ISSN 2394 - 7837

ARTICLE

Morphological and Biochemical Comparison of three species of Sida, *Sida cordifolia* L., *Sida acuta* Burm.F. and *Sida Rhombifolia* L.

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Received 12/04/2018 Accepted 25/06/2018

Abstract

Plant biochemistry is one of the rapidly expanding areas of plant science and seeks chemical characters to study and explain nature of plants or tissue of a cell. From its origin, till now this branch contributes a lot to its sister areas of science. Present study gathered information about the morphological and biochemical status of three species of Sida (Sida cordifolia, L.; Sidaacuta, Burm.f., and Sidarhombifolia, L.). The results were compared and analysed. On analysis, it was found that Sidacordifolia, L. contains more amount of proteins and lipids. Sidarhombifolia, L. showed least value in protein analysis. All the three species showed almost equal amount of carbohydrate content. To substantiate the biochemical study, analysis of photosynthetic pigments was also carried out which showed a high chlorophyll content in Sidaacuta, Burm.f. with a peak absorbance at 440 nm and 650 nm. Sidarhombifolia, L.showed least amount of chlorophyll a and Sida cordifolia, L. contains least chlorophyll b. These two plant species absorbs light on 420 nm and 650 nm at maximum rate. These variations in biochemical characters in the same taxa may be due to various reasons which can be revealed through further investigation using advanced analytical methods.

Keywords: *Sida cordifolia,* L.,*Sida acuta,* Burm.f, *Sida rhombifolia,* L.

Introduction

From time immemorial, man has a deep interest in different things that happening around as well as in side an organism. Years later, this interest leads to the opening of a scientific discipline known as "life sciences". Biochemistry, sometimes called biological chemistry, is the study of chemical processes in living organisms, including living matter. Biochemistry governs all living organisms and living processes. By controlling information flow through biochemical signaling and the flow of chemical energy through metabolism, biochemical processes give rise to the incredible complexity of life. Over the last 40 years biochemistry has become so successful at explaining living processes that now almost all areas of the life sciences from botany to medicine are engaged in biochemical research. Today the main focus of pure biochemistry is in understanding how biological molecules give rise to the processes that occur within living cells, which in turn relates greatly to the study and understanding of whole organisms.

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient with little or no side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs (Dewick, 1996), antimicrobial drugs (Phillipson, 1996), antihepatotoxic compounds (Ncubeet al., 2008). According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Mann, 1978).

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga, 2005;Mann, 1978). These compounds are synthesized by primary or rather secondary metabolism of living organisms. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999). Phytochemicals have been recognised as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world in the search for phytochemicals that may be benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region. The presence of phytochemical of interest may lead to its further isolation, purification and

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characterization. Then it can be used as the basis for a new pharmaceutical product.

Taking all these facts in consideration, a study was planned which includes the estimation and comparison of biochemical composition in three different species under the genus Sida. The group of biochemical compounds includes total carbohydrates, total proteins and total lipids. To substantiate the results, analysis of photosynthetic pigments were also planned. It is expected that the study bring forth results which is relevant and helps to know more about this particular scientific area.

Materials and Methods

Morphological Studies

The parts like root, stem, leaf, etc. of collected plants were separately taken and each of the above analysed for morphological characters like branching, phyllotaxy, venation, pubescency, floral features etc. Then the dissected parts were analysed under stereomicroscope – Leica EZ4D.

Biochemical Studies

The leaf, stem and roots of three species of Sidacordifolia, L;Sidaacuta, Burm.f.andSidarhombifolia, L.were collected. Surface sterilization of the specimen was done using 0.1% Sodium hypochloride. The selection procedure was kept constant for all the parameters.

Determination of total Carbohydrates

The amount of total carbohydrates present in leaves of three species of Sida - Sidacordifolia, L.;Sidaacuta, Burm.f.andSidarhombifolia, L.were estimated by Phenol Sulphuric acid method (Dubois et al, 1956). 1 gm of fresh tissue of the sample was taken and homogenized in 10 ml distilled water. The homogenate was filtered through a cheese cloth and was centrifuged at 10,000 g for 10 min. The supernatant was collected and the volume was made up to 10 ml. An aliquot was pipetted out and made up to 1 ml using distilled water. To this , 1 millilitre of 5% phenol was added and shaked vigorously. After 10 minutes of vigorous shaking, 5 millilitres of 96% sulphuric acid was added. The whole mixture was placed in a water bath of 30°C. After 30 minutes, the absorbance was read at 630 nm. The total Carbohydrate level was then calculated using the standard graph of glucose.

Estimation of total proteins

The amount of total proteins present in leaves of three species of Sida - Sidacordifolia, L.;Sidaacuta, Burm.f.andSidarhombifolia, L. were estimated following Lowry et al. (1951).1 gm of Fresh tissue of the sample was homogenized in 10 ml of 0.1 M phosphate buffer of pH 07 in a pre-chilled mortar and pestle. The homogenate was filtered and centrifuged at 10,000 g for 10 min. The supernatant was collected and the volume was made up to 10 mlwith Phosphate buffer. An aliquot was taken and made up 1 ml with phosphate buffer. 5 ml of the reagent C (2% Na2CO3 in 0.1 N NaOH and 5% CuSO4. 5 H2O in 1% Potassium Sodium Tartrate in 50:01 ratio) was added to the sample, mixed thoroughly and kept for 10 min. 0.5 ml of the Folin's reagent was added and kept for 30 min. The absorbance was recorded spectrometrically at 650 nm against a properblank. The protein content was calculated from the standard graph of BSA and presented in mg/gm tissue.

Estimation of total lipids

The amount of total proteins present in leaves of three species of Sida, Sidacordifolia, L.;Sidaacuta, Burm.f.andSidarhombifolia, L. were estimated by the method proposed by Bligh and Dyer (1959).A known amount of the sample was extracted with 15 ml of CHCl3 : Methanol (2:1 v/v) mixture. The extract was cooled and centrifuged for 10 min. at 2000 rpm. It was purified by transferring it into a separating funnel followed by the addition of CHCl3, distilled water and saturated NaCl. The lower layer separated was collected in a clean, pre-weighed petridish. The solvent was evaporated in a hot air oven at 60 C until a constant weight was obtained. The petridish with lipid was weighed. The amount of lipid was calculated and presented in percentage weight.

Estimation of chlorophyll

The total chlorophyll present in leaves of three species of Sida - Sidacordifolia, L.;Sidaacuta, Burm.f..andSidarhombifolia, L.were estimated using Arnon's formula (Arnon, 1949).2 gm of fresh leaf tissue was taken and homogenized in 16 ml of 80% acetone using a pre-chilled mortar and pestle. The homogenate was filtered and centrifuged at 5,000 g for 5 min. The supernatant was collected and made up to 100 ml using 80% acetone. The absorbance was read at 645 nm and 663 nm using spectrophotometer. The total chlorophyll comprising chlorophyll a and chlorophyll b were computed on mg/l basis using the following formula.

Total Chlorophyll	:	(D645 X 0.0202) + (D663 X 0.00802) X 1000 mg/l
Chlorophyll a	:	(D663 X 0.0127) + (D645 X 0.00269) X 1000 mg/l
Chlorophyll b	:	(D645 X 0.0229) + (D663 X 0.00468) X 1000 mg/l

Absorption spectrum of Chlorophyll

Analysis of chlorophyll pigment in leaves of the three species of Sida - Sidacordifolia, L.;Sidaacuta, Burm.f.andSidarhombifolia, L.were done spectrometrically by plotting absorption spectrum.2 gm of fresh mature leaves were taken and homogenized in 10 ml of 80% acetone using a pre-chilled mortar and pestle. The homogenate was filtered and centrifuged at 5,000 g for 5 min. The supernatant was collected and diluted by adding 5 ml of 80% acetone to 5 ml of the extract. The absorbance was read at different wavelengths ranging from 420 – 720 nm against the acetone blank solution using spectrophotometer. The

absorption spectrum of the chlorophyll extract was constructed computationaly by plotting the absorbance against wavelength.

Results and Discussion

Morphological Analysis

Sida cordifolia, L.:-

Commonly known as Country mallow is widely distributed along with other species are common throughout the tropical and sub-tropical plains all over India and Srilanka up to an altitude of 1050 m, growing wild along the roadside. Sidacordifolia is an erect perennial that reaches 50 to 200 cm (20 to 79 in) tall, with the entire plant covered with soft white felt-like hair that is responsible for one of its common names, "flannel weed". The stems are yellow-green, hairy, long, and slender. The yellow-green leaves are oblong-ovate, covered with hairs, and 3.5 to 7.5 cm (1.4 to 3.0 in) long by 2.5 to 6 cm (0.98 to 2.36 in) wide. The flowers are dark yellow, sometimes with a darker orange center, with a hairy 5-lobed calyx and 5-lobed corolla. The plant flowers from August to December and fruiting occurs from October to January.

Sida acuta, Burm.f.:-

Commonly known as wire weed. Species is aUndershrub, with mucilaginous juice with erect, cylindrical, branched, green and solid stem. Stem possess lanceolate to linear

Table 1. Biochemical composition of leaf, stem and root tissue (mg/gm) collected from three different species of Sida.

Plant Species	Part	Total Carbohydrates	Total Proteins	Total Lipids
Sida cordifolia, L.	Leaf	27.38	76.13	36.11
	Stem	21.71	52.87	34.12
	Root	12.34	32.39	35.87
Sida acuta, Burm.f.	Leaf	29.90	61.29	31.78
	Stem	23.33	44.44	18.32
	Root	18.19	37.16	19.88
Sida rhombifolia, L.	Leaf	31.54	35.16	34.84
	Stem	28.48	41.45	27.31
	Root	20.38	34.21	29.43

Table 2. Chlorophyll composition (mg/gm) in leaves collected from three species of Sida.

Plant Species	Total Chlorophyll	Chlorophyll a	Chlorophyll b
Sida cordifolia, L.	6.31	2.14	3.25
Sida acuta, Burm.f.	9.25	3.33	4.12
Sida rhombifolia, L.	6.82	1.75	3.72

leaves which are alternate, acute at apex and coarsely and remotely serrate leaves. Flowers are Small and axillary, seen in cymose clusters of 2 or 3. Pedicels jointed at the middle, epicalyx absent, complete, bisexual, regular, actinomorphic, hypogynus, pendamerous, and yellow. Calyx is 5-lobed and campanilate. Corolla is with 5 petals, polypetalous and slightly connate below. Stamens many and monadelphous with monothecous, extrorse and reniform anthers. Carpals five, syncarpous with petalocular superior ovary showing axile placentation. Style one and carpels correspond to the number of carpels. Fruit is a schizoczrp.

Sida rhombifolia, L.:-

Commonly called bala or atibala is a perenial or sometimes

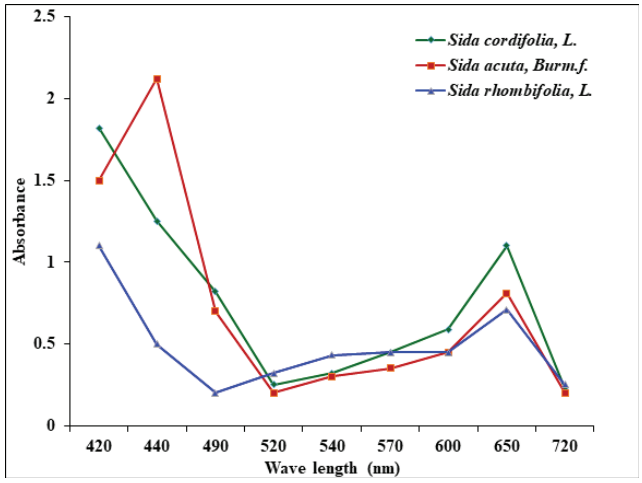

Figure 1- Absorption spectra of chlorophyll pigment in leaf tissue collected from three species of Sida.

annual plant in the family Malvaceae. The species is native to tropics and subtropics and usually confined to waste ground such as roadsides and rocky areas. Synonyms include Malvarhombifolia. It is used in ayurvedic medicine, where it is known as Kurumthotti.The stems are erect to sprawling and branch growing 50 to 120 cms in height, with the lower sections being woody. The dark green diamond shaped leaves are arranged alternatively along the stem, 4 to 8 cms long with short petiole. They are pale below, with short greyish hairs. The leaves have toothed or serrate margins. The petioles have small spring stipules at their bases. The moderately delicate flowers occurs singly on flower stalks that arise from the area between the stems and leaf petioles (axillary position). Flowers are pentamerous with creamy to orange – yellow petals and monadalphous androecium. Gynoecium is pentacarpellary with five capitate stigma.

Total Carbohydrates

Table 1 demonstrates the biochemical content of leaf tissue collected from three different species of Sida. The distribution of total carbohydrates showed a gradual decrease from Sidarhombifolia, L. to Sidacordifolia, L.through Sidaacuta, Burm.f.The carbohydrate content in leaves of Sidarhombifolia, Linn. was higher than that of other two, while in Sidacordifolia, L. it is comparitatively low. In Sidaacuta, Burm.f.,the carbohydrate content was recorded as 29.90 mg/gm tissue. This results points to the physiological efficiency of Sidarhombifolia, L.Biochemical importance of carbohydrates has been studied by several workers. In Apocynaceae 12 lacticiferous members of closely related taxa could be distinguished by their carbohydrate content (Marimuthu, 1986). Therefore the results of present study could be considered as a distinguishing character for comparing the three species of Sida.

Total Proteins

Analysis of total proteins in the leaf tissue of three species of Sida indicated that Sida cord folia had the higher content of proteins than other two species. The distribution of total proteins was demonstrated in table 1. A gradual reduction of total protein content was noticed from Sidacordifolia, L. Sidaacuta, Burm.f. Sidarhombifolia,L.Analysis of proteins from different taxa had provided a powerful tool for evolutionary and systematic study (King, 1977). Certain types of proteins are present in almost all photosynthetic plants. They show variations in different groups and the degree of similarity between these proteins is directly proportional to their genetic relationship. The protein content and the amino acid sequence had been elaborately used to elucidate the phylogeny and taxonomy of angiosperm families, such as Malvaceae, Ranunculaceae, Magnoliaceae, Polygalaceae and Solanaceae (Martin and Dowd, 1984; Martin et al., 1985).

Total Lipids

Table 1 shows lipid profile of all the three species of Sida. Lipid content shows a graded decrease from Sidacordifolia. L. to Sidaacuta, Burm.f.throughSidarhombifolia, L. Leaves of Sidarhombifolia, L. showed a highest lipid content of 36.11 mg/gm, where as that of Sidarhombifolia, L. and Sidaacuta, Burm.f.shows 34.84 mg/gm and 31.78 mg/gm of lipids respectively. The role of lipids in taxonomic studies was first studied by Hilditch in 1956. Handa and Vasedu (1956) analysed several genera of family Acanthaceae and grouped into four groups by studying fattyacid constitution in lipids. So in present study, the variations in total lipids could be considered as a distinguishing character for systematic study.

Analysis of photosynthetic pigments

To substantiate the morphological and biochemical studies, estimation of photosynthetic pigment, chlorophyll and its absorption spectra were carried out.Estimation of total chlorophyll pigment in three species of Sidashowed that total chlorophyll content was high in Sidaacuta, Burm.f. Oth-

er two species showed almost equal level of total chlorophyll content. The fractions of chlorophyll such as chlorophyll a and chlorophyll b also showed a varying distribution in all the three species. Both chlorophyll a and chlorophyll b were high in Sidaacuta, Burm.f.A sharp reduction in chlorophyll a was seen in Sidarhombifolia, L. In the case of chlorophyll b, both Sidacordifolia, L.andSidarhombifolia, L.showed almost equal level. The a/b ratio also high in Sidarhombifolia, L. and Sid cordifolia, L. and Sidaacuta, Burm.f.showeda ratio of 1.5 and 1.23 mg/gm respectively. The chlorophyll pigment plays a crucial role in the phenomenon of photosynthesis. The two fractions of chlorophyll such as chlorophyll a and chlorophyll b absorb radiant energy, which is utilized in the reduction of CO2. Chlorophyll a accounts for the absorption of red light (600 – 700 nm), while chlorophyll b accounts for the absorption of blue light (400 – 500 nm). Higher concentration of total chlorophyll and its fractions in Sidaacuta, Burm.f. shows its physiological efficiency, compared to Sidacordifolia, L.andSidarhombifolia, L.

Absorption Spectra

The chlorophyll pigments of the three plant species were analysed with the help of absorption spectra in the range of visible wavelength from 420 – 720 nm (Fig. 1). The pigment extract of Sidacordifolia, L. showed an absorption maximum of the blue region at the wave length of 420 nm with a narrow sholder at 490 nm. The peak absorbance at red region was represented the wave length 650 nm, with a broad base in between wave lengths 570 – 650 nm. In Sidaacuta, Burm.f.,the peak absorption was noticed at 650 nm and 440 nm in red and blue regions respectively. In Sidarhombifolia, L.the absorption was similar to that of Sidacordifolia, L. The increase in absorbance showed by Sidaacuta, Burm.f.andSidacordifolia, L. may be due to the elevated concentration of chlororophyll a pigment in both these plant species.Recent developments in the techniques of isolating and separating plant pigments, coupled by action and absorption spectra are proved to be useful tools in physiological research (Buchanan, 1980). In present study it was conformed that the rate of absorption increases to the increase in concentration of photosynthetic pigments.

The results of the present study gains significance predominance in biochemical status of the three species of Sida. The study on biochemical characteristics, yield a high contribution in solving phylogenic issues among plants. It helps to evaluate not only the therapeutical, ethnomedical and antimicrobial characters, but also contributes in analysing the biodiversity among plant species. So specific attention should be provided to this particular taxa which includes a detailed and elaborate investigations. This will help the present and future generations, to utilize these plants as an effective tool in many branched of botany and medicine.

Conclusion

Scientific world is always curious about the chemical properties of different cell types. This curiosity leads the emergence of Plant biochemistry which seeks chemical characters to study and explain nature of plants or tissue of a cell. Present study deals with the morphological and biochemical status of three species of Sida. The results were compared and analysed. From the study showed more protein and lipid content in Sidacordifolia, L.and Sidarhombifolia, L. showed least value. All the three species showed almost equal amount of carbohydrate content. Sidaacuta, Burm.f.contains high amount of chlorophyll pigments, On analysing the absorption spectra it is seen that Absorption spectrum of these two plant species absorbs light on 420 nm and 650 nm at maximum rate and Sidaacuta, Linn. show showed with a peak absorbance at 440 nm and 650 nm.These variations in biochemical characters in the same taxa may be due to various reasons which may include both internal and external factors that can be revealed through further investigation using advanced analytical methods.

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