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Analysis of primary and secondary metabolites in root extract of *Sterculia urens* Roxb., a traditionally important medicinal tree

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Abstract: *The present study evaluates the quantitative analysis of primary (proteins, total soluble sugars, starch, amino acids) and secondary metabolites (phenolics and tannins) in the root extract of Sterculia urens Roxb. Quantitative analysis of root extract contains the proteins (0.35±0.02 mg/gram), total soluble sugars (36.8±1.39 mg/gram), starch (0.25±0.02 mg/gram), amino acids (0.65±0.02 mg/gram) phenolics (0.15±0.02 mg/gram) and tannins (0.27±0.04 mg/gram) respectively. Presence of primary and secondary metabolites in root extract justifies the use of plant species as traditional medicine for treatment of various diseases. Further studies are required to isolate, characterize the bioactive compounds from root extract of S. urens.*

Keywords: *Sterculia urens, root extract, primary metabolites, phenolics, tannins*

Introduction

Medicinal plants play a significant role in day-to-day lives of people living in developing countries of Asia and Africa. Medicinal plants not only serve as substitutes for modern medicine but also enhance the health and security of the people. They play essential roles in daily life and are connected to diverse social, cultural, and economic events associated with life, aging, illness, and death. From ancient times, plants have been rich sources of effective and safe medicines. Approximately 75-90% of the rural population in the world depends on traditional medicine for their health care system (Agidew, 2022).

The phytoconstituents derived from bark, leaves, flowers, roots, fruits, seeds etc are grouped into primary and secondary metabolites. Primary metabolites include amino acids, common sugars, proteins and chlorophyll etc., and secondary metabolites are tannins, alkaloids, saponins, steroids, glycosides, terpenoids, flavonoids, anthra quinones etc. Generally the medicinal property of the plant species is due to the bioactive components that causes the definite pharmacological action on the body (Naseem, 2014). Some phytochemicals such as phenolic acids and flavonoids act as antioxidant and prevents the onset or the progression of many diseases. Knowledge on the phytochemicals of the plant is desirable for the synthesis of complex chemical substances. These phytochemicals work with nutrients and fibres to form an integrated part of defense system to fight against various diseases (Daniel and Krishnakumari, 2015).

Sterculia urens is a medium to large sized tree belongs to the family Sterculiaceae and grows wildly on deciduous forests of dry rocky hill lands.

The tree yields a gum known as gum karaya and this gum has numerous applications in food, dairy, beverages, cosmetic, textile, oil, and pharmaceutical sectors. Gum exudate of this plant is having large market value globally (Dhiman *et al*., 2019). Bark, seed and leaf are used for wound healing and throat infections (Oak *et al*., 2015). Barks are also used to treat rheumatoid arthritis (Rao *et al*. 2016) and oligospermia (Dhiman *et al*., 2019). Root powder of *S.urens* along with bark powders of *Ailanthus excelsa* and *Madhuca longifolia* var. *latifolia* and leaf powder of *Vitex negundo* was used reduce body swellings used by Korku tribe of Amravati district of Maharashtra (Jagtap *et al*. 2006).

Good amount of work was done and reported on karaya gum of *Sterculia urens*. But little information available on primary and secondary metabolites in root extract of *Sterculia urens* Roxb. The main objective of the present study is to evaluate primary (proteins, total soluble sugars, starch, amino acids) and secondary metabolites (phenolics and tannins) in root extract of *Sterculia urens* Roxb. This information introduces a new arena for the discovery of bioactive ingredients useful for the manufacturing of new drugs.

Materials and methods

Fresh healthy root material was collected from Kovela foundation, a Non Governmental Organization (NGO), Visakhapatnam, Andhra Pradesh, India.

Extraction

One gram of fresh root sample was macerated in pre-chilled phosphate buffer (0.1 M, pH 7.6), containing 0.1 mM EDTA using motor and pestle. The homogenate was centrifuged at 10000 rpm for 30minutes. The supernatant was collected and used for the assay of proteins, phenols and tannins.

Estimation of proteins

1ml of above extract was taken and 1ml of ice cold 20% TCA was added. Centrifuged the extract and supernatant was used for phenol estimation and the pellet was washed twice with acetone and again centrifuged at 8000rpm. The pellet was dissolved in 5ml of 0.1N NaOH and used for protein estimation. Total proteins were estimated by the method of Lowry *et al*. (1951) using BSA (100μg/ml) as standard. Each experiment was repeated thrice.

Total phenolic content

The total phenolic content was determined spectrophotometrically by the method described by Sadasivam and Manickam (1996). A calibration curve was constructed by using standard Gallic acid (250μg/ml) solution. To 2 ml of protein free supernatant, 1 ml of Folin-ceocalteau reagent was added. After 3 min, 13 ml of distilled water was added. Later 2 ml of sodium carbonate (7.5%) solution was added and the volume was adjusted to 20 ml. The above mixture was kept for 1 hour for colour development and absorbance was recorded at 630 nm. The amount of phenols in root extract was calculated from the gallic acid calibration curve and it was expressed as mg of gallic acid equivalents/gram fresh weight. Each experiment has three replicates and the experiment was repeated thrice.

Tannin content

Tannin content was determined by Folin Denis method (Karaogul *et al*., 2017). To 0.1 ml of supernatant, 6.9 ml of distilled water was added and the contents were mixed with 1.5 ml of 20% sodium carbonate and 0.5 ml of folin-phenol reagent. The mixture was shaken well, kept at room temperature for 1 hour

and absorbance was measured at 725 nm in a spectrophotometer. Standard was constructed using standard tannic acid (250μg/ml) and results of tannins were expressed in terms of tannic acid in mg/ml of extract.

Total soluble sugars

One gram of fresh root was rinsed with water and dried between filter paper and were grounded in a mortar and pestle in 10ml of 80% ethanol (v/v) and the mixture was boiled for 10 minutes and centrifuged at 2000rpm for 10 minutes. The supernatant was collected and pellet was reextracted in 10 ml of hot 80% ethanol. Supernatant from both extractions were combined, and combined supernatants were evaporated till the volume was reduced to 90%. 2ml of water was added, evaporation was continued until no trace of alcohol. After evaporation the volume was made up to 5ml with distilled water at room temperature. The above extract was used for estimation of total soluble sugars (Dubois *et al*., 1956). Standard graph was constructed by taking standard glucose in the range of (10μg-100μg/ml). One ml of extract was taken, 1ml of phenol solution was added. 5ml of 96% sulphuric acid was added to each tube and shaken well and kept in a boiling water bath at 25° C to 30° C for 20 minutes. Absorbance was measured at 490 nm. The amount of total soluble sugars present in the test sample was calculated using a standard graph and results were expressed per gram tissue.

Estimation of starch

0.5 grams of the sample was homogenized in hot 80% ethanol to remove sugars. Extract was centrifuged and the residue was repeatedly washed with hot 80% ethanol till the washings do not give colour with anthrone reagent. Residue was dried well, and to the residue 5ml of water and 6.5 ml of 52% perchloric acid was added. Extract was kept at 0°C for 20 min. The extract was centrifuged and the supernatant was collected. Extraction was repeated using fresh perchloric acid and the supernatants were pooled. 0.2 ml of the supernatant was taken made up to volume to 1 ml with water. The standard graph were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up the volume to 1 ml in each tube with water. Four ml of anthrone reagent was added and kept in boiling water bath for eight minutes and the tubes were rapidly cooled and the intensity was measured at 630 nm (Hodge and Hofreiter, 1962).

Estimation of amino acids by ninhydrin method

0.5 grams of the sample was weighed and grounded in a mortar and pestle. 5 ml of 80% ethanol was added to the homogenate and centrifuged at 3000 rpm. Supernatant was collected and the extraction was repeated twice with the residue and the supernatants were pooled. Finally the volumes were reduced by evaporation and used for qualitative and quantitative estimation of free amino acids. Free amino acids were determined in the final supernatant by ninhydrin method (Moore and Stein, 1954) using leucine as standard. Standard graph was constructed by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of standard amino acid solution and 0.2 ml of the supernatant to the respective labelled test tubes and made up the volume to 1 ml with distilled water. One ml of ninhydrin reagent was added and the contents were mixed by vortexing/shaking. Covered the mouth of the tubes with aluminum foil. All the test tubes were kept in boiling water bath for 15 minutes. Test tubes were cooled and 1ml of ethanol was added to each test

tube and mixed well. Now the absorbance was recorded at 570 nm using a colorimeter.

Statistical analysis

Each experiment has three replicates and the experiment was repeated thrice and the data was subjected to one way ANOVA using Minitab version 15. A significance level of 0.05 was used for all statistical tests.

Results and discussion

The amount of primary (proteins, total soluble sugars, starch, amino acids) and secondary metabolites (phenolics and tannins) present in the root extract of *S.urens* were shown in Table 1. Root extract contains the proteins (0.35±0.02 mg/gram), total soluble sugars (36.8±1.39 mg/gram), starch (0.25±0.02 mg/gram), amino acids (0.65±0.02 mg/gram) phenolics (0.15±0.02 mg/gram) and tannins (0.27±0.04 mg/gram) respectively (Table 1).

Table 1: Protein, phenolics, tannins, soluble sugars, starch and amino acid content in root extract of *S.urens*.

S. No	Type of metabolites	Concentration (mg/gram)
1.	Proteins	0.35±0.02
2.	Total soluble sugars	36.8±1.39
3.	Starch	0.25±0.02
4.	Amino acids	0.65±0.02
5.	Phenolics	0.15±0.02
6.	Tannins	0.27±0.04

Each value represents Mean±SE of three independent experiments.

Traditional medicine plays a significant role in healthcare of the people around the globe and the medicinal plants provide a valuable contribution to this practice. Different plant parts such as root, leaves and fruit have many traditional values and pharmacological properties due to the presence of various phytochemical constituents (Tesfahuneygn and Gebreegziabher, 2019). *S.urens* has traditional significance and it's roots are used for facilitating child birth, relieving constipation and reduces the body swelling (Anjali *et al*., 2020). For utilizing this plant in modern therapeutic systems, rigorous experimental evidences, toxicity analysis and clinical trials are required. Hence, the present study was initiated to evaluate the medicinal importance of *S. urens*. The genus *Sterculia* contain significant amounts of flavonoids, terpenoids, phenolic acids, alkaloids, and other metabolites such as carbohydrates, lipids, lignans, and lignin (El-Sherei *et al*., 2016).

Quantitative analysis of primary metabolites shows that high total soluble sugar content, followed by amino acids, protein and starch content. Sugars can be used as artificial sweeteners and they help in preventing diabetes (Daniel and Krishnakumari, 2015). The presence of proteins in different plant parts shows their increase in food value (Daniel and Krishnakumari, 2015).

The phenolic content (0.15±0.02 mg/gram) is important in the regulation of plant growth, development and disease resistance. Phenolic compounds acts as antioxidant, which is crucial for radical scavenging, metal ion chelator, reduce lipid peroxidation, prevent DNA damage, and scavenging reactive oxygen species (Annapandian and Rajagopal, 2017). Polyphenolics exhibit the strong antioxidative, hepatoprotective, cytoprotective and antimicrobial activities (Ghaffari *et al*., 2012; Pham *et al*., 2018). Tannins exhibit the antimicrobial, anti-inflammatory, anti-viral, anti-parasitic and astringent activities. Tannin can evoke an antidiarrheal effect and these substances

may precipitate proteins on the enterocytes reducing peristaltic movement and intestinal secretion (Auwal *et al*., 2014).

Conclusion

Presence of higher concentration of both primary and secondary metabolites implies that the root extract of *S.urens* has several medicinal applications. Presence of phenolics and tannins denotes the antioxidant property. This primary information will be useful in further studies on the discovery of bioactive ingredients and to resolve their efficacy by in vivo studies and validate their safety and effectiveness in clinical trials.

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Conflict of interest

The authors have no conflict of interest.

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