid was added again left overnight at 0°C and the extract was saved (R_1). To these residues 0.5 ml of 1 M perchloric added in each case left at 0°C for 4 h, centrifuged again extract removed (R_2). Both the extracts (R_1 + R_2) were taken together and the volume was raised to 100 ml with 1M perchloric acid (constituted RNA fraction).

For the extraction of DNA, to the residual tissue left after processing, 5 ml of 0.5 M perchloric acid was added in each case and heated at 70°C for 20 min, cooled, centrifuged and extract was saved (D_1). This step was repeated again and the supernatant (D_2) was removed. Both the extracts (D_1 + D_2) of each of the test sample(s) were pooled together and the volume or each was raised to 100 ml with 0.5 M perchloric acid (constituted the DNA fraction).

Quantification

Using the method of Jensen (1956) the individual levels of nucleic acids (RNA/DNA) were quantitatively determined, where the regression curves of the standard RNA or DNA (Sigma Chemicals Co.) were prepared by dissolving 1 mg each separately in 1 m1 of 0.04 M Na_2CO_3 solution. Later, these were neutralized with 0.1 HCl and diluted with distilled water to prepare stock solutions (50 g/ml). From this 1 to 9 ml of both the nucleic acids were transferred to separate test tubes and the volume of each 01 was raised 10 m1 with 0.5 M perchloric acid in DNA and with 1 M perchloric acid in RNA as stock solutions. The ODs were recorded at 260 nm. in a UV spectrophotometer against the respective blank(s). Three replicates were examined and the average values were plotted against their respective concentrations to compute regression curves for DNA and RNA separately which followed the Beer's Law.

Similarly, the ODs of the experimental test extract were recorded as described above and the concentration of the nucleic acids (RNA/DNA) was calculated by referring the ODs of the unknown the known regression curve. Five replicates were in each case and their mean values were recorded.

PROTEINS

Extraction Procedure

Each of the dried test samples (60 mg) was measured in 10 ml of cold 10% TCA solution (30 min), kept at 4°C overnight and centrifuged. The supernatants were discarded in each case and the resultant pellet of each was re-suspended in 10 ml of 5% TCA solution and heated at 80°C in a water bath for 30 min. These samples were cooled, re-centrifuged and the supernatant so obtained were discarded each time. The pellet was then washed with distilled water and centrifuged. Each of the residues left after the centrifugation was dissolved in 10 ml of 1 N NaOH and left overnight at room temperature (Osborne, 1962).

Quantification

Using 1 ml aliquot of extract, total phenol contents were estimated following the method of Lowry et. al., (1951).

A stock solution of Bovine serum albumin (BSA; Sigma chemical Co., St. Louis, USA) was prepared in 1N NaOH (1 mg/ml), out of which 0.1 to 0.8 ml of the solution was separately pipetted in the test tubes and the volume in each case was raised to 1 ml by adding distilled water. To each, 5 ml of the alkaline solution (prepared freshly by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4.5H_2O$ in sodium potassium tartarate) was added and kept at room temperature (10 ml). Later, to each of these tubes 0.5 ml of Folin-Ciocalteau reagent (CSIR Centre for Biochemicals, Delhi, India; diluted with equal volume of distilled water, just before use) was added rapidly with immediate mixing and after 30 min, the ODs were measured at 750 nm of using a spectrophotometer against the appropriate blank, Five replicates of each concentration were taken and their mean values plotted against their respective concentration to compute a regression curve.

All the test samples were similarly processed as above and the level of total proteins individually was calculated by referring the ODs of the test sample with the standard curve (BSA). Five replicates were examined in each case and the mean values were recorded.

PHENOLS

Extraction Procedure

Each of the deproteinized test samples (200 mg) was homogenized in 10 ml of 80% ethanol for 2 hrs. and left overnight at the room temperature. Each of these was centrifuged and the supernatant was collected separately, the volume of which was raised 40 ml with 80% ethanol in each case

Quantification

Total phenols were quantified in each test samples, following the protocol of Bray and Thorpe, (1954) which included the preparation of a regression curve of standard phenol (Caffeic acid).A stock solution of the standard phenol (Caffeic acid) was prepared in 80% ethanol (400 mg/ml) out of which 0.1 to 0.9 ml was taken into separate test tubes and the volume of each was raised to 1 ml with 80% ethanol, To each tube, 1 ml Folin-Ciocalteau reagent (diluted with distilled Water in 1:2 ratio, just before use) was added followed by 2 ml of 20% Na_2CO_3 solution and this mixture was shaken vigorously. Such samples were placed in a boiling water bath for exactly 1 min and later, cooled under 3 times running tap water. Each of the reaction mixture was diluted to 25 ml with distilled water and ODs were taken at 750 nm against a blank using a spectrophotometer. Five replicates were taken for each concentration and the average OD was plotted against the respective concentration to compute a regression curve which followed the Beer's Law. Similarly various experimental plant samples were processed and the ODs were measured. From the mean values, total levels of phenols were calculated (with reference to caffeic acid) by referring the ODs experimental samples with the standard regression curve.

PHOTOSYNTHETIC PIGMENTS

(CHLOROPHYLLS AND CAROTENOIDS)

Extraction Procedure

The fresh experimental materials (1 g each) were homogenized in 40 ml of 80% acetone, to which a pinch of $NaHCO_3$ was added to prevent any pheophytin formation (Sunderland, 1966). This extraction was carried out in the dim light conditions to avoid any photobleaching (Holden, 1976). Each sample was centrifuged and the supernatant was collected separately. For the complete extraction of pigments, this step was repeated thrice and the supernatants of each were pooled separately. For the volume of which was raised to 80% acetone individually.

Quantification

The ODs of each of the, above extracts were recorded at 652, 663 and 480 nm with a spectrophotometer against 80% acetone as the blank. Five such replicates at each wavelength were chlorophyll a band the total present in the samples were calculated in mg/g of plant material from the equations derived by Arnon, (1949) as follows -

$$\text{Chlorophyll a} = \frac{11.3A_{663} - 0.96A_{645}}{\alpha \times 1000 \times w} \times V$$

$$\text{Chlorophyll b} = \frac{18.3A_{645} - 3.9A_{663}}{\alpha \times 1000 \times w}$$

$$\text{Total Chlorophyll} = \frac{A_{652} \times V}{34.5 \times w}$$

(Where, A = Absorbance; V = Volume of each extract; w = weight of the plant material used; α = the length of light path in the cell, which is usually 1 cm).

Similarly, the level of total carotenoids (mg/g) was calculated using the equation given by Kirk and Allen (1965), where E is determined as follows "

$$\Delta E\ Car_{480} = [\Delta E_{480} + (0.114 \cdot \Delta E_{663})] - (0.638 \Delta E_{645})$$

($\Delta E\ Car_{480}$ = increase in absorbance at 480 nm due to carotenoids; ΔE_{480} = extinction at 480 nm; ΔE_{645} = extinction at 645 nm; ΔE_{663} = extinction at 663 nm).

RESULT AND DISCUSSION

Biologically active compounds contain a remarkably diverse assay of organic compounds and the carbohydrates are not only the first formed organic compounds in the plants as a result of photosynthesis but, also a major source of energy. Not only this, all the biochemical compounds are directly/indirectly derived from them for the important framework or they also modify the physico-chemical characters of other groups of compounds by combining with them. In the present study, higher levels of starch was observed in stem (C. nodosa; 10.5 mg/gdw > C. renigera; 10.2 mg/gdw > C. pumila; 9.04 mg/gdw) and lowest concentration level was measured in flowers (C. pumila; 2.86 mg/gdw < C. nodosa; 3.48 mg/gdw < C. renigera; 3.76 mg/gdw). However rich concentration of starch was present in stem comparatively than other plant parts. Similarly higher concentration of soluble sugars were observed in pods (C. nodosa; 10.30 mg/gdw > C. renigera; 10.27 mg/gdw > C. pumila; 8.39 mg/gdw) and minimum level was observed in stem (C. pumila; 2.03 mg/gdw < C. renigera; 2.07 mg/gdw < C. nodosa; 2.08 mg/gdw), overall higher concentration of total soluble sugars was found in pods and leaves of selected Cassia species(Table 1). A glance to the review indicates that studies on the carbohydrates has centred on their isolation and characterization from C. tora, C. marylandica (Srivastava et al., 1982), C. javanica (Singh and Jindal, 1983), C. laevigata (Alam et al., 2008), C. occidentalis , C. absus (Kapadia and Fernandez, 1986) and C. suratlensis (Tona et al., 2004; Borrelli et al., 2005) but, no attempts have been made to assess the levels of carbohydrates quantitatively in the selected Cassia species.

Ascorbic acid (vitamin C), an essential dietary requirement in man, is widely distributed in the plants and due to its universal presence

in the actively metabolizing cells. Isherwood and Mapson (1962) have suggested the role of ascorbic acid in the plant growth and metabolism. Later, Chinoy (1984) also suggested the important role of ascorbic acid during the juvenile phase of growth of a plant .The results(Table-1) shows that higher concentrations of ascorbic acid was measured in leaves (C. renigera; 0.867 mg/gdw > C. nodosa; 0.764 mg/gdw) but in C. pumila it was found higher in flowers(0.390 mg/gdw) as compared to other plants parts with the minimum levels in roots(C. pumila; 0.039 mg/gdw < C. nodosa; 0.04 mg/gdw < C. renigera; 0.089mg/gdw) was measured.

In higher plants, generally the lipids constitute ~ 7% on dry weight basis. In the present study, in intact plants higher levels of lipids were estimated in pods (C. nodosa; 44.60 mg/gdw > C. renigera; 32.36 mg/gdw > C. pumila; 31.68 mg/gdw) and minimum levels was observed in stem of (C. nodosa; 0.307 mg/gdw > C. renigera; 3.48 mg/gdw C. pumila; 3.89 mg/gdw). As result shows that concentrations higher of lipids were observed in pods comparatively other plant parts of selected Cassia species(Table 1). Earlier, Zako et al. (1986) studied the seed oils (unsaturated fatty acids) of C. absus (4.8%), C. fistula (4.0%) and C. occidentalis (3.55%) by Von Rudolff's technique . Thus, higher levels of lipids in the selected plant species in the present investigation indicates their probable drought tolerance capacity and physiological characteristics under necessarily needed in the ecological adaptation to the semi-arid environment.

Proteins occur throughout the plant cells, both in extrinsic and intrinsic, simple and conjugated forms. In many plant species the exhibited bioactivity, viz. antiviral and others has been attribute to the proteinaceous substances present in their tissues. As a result studies on the quality and quantity of proteins has been undertaken in a large number of plants. In the present investigation, therefore, the selected plant species were assessed for the total protein content, where higher levels of proteins were observed in pods (C. renigera; 90.26 mg/gdw < C. nodosa; 86.74 mg/gdw < C. pumila; 76.46 mg/gdw) and low concentrations of proteins were observed in leaves (C. pumila; 13.33 mg/gdw < C. renigera; 15.00 mg/gdw < C. nodosa; 16.00 mg/gdw). Therefore, rich concentration of proteins were observed in pods comparatively other plant parts of selected species(Table-1). Earlier, protein as pentosan contents of the mucilage of C. grandis (Kapoor et al., 1980) and protein contents only in C. auriculata (10.4%), C. fistula (21.8%) C. gluca (24.1%), C. mimosides (29.9%) and C. spectabilis (31.1%) have been estimated. The results of present study shows that the higher percentage of proteins have been measured 55.55% in pods of C. renigera that is higher than the protein concentration observed previously in C. spectabilis (31.1%).

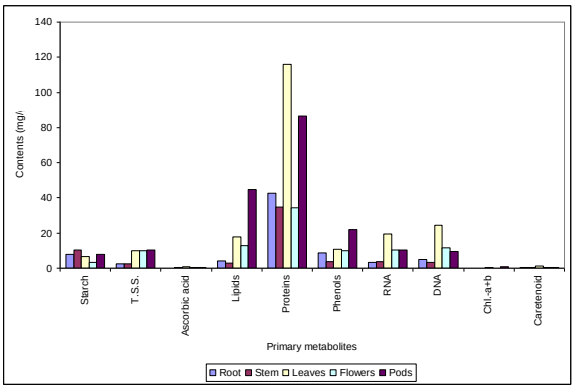
Phenols, exhibit important role in the regulation of plant growth and development. In the present study, phenols have been studied in three Cassia sp. In the intact plants, in general higher levels were recorded using various plant parts in leaves (C. nodosa; 22.00 mg/gdw > C. renigera; 20mg/gdw) but in C. pumila higher concentration of phenols was measured in pods(4.56 mg/gdw). The minimum levels of phenol was observed in stem (C. nodosa; 3.84 mg/gdw < C. renigera; 4.67 mg/gdw) but in C. pumila minimum concentration of phenol was recorded in roots (2.68 mg/gdw, Table 1). Relatively higher levels of phenolic compounds in the intact plants is perhaps to provide the chemical resistance (allelopathic effect) to invading microorganisms (Harborne, 1980).

Nucleic acids, being the key component of the biochemical processes of all the living organisms, have been estimated in the selected Cassia species, where result, shown in Table 1, These results indicates that generally the higher concentration of DNA is present in various plant parts than that of RNA. It was also observed that higher levels of RNA & DNA were found in the leaves of C. nodosa (DNA: 14.32 mg/gdw; RNA: 6.32 mg/gdw) and C. renigera (DNA: 10.64 mg/gdw; RNA: 4.60 mg/gdw) but in C. pumila higher levels of DNA and RNA were recorded in flowers (DNA: 10.78 mg/gdw; RNA: 4.48 mg/gdw). The minimum levels of DNA and RNN was observed in root of C. nodosa (DNA : 2.86 mg/gdw; RNA : 0.46 mg/gdw). Out of the selected Cassia species maximum concentration of RNA was found in the leaves of C. nodosa (6.62 mg/gdw) and maximum DNA concentration was also measured in the leaves of C. nodosa (14.32 mg/gdw, Table-1). A review of literature revealed that no such study on the DNA and RNA levels in Cassia species has been carried out earlier and hence, the present study is noteworthy.

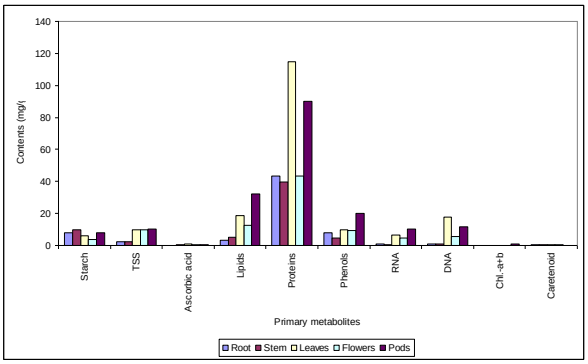
Chlorophylls are the main photosynthetic pigments in the green plants and hence, their determination is frequently required in most of plant analysis. In the present study, levels of total chlorophylls were found to be higher in leaves (C. pumila; 0.924 mg/gdw > C. renigera; 0.214 mg/gdw < C. nodosa; 0.211 mg/gdw), as compared to other plant parts but chlorophyll (a+b) completely absent in stem and root of C. nodosa and C. renigera. Similarly, carotenoids were also measured to be higher in leaves of (C. nodosa; 1.116 mg/gdw > C. pumila; 0.846 mg/gdw > C. renigera; 0.554 mg/gdw), with the minimum levels of caretenoid was present in root of (C. pumila; 0.30 mg/gdw < C. nodosa; 0.34 mg/gdw < C. renigera; 0.40 mg/gdw) as compared to other plant parts of slected Cassia species(Table 1). Earlier, (Zako et al., 1987) studied the change in the carotenoids and tocopherols during seed maturation of Cassia species but, no such estimations have been reported. Hence, the present study on the chorophylls and carotenoids is first of its kind in the selected Cassia species.

Table1 : Isolated primary metabolites contents (mg/gdw) from different plant parts

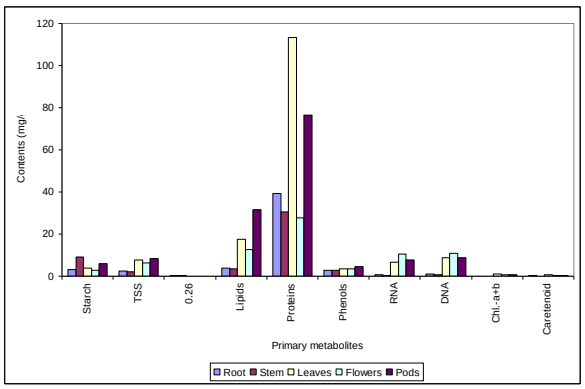
Plant species	***C. nodosa***					***C. renigera***					***C. pumila***				
Primary metabolites	**Root**	**Stem**	**Leaves**	**Flowers**	**Pods**	**Root**	**Stem**	**Leaves**	**Flowers**	**Pods**	**Root**	**Stem**	**Leaves**	**Flowers**	**Pods**
Starch	8.07	10.5	6.8	3.48	7.69	8.074	10.02	6.04	3.76	7.89	3.08	9.04	3.84	2.86	5.84
Total soluble sugar	2.45	2.48	9.76	9.86	10.3	2.45	2.47	9.76	9.73	10.27	3.34	2.03	7.86	6.48	8.39
Ascorbic acid	0.04	0.36	0.764	0.368	0.264	0.089	0.40	0.867	0.376	0.254	0.09	0.26	0.245	0.326	0.096
Lipids	4.06	3.07	17.67	12.68	44.6	3.48	4.96	18.69	12.76	32.36	3.89	3.48	17.67	12.68	31.68
Proteins	42.65	34.69	116.00	34.38	86.74	43.60	39.48	115.00	43.40	90.26	39.40	30.43	13.33	27.68	76.46
Phenols	8.64	3.84	10.69	9.86	22.00	7.84	4.67	9.87	9.27	20.00	2.68	2.74	3.67	3.64	4.56
RNA	0.46	0.78	6.32	5.36	1.49	0.79	0.49	4.60	2.60	3.34	2.74	1.46	6.60	4.48	2.06
DNA	2.86	3.20	14.32	11.46	9.46	1.89	1.76	10.64	5.48	11.78	2.89	2.78	8.67	10.78	4.08
Chl.-a+b	-	-	0.211	0.186	0.94	-	-	0.214	0.186	0.94	0.13	0.14	0.924	0.846	0.746
Caretenoid	0.34	0.48	1.116	0.56	0.346	0.40	0.47	0.554	0.430	0.101	0.30	0.146	0.846	0.246	0.301



Graphical presentation of Primary metabolites in different plant part of *C. nodosa*



Graphical presentation of Primary metabolites in different plant part of C. renigera



Graphical presentation of Primary metabolites in different plant part of *C. pumila*

CONCLUSION

During the present research work, all the parts of respective plants were analyzed for their chemical composition. It was found that the higher level of starch, lipids, chlorophylls and nucleic acid was observed in *Cassia nodosa* leaves and phenols contents was higher in leaves of *C. nodosa* as well as in pods of *C. pumila.* The protein and lipid were found to be higher in pods of *C. renigera.* The results revealed that the Cassia species are rich in primary metabolites and the quantities of these primary metabolites are differs with specific plant part.

REFERENCES

1. Alam M. T., M. M. Karim and S. N. Khan. Antibacterial Activity of Different Organic Extracts of *Achyranthes sspera* and *Cassia alata.* Phytother Res. 2008, 24(2):786-789
2. Arnon D. I. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. Plant. Physiol. 1949,24 : 1-5.
3. Barthakur N. N, N. P. Arnold and I. Alli. The Indian Labernum (*Cassia fistula* L.) fruit: an analysis of its chemical constituents. Plant Foods Human Nutr. 1995,47: 55-62.
4. Borrelli F., R. Capasso, G. Aviello, G. Di Carlo, A. A. Izzo, N. Mascolo and F. Capasso. *Senna* and the formation of aberrant crypt foci and tumors in rats treated with azoxymethane. Phytomedicine. 2005,2(6-7): 501-505
5. Bray, H. G. and W. V. Thorpe. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. 1954,1 : 27-52.
6. Chinoy J. J.. The role of ascorbic acid in growth, differentiation and metabolism of plants. Martinus Nijhoff, Dr. W. Junk Publishers, Hague. 1984, A Flavone from the Leaves of *Cassia Alata* 2006.
7. Dubois M., K. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith. A colorimetric method for the determination of sugar. Nature, 1951,168 : 167.
8. Farnsworth, N. R.. Biological and phytochemical plants. J. Pharm. Sci. 1966, 55 : 225-276
9. Fairbairn J. W. and A. B. Shrestha. The distribution of anthraquinone glycosides in *Cassia senna* L. Phytochemistry, 1967, 6: 1203-1207.
10. Govaerts R. How many species of seed plants are there? – Taxon, 2001,50: 1085–1090.
11. Harborne J. B. 1980. Plant phenolics. IN: Secondary Plant products. Bell, E.A. and B.V. Charlwood (eds.), Springer-Verlag. Berlin, pp. 320-402.
12. Holden M. Chlorophylls IN: Chemistry and Biochemistry of Plant Pigments. Good win, T. W . (ed.), Academic Press, London, 1976,pp. 462-488.
13. Isherwood F. A. and L. W. Mapson 1962. Ascorbic acid metabolism in plants. Part II. Biosynthesis. Ann. Rev. Plant Physiol. 13: 329-350.
14. Jayaraman J. Laboratory Manual in Biochemistry. Wiley Eastern Limited, New Delhi, 1958, pp. 96-97.
15. Jensen W. A. 1956. On the distribution of nucleic acids in the root tip of *Vicia faba*. Exp. Cell. Res. 10: 222-226.
16. Kapadia Z. and L. Fernandez. Isolation of rafinose from *Cassia absus* Linn. J. Chem. Soc. (Pak.) 1986, 8: 429-430.
17. Kapoor V. P., M. L. H. Farooqi and L. D. Kapoor 1980. Chemical investigation of seed mucilages from *Cassia* species. Indian Forest. 106 : 810-812.
18. Kashiwada Y., K. Toshika, R. Chen, G. Nonaka and I. Nishioka. Tannins and related compounds. XCIII. Occurrence of enantiomeric proanthocyanidins in the Leguminosae plants, *Cassia fistula* L.; *Cassia Javanica* L. Chem. Pharm. Bull. 1996, 38: 888–893.
19. Kirk J. T. O. and R. L. Allen. Dependance of chloroplast pigment synthesis on their protein synthesis. Effect of actidione. Biochem. Biophys. Res. Commun. 1965,21: 523-533.
20. Kumar A., C. S., Pande and R. K. Kaul. Chemical examination of *Cassia fistula* flowers. Indian J. Chem. 1966, 4: 460.
21. Lange D.The role of east and southeast Europe in the medicinal and aromatic plants' trade.– Medicinal Plant Conservation 2002,8: 14–18
22. Loomis, W.E. AND C.A. SHULL. 1937. Methods in Plant Physiology. McGraw- Hill, New York.
23. Lowry O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 1951,193: 265-275.
24. Mccready R. M., J. Guggoiz, V. Silviera and H. S. Owens. Determination of starch and amylase in vegetables. Anal. Chem. 1950, 22: 1156-1158.
25. Ogur M. and G. Rosen. The extraction and estimation of deoxypentose nucleic acid. Biochem. 1950,25 : 262-275.
26. Osborne D. J. 1962. Effect of kinetin on protein and nucleic acid metabolism in Xanthium leaves during senescence. Plant Physiol. 37 : 595-602.
27. Paech K. and M. V. Tracey. Modern Methods of Plant Analysis. 1955, Vol. III. Springer-Verlag, Berli.
28. Pari L. and M. Latha 2002. Antidiabetic Activity of *Cassia auriculata* Flowers: Effect on Lipid Peroxidation in Streptozotocin Diabetes Rats Pharmaceutical Biology. 40(7) : 512-517.
29. Roe, J. H. and C. A. Kuenther 1943. The determination of ascorbic acid in whole blood and urine through the 2,4-

dinitrophenylhydrazine derivative. J. Biol. Chem. 147: 399-407.

30. Sambaiah K. and B. R. Lokesh. Antioxidants and lipid oxidation in foods: A critical appraisal. J. Food Sci. & Technol. 1998,35: 283-298
31. Sayeed Abu M., M. Abbas Ali, G. R. M. Astaq Mohal Khan and M. S. Rahman. Studies on the characterization and glyceride composition of *Cassia fistula* seed oil. Bangladesh J. Sci. Indust. Res. 1999, 34: 144-148
32. Singh R. B. and V. K. Jindal. Galactomannan from *Cassia javanica* seeds - methylation a perioditic oxidation studies. Indian J. Chem. 1983, 22B : 934-935.
33. Soukup J. Vocabulary of the Common Names of the Peruvian Flora and Catalog of the Genera. Editorial Salesiano, Lima , 1970,436 pp
34. Srivastava H. C., S. U. Gupta and N. Misra. Structure of galactomannan from *Cassia marylandica* seeds. J. Sci. Res. Planta Med. 1982, 3 : 35-43.
35. Sunderland N. Pigmented plant tissues in culture. Auxins and pigmentation in chlorophyllous tissues. Ann. Bot. 1966,30 : 253-268.
36. Tona L., R. K. Cimanga, K. Mesia, C. T. Musuamba, T. De Bruyne, S. Apers, N. Hernans, S. Van Miert, L. Pieters, J. Totte and A. J. Vlietinck, *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J. Ethnopharmacol. 2004, 93(1): 27-32.
37. Zako S. M., W. Akht A. R., S. A. Khan and M. K. Bha Tty 1986. Characterization of *Cassia* seed oil. Proc. Pakistan Acad. Sci. 23 : 167-172.