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# Estimation of primary metabolites and enzymatic studies in withania

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## ABSTRACT

Biochemical studies of the individual plant parts is a necessary prerequisite in order to evaluate their importance in the over all metabolism of the plant. The use of traditional medicines and medicinal plants in most developing countries, is basis for the maintenance of good health. Laboratory evaluations were made to assess the phytochemical screening and quantification of primary metabolites in *Withania somnifera* and *Sida cordifolia*. It contains higher soluble sugars in leaves, starch in stem, lipid in stem, phenol in leaves as compared to other parts of the plant. Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears to be the fundamental mechanism underlying a number of human neurodegenerative disorders.

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## Introduction

Plants have been an important source of precursors and products used in a variety of industries, including those of pharmaceuticals, food, cosmetics and agrochemicals. The continuing search for new drugs has seen researchers looking towards the natural world for potential products. Medicinal plants are the most important source of life saving drugs for the majority of the world population (Swami Nathan, 2001). There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies .Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds. Plants have formed the basis of sophisticated traditional medicine systems among which are Ayurvedic, Unani, and Chinese. These systems of medicine have given rise to some important drugs which are still in use (Tiwari and Lindsay, 1995).

Withania somnifera L. (Dunal) is a very important shrub. It belongs to family Solanaceae and is classically known for its rejuvenative benefits. It has recently been referred to as Indian ginseng for its reputed restorative benefits. Withania somnifera L. (Dunal) is distributed in varied types of habitats (Chopra et al., 2006). It is commonly found in India, Pakistan, Afghanistan, Egypt, Spain, Congo, North, East and South Africa. In India, total cultivation is about 5000 hect. in Rajasthan and A.P. but it also is found in North Western parts i.e. Bombay, Gujarat, forest lands of Madhya Pradesh, Rajasthan, Himachal Pradesh, Andhra Pradesh, J & K (Kothari *et al.*, 2003). Roots are used to cure hiccups, cough dropsy rheumatism and male disorders, inflammation, ulcers and scabies in the form of external application.. The tribals especially Bheel and Garashia give root powder orally to the male patients of asthma and bronchitis (Singh and Pandey, 1998). Methanolic extract of the plant was found to reduce leucopenia induced by radiation (Kuttan, 1996). It has been in use for a very long time for all age groups and

both sexes and even during pregnancy without any side effects.. It is useful adjunct for patients undergoing radiation and chemotherapy. Recently *W. somnifera* L. was also used to inhibit the development of tolerance and dependence on chronic use of various phytotropic drugs (Gupta and Rana, 2007).

Sida cordifolia (L) belongs to family Malvaceae. It is an annual or biennial small shrub In this plant, most useful medicinal secondary metabolites are found in leaf, root and flowering buds (Florence, 2004). Sida cordifolia L is an extremely variable plant. It is a single genus, but note that it "may represent a species complex". Individual plants of this species vary greatly in height density of hairs, leaf size and shape and flower color and size.

Sida cordifolia L. is native of tropical America and subtropical region of both hemispheres. The plant is reported to have been introduced in Asia and Africa by the portuguese as medicinal plant species. The centre of origin of Sida cordifolia L. is the forest island (Florence, 2007). Other authors believed that this shrub plant may have originated from forest of the Shivalik hills. It grows commonly in India, Pakistan, America north and East tropical Asia. It is found in North western part i.e. Maharashtra, Madhya Pradesh and chiefly mountain region of Mount abu and Udaipur hills and other common hilly areas . Sida cordifolia is commonly found in Rajasthan University campus, Jaipur, Rajasthan. Sida cordifolia was declared noxioius weed in western Australia and the northern territory (Smith, 2002; Henty et al., 2007).

*Sida cordifolia* L. is most commonly used herb in Ayurvedic system of medicine, because this plant balances all the three laws of physiology i.e. *Vata*, *Kapha* and *Pitta*, it provides nourishment for all the aspects of natural intelligence. This plant exhibits properties which can be useful against AIDS and cancer.

It is considered to have diphortic diuretic, CNS (central nervous system) stimulating and antiasthmatic activity. According to Dr.Albert Leung Plant extract and parts are used as food, drugs and cosmetic. The root is considered diuretic and depressive. They are given in the treatment of gonorrhoea and during worm infestation (Sharma *et al.*, 2007). The present investigation deals with estimation of primary metabolites and

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### enzymes in *W. somnifera* and *S. cordifolia* Materials and Methods Carbohydrate

The dried and milled test sample 50 mg each was macerated in a grinder with 20 ml of ethanol and centrifuged (1200 rpm) for 15 min, the supernatants were removed and were concentrated on a water-bath. The volume was raised to 50 ml with distilled water and processed further by following the method of Loomis and Shull (1937) for soluble sugars. However, the residual pellet obtained by centrifugation was used for the estimation of starch. Aliquot (1 ml) of each of the test sample from were used to quantifying the total levels of carbohydrates using phenols-sulphuric acid method (Dubois *et al.*, 1951).

A stock solution of glucose (100 mg/ ml) was prepared in distilled water, out of which 0.1 to 0.9 ml was transferred to test tube and the volume was raised to 1 ml with distilled water. To each of these, 1 ml of 5% aqueous phenol was added rapidly having kept in an ice chest and shaken gently. Later 5 ml of Conc. H2SO4 was rapidly added by agitating gently during the addition of the acid subsequently, the tube was kept on a waterbath ( $26^{\circ} - 30^{\circ}$  C) for 20 min, and the optical density (ODs) of the yellow orange colours thus developed were taken at 490 nm in a Spectrophotometer after having set it for 100% transmission against the blank. Four replicates of each sample were run and there mean values were calculated.

A regression was computed between its known concentrations and their respective ODs. which followed the Beer's Law. The concentration (mg/gdw) of the total soluble sugars was directly worked out from the regression curve of the standard glucose. Three replicates of each experimental sample were taken and their mean values recorded. The sugar content 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.



Fig. 1. L=Leaf, St=stem, C= Callus, S=Seed, D.C=.Differentiating callus



Fig. 2. L=Leaf, St=stem, C= Callus, S=Seed, D.C=.Differentiating callus



Fig. 3. L=Leaf, St=stem, C= Callus, S=Seed, D.C=.Differentiating callus

## **Total Phenol**

Each of 200 mg dried and milled test samples was homogenized in 80% ethanol (10 ml) for 2 hrs and left over night at room temperature. It was centrifuged, the supernatants were collected individually and the volume of each was raised to 40 ml with 80% ethanol. To estimate total phenols in each of the test sample, the protocol of Bray and Thorpe (1954) was followed, wherein a standard curve of caffeic acid (a phenol) was prepared.

A stock solution (40 mg/ml) of caffeic acid was prepared in 80% ethanol, from which 0.1 to 0.9 ml was transferred into testtubes separately and the volume in each case was raised to 1 ml with 80% ethanol. To each of these tubes, 1 ml of Folin– Ciocalteau reagent (prepared by diluting the reagent with distilled water in 1:2 ratio just before use) accompanied by 2 ml of 20% Na2CO3 solution was added and the mixture was shaken vigorously. Each of these were boiled on a water bath (1 min), cooled and diluted to 25 ml with distilled water. The OD was taken at 750 nm using a spectrophotometer against a blank. Three such replicates were taken for each concentration and the average OD was plotted against the respective concentration to compute a regression curve.

#### Amino Acid

Amino acid was determined by the protocol as described by Lee and Takahashi (1966)

#### Ascorbic Acid

The experiment has been performed using the protocol of Roe JH and Kuether CA (1943).

## **Enzyme Activity**

POLYPHENOLOXIDASE and INDOLE ACETIC ACID Oxidase

The enzyme from the plant tissues were evaluated with the established protocols of Shinshi and Noguchi (1975) for Polyphenol oxidase.

#### Acid Phosphatase

The estimation of this enzyme in fresh plant tissues of King E.J. and Jagatheesan K.A. (1959)



Fig. 4. L=Leaf, St=stem, C= Callus, S=Seed, D.C=.Differentiating callus



Fig. 5. L=Leaf, St=stem, C= Callus, S=Seed, D.C=.Differentiating callus

#### **Observervations and Results**

Carbohydrate contents were found maximum amount in simple callus followed by leaf, stem and differentiating callus and minimum content in seed in case of *Withania somnifera*. However carbohydrates contents were found to be maximum in stem followed by leaf, callus and differentiating callus minimum in seed in case of *Sida cordifolia* L. (Graph 1).

Maximum amount of total phenols were recorded in leaf followed by stem, differentiating callus, seed and minimum in callus of *Withania somnifera*. L. while maximum amount of total phenol contents were observed in stem and minimum in seed in case of *Sida cordifolia* L (Graph-2)

Differentiating callus showed maximum amount of amino acid, while callus exhibited minimum in case of *Withania somnifera* L. (Dunal) and maximum amount of amino acid in leaf while seed had negligible content in case of *Sida cordifolia* L(Graph -3).

Maximum amount of ascorbic acid was found in leaf and minimum in seed in Withania *somnifera* L. While maximum amount of ascorbic acid was observed in differentiating callus and seed had no content (present in ascorbic acid amount nil) in case of *Sida cordifolia* L. (Graph- 4).

Maximum activity of polyphenol oxidase was observed in leaf and minimum in differentiating callus of *Withania somnifera* L. Also maximum enzyme activity was observed in leaf while seeds had no content (polyphenol oxidase enzyme activity present in nil) in case of *Sida cordifolia* L (Graph-5)

IAA oxidase activity was recorded as follows :- stem had maximum and seeds had minimum content in *Withania* somnifera L. while in case of *Sida cordifolia* L. IAA oxidase activity was observed almost maximum in stem and negilable in seed (Graph-6).

In *Withania somnifera*, acid phosphatase activity was found to be maximum in seeds and minimum in callus. But in *Sida cordifolia* acid phosphatase activity was found to be maximum in stem. and low content in differentiating callus (Graph-7).



Fig. 6. L=Leaf, St=stem, C= Callus, S=Seed, D.C=.Differentiating callus



Fig. 7. L=Leaf, St=stem, C= Callus, S=Seed, D.C=.Differentiating callus

#### Discussion

Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds. Plant synthesizes primary metabolites (lipid, protein, starch, sugars, phenol etc.) for the normal growth and development of itself. Plants are the living beings, because of value whole life on earth can be sustained, as they supply oxygen, food, energy and many valuable metabolites. Similar results were observed by others who have evaluated the primary metabolites of different medicinal plants (Schulz and Baranska, 2006; Vijayvergia and Kumar, 2007; Vijayvergia and Tanwer, 2010).

As much work has been done on plants by synthetic chemistry application in the last four decades, there is still much left to be done by general sectors of chemical industry. Some unique reactions are found in some plants apart from primary metabolic pathway, which results in formation of a special compound unique to a few species or to a single cultivar. These unique compounds are considered as secondary metabolites and, are of high medicinal value. These secondary metabolites do not function in host plants as primary metabolites, but are of great therapeutic importance for the plant to fight against various stresses (Shetty *et al.*, 2004, Ramchandra and Ravisankar, 2002 ., Huber *et al*, 2011 )

Proteins are formed from monomers known an amino acid. Carbohydrate once recognized for its role in food and nutritional applicatoin, is now found to act on other physiological process and diseases, including inflammation, cancer, cell metastasis and diabetes etc. In the present work, callus showed maximum amount carbohydrate of which gradually decreased in callus, leaves, stem, differentiation callus and seed of *Withania* somnifera L. But in *Sida cordifoliaL* carbohydrate content was found maximum in stem, but declined in leaves, callus, differentiating callus and seed (Boraston *et al.*, 2004).

Phenols are the compounds with a benzene ring with one or more hydroxyl groups attached to it. These are natural and synthetic compound. These are basically found in all foods. During the present work maximum phenol content was found in leaf and minimum in callus of *Withania somnifera* L. In *Sida cordifolia* L it was found maximum in stem part and minimum in seed. Same results were reported by Zheng and Shetty (2000). Amino acids are monomers of proteins. There are hundred of thousand of different protein that exist in nature. In the present work, protein was found to be maximum in stem of W. *somnifera* and in leaves of *Sida cordifolia*. Shekhawat (2002) reported variation in protein status in various parts of diverse plant species. In *Withania somnifera* maximum amino acid was observed in differentiating callus followed by leaf, seed, stem and minimum in callus. In *Sida cordifolia* maximum amino acid was noted in leaves and it decreased in stem, differentiating callus, callus and then no amount in seed.

Ascorbic acid is vital for immune and nervous system because it strengthens blood vessels. It is also needed for the manufacture of collagen, which is a must for tissue repair. It functions as antioxidant and participates in oxidation reduction reactions. During present work maximum amount of ascorbic acid was found in leaves and differentiating callus followed by callus and seeds of *Withania somnifera L*. However is *Sida cordifolia L* maximum amount of ascorbic acid was found in differentiating callus and its amount gradually decreased in simple callus, leaf and stem. Production of virus free endogenous ascorbic acid and effect of exogenous ascorbic acid on growth and metabolism of some important plant species was reported (Rajor, 2002; Shekhawat, 2002).

Polyphenol oxidase is an copper containing enzyme and catalyzes the oxidation of phenols in plant cells its presence in chloroplast, chromoplast, leucoplast, amyloplast or other plastids. In the present set of experiments polyphenol oxidase enzyme activity was found to be maximum in leaf followed by seed, stem and callus in case of *Withania somnifera*. Its amount was reported maximum in leaf then followed by simple callus and stem of *Sida cordifolia*. L.

Enzyme IAA oxidase catalyses various biochemical and physiological activities. In the present experiment IAA oxidase activity was observed maximum in stem and gradually decreased in differentiating callus, callus and leaves in case of *Withania somnifera* L. In *Sida cordifolia* L. maximum activity of IAA oxidase was seen in stem, leaf, callus and then it declined in seed. Mathur (2002) observed maximum enzyme activity in stem, pericarp and shoot tip but (Chauhan and Mehta, 1975) observed maximum IAA oxidase activity in leaves of *Sida cordifolia*.

Orthophosphoric monoester phosphohydrolyse (acid phosphate) helps in transport and production of inorganic phosphates which are indeed a must for large number of metabolic reactions. In the present research work acid phosphatase activity was found to be maximum in seeds and then in leaves, stem, differentiating callus and callus in *Withania somnifera*. Acid phosphatase activity was found maximum in stem, which then gradully decreased in leaves, seed and callus in *S. cordifolia* showed activity of acid phosphatase in callus.

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