Phytochemical estimation of two primary metabolites from medicinally useful plant, *Terminalia arjuna* (Roxb.) Wt. and Arn.

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Plants give us varieties of bounties, as nature is a rich store house of herbal remedies. They are known more and more for their vital usages in many areas, including medicinal purpose. The medicinal properties of plants attributed to their chemical constituents. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. Medicinal plants play a significant role for the development of new drugs for man's well being and his survival. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments. Around 20,000 medicinal plant species have been recorded (Dev, 1997), about 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti *et al.* 2008).

*Terminalia arjuna* (Roxb.) Wt. and Arn. (commonly known as ‘Arjuna’) considered as cardiac tonic and stimulant. Its bark provides nutrition to heart muscles thereby strengthening. It is widely used as astringent, cooling, asphrodisiac, cardiotonic and in fractures, ulcers, spermatorrhoea, leucorrhoea, diabetes, cough, tumor, asthma, skin disorders and juice of fresh leaves of Arjuna can be used beneficially in earaches (Paarakh, 2010; Biswas *et al.*, 2010).

Primary metabolites are responsible for growth and development of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs. Advancements in laboratory techniques were made to assess the study of primary metabolites of various plant parts in selected plant species *Terminalia arjuna* (Combretaceae). The present study deals with the estimation of two basic primary metabolites (protein and ascorbic acid) of *T. arjuna*.

Fresh plant parts were collected from local areas of Bikaner region, Rajasthan, India and used for estimation of two primary metabolites (protein and ascorbic acid). Two different protocols were used for the quantitative estimation of primary metabolites.

Root, stem, bark, leaves, flowers and fruits of the mature plant were collected from local areas of Bikaner in the year of 2011-12, washed with distilled water, shade dried and powdered. The plant parts- leaves and fruits were collected during the months of April-October; flowers during April-May and bark collected during March-July. The powder was used for analysis of protein (Lowry, 1951) and ascorbic acid (Chinoy, 1962) of the selected plant species.
Estimation of proteins

Protein Extraction:

Each of the plant parts were homogenized separately in 10% cold Tri Chloro Acetic acid TCA (10 mg: 5 ml) and were centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and pellets were saved. Pellets were again suspended in 5 ml of 10% cold TCA and recentrifuged for 10 minutes. Supernatant was again discarded and the precipitate was dissolved in 10 ml of 0.1 N NaOH. 0.1 ml of this solution was used for protein estimation.

Quantitative estimation of Protein:

In each of 1 ml extract, total protein content was estimated using the protocol of Lowry et al., 1951. A stock solution (1mg/ml) of bovine serum albumin was prepared in 1 N NaOH, five concentrations (0.2, 0.4, 0.6, 0.8 and 1ml) from the working standard solution were taken in series of test tubes. In another set of test tubes 0.1 ml and 0.2 ml of the sample extracts were taken and the volume was raised up to 1 ml in all the test tubes. To each test sample, 5ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na$_2$CO$_3$ in 0.1 N NaOH and 1 ml of 0.5% CuSO$_4$. 5H$_2$O in 1% sodium potassium tartrate) was added at room temperature and left undisturbed for a period of 10 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocalteau reagent (diluted with equal volume of distilled water just before use) was rapidly added and incubated at room temperature (about 25°C) for 30 minutes until the blue colour developed. The spectronic colorimeter (Bausch and Lomb) was adjusted at wavelength of 750 nm and set at 100% transmittance using a mixture of 1ml of the extract, 2ml of 5% MPA, 5 ml n-amyl alcohol and 3.2ml distilled water (blank solution) before taking test samples. Ascorbic acid content present in 1ml of extract was measured by using the regression formula:

\[ Y = 0.1103 - (0.14 \times \text{O.D}) \]

Where, \( Y = \text{Concentration of ascorbic acid in mg, O.D. = Optical Density} \)

Ascorbic acid was estimated using the protocol of Chinoy (1962). Dried plant parts were weighed separately crushed in a mortar in 2% Meta Phosphoric Acid (MPA) (100 mg tissue and seed sample in 1 ml of MPA) and allowed to macerate for one hour. These were then centrifuged separately at low speed (2500 r.p.m.) for fifteen minutes, the residues were discarded and the supernatants were used for the estimation of ascorbic acid following the procedure of Jensen (1962). Each of the 1 ml test solutions were mixed with 2ml of 5% MPA and kept for 30 minutes without stirring at room temperature. 5ml of n-amyl alcohol and 3.2 ml of dye (5mg/100ml, 2, 4-dichlorophenol indophenol) were added and air bubbled through the lower layer. Each of the test tubes was stoppered tightly, the mixture was shaken vigorously and the upper layer was used for the estimation of ascorbic acid. The Spectronic-20 colorimeter (Bausch and Lomb) was adjusted at wavelength of 546nm and set at 100% transmittance using a mixture of 1ml of the extract, 2ml of 5% MPA, 5 ml n-amyl alcohol and 3.2ml distilled water (blank solution) before taking test samples. Ascorbic acid content present in 1ml of extract was measured by using the regression formula:

\[ Y = 0.1103 - (0.14 \times \text{O.D}) \]

Where, \( Y = \text{Concentration of ascorbic acid in mg, O.D. = Optical Density} \)
Ascorbic acid content per 100 gm dry weight was calculated as follows:

\[ \text{Free ascorbic acid} = \frac{A \times V}{W} \times 1000 \times 100 \]

Where, \( A = Y \) = mg ascorbic acid / ml of original extract  
\( V \) = total volume of the original extract (in ml)  
\( W \) = weight of the plant tissue sample (in mg) used for analysis

The various plant parts (root, stem, bark, leaves, flowers and fruits) of \( T. \) arjuna varied in composition of primary metabolites studied (Rishi et al., 2009; Sagwan et al., 2010; Talreja, 2011; Yadav et al., 2012). In the present investigation, \( T. \) arjuna was evaluated quantitatively for the analysis of total soluble protein and ascorbic acid. The results are presented in Table-1.

Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future (Thomsen et al., 1991). Total levels of protein were found to be higher in fruits i.e. 0.27mg/g.d.w. and lower amount in flower i.e. 0.10mg/g.d.w. (Fig. 1). Protein content of leaves (0.23 mg/g.d.w.) is little less than fruits but higher than stem (0.16 mg/g.d.w.), bark (0.18 mg/g.d.w.) and flowers (0.10 mg/g.d.w.) (Table 1).

Ascorbic acid (vitamin C) is a familiar molecule because of its dietary significance, it is not only an important antioxidant, it also appears to link flowering time, developmental senescence, programmed cell death and responses to pathogens through a complex signal transduction network (Mapson, 1958; Nicholas, 1996). The amount of endogenous ascorbic acid increased in the order root<flower<bark.stem<leaves<fruits in \( T. \) arjuna. Total levels of ascorbic acid were found to be maximum in fruits i.e. 6.08 mg/100g.d.w. and minimum in roots i.e. 5.26mg/100g.d.w.(Fig. 2). Flowers (5.31 mg/g.d.w.) contain less amount of ascorbic acid than all plant parts but higher than root (5.26 mg/g.d.w.) (Table 1).

CONCLUSION

\( T. \) arjuna is quantified as a rich source of primary metabolites like proteins and ascorbic acids. The highest amount of protein (0.27mg/ 100g.d.w.) and ascorbic acid (6.08 mg/100g.d.w.) was observed in fruits. Similarly lowest amount of protein (0.10 mg/100g.d.w) was in flowers, ascorbic acid in roots (5.26mg/100g.d.w.). These results are suggestive of primary bioactive compound of commercially importance and may result in great interest in plants pharmaceuticals. Primary metabolites analysis is necessary for knowing the nutritional potential of plants and also the precursors for the synthesis of secondary metabolites (Vijayvergia and Kumar, 2007).

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Table 1: Estimation of primary metabolites in different parts of *T. arjuna* (mg/100gdw)

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<thead>
<tr>
<th>Primary metabolites</th>
<th>Plant parts</th>
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<tbody>
<tr>
<td></td>
<td>Root</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>5.26±</td>
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<td></td>
<td>0.12</td>
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Fig. 1: Protein content of *T. arjuna* (mg/100gdw)

Fig. 2: Ascorbic acid content of *T. arjuna* (mg/100gdw)
REFERENCES


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