ORIGINAL ARTICLE

Quantitative analysis of some secondary metabolites of *Svensonia hyderobadensis* (Walp.) mould: A rare medicinal plant

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Background: The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. **Aim:** The present study intended to find out the total content of some important phytochemical constituents like phenols, flavonoids, tannins, non-tannin phenols, condensed tannins and hydrolysable tannins in different parts of *Svensonia hyderobadensis.* **Materials and Methods:** By using Spectrophotometric methods. **Results:** The results showed that the plant is rich in phenols. Among the selected parts of the plant, the root showed maximum content of selected secondary metabolites, followed by leaf and stem. Roots are found to be rich in phenols [160.87 mg tannic acid equivalent (TAE)/g] and tannins (145.99 mg TAE/g); stem and leaf are rich in flavonoids (67.65 mg TAE/g and 133.95 mg TAE/g) followed by selected secondary metabolites. **Conclusion:** The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Key words: Quantitative analysis, secondary metabolites, *Svensonia hyderobadensis*

INTRODUCTION

Svensonia hyderobadensis is a rare medicinal plant belonging to the family Verbenaceae. The plant is used to treat hepatotoxic diseases[1] and has also been tested for its antimicrobial activity[2] and phytochemistry.[3] Herbal medicines have become more popular in the treatment of many diseases due to the popular belief that green medicine is safe, easily available and has less side effects. Secondary metabolites of plants serve as defence mechanisms against predation by many microorganisms, insects and herbivores.[4] Several plants have been studied for quantification of secondary metabolites, such as *Jatropha*,[5] *Clerodendron colebrookianum* and *Zingiber cassumunar*,[6] *Spondias mombin*[7] and leaves of *S. hyderobadensis*.[8]

Natural antioxidants such as phenols, flavonoids and tannins are increasingly attracting attention because they are having natural qualities of disease-preventing, health-promoting and anti-ageing substances.[9] Antioxidants may serve the task of reducing oxidative damage induced by free radicals and reactive oxygen species under oxidative stress conditions in humans. These conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity.[10] Recently, there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue injury.[11] Besides, well-known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices have been already exploited commercially either as antioxidant additives or as nutritional supplements.[12] A number of plant species have been investigated in search for novel antioxidants.[13] Still, there is a demand to find more information concerning the antioxidant potential of plant species. Hence, the present study was undertaken to quantify some of the important phytochemicals from various parts of *S. hyderobadensis.*

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MATERIALS AND METHODS

Plant Materials

The fully mature healthy plant materials, i.e. root, stem and leaves of *S. hyderobadensis*, were collected from Mamandur Forest, Chittoor District, Andhra Pradesh, India, during March 2011. The materials were washed thoroughly and shade dried.

Sample Preparation and Extraction

The samples were ground to pass through a sieve of 1 mm diameter. Tannins were extracted by taking

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400 mg ground sample in a conical flask with 40 ml diethyl ether containing 1% acetic acid (v/v) and mixing to remove the pigment material. The supernatant was carefully discarded after 5 min and 20 ml of 70% aqueous acetone was added. The flask was sealed with cotton plug, covered with aluminium foil and kept in an electrical shaker for 2 h for extraction. Then, it was filtered through Whatman filter paper No. 1 and the sample was kept refrigerated at 4°C until analysis.

Total Phenols and Tannins Determination

Tannins were estimated according to the procedure of Makkar *et al*.[14] The standard was prepared from the stock solution of tannic acid (0.5 mg/ml) taking 0, 10, 20, 30, 40 and 50 µl in test tubes and the volume was made up to 1.0 ml. It gives a tannic acid concentration of 0, 5, 10, 15, 20 and 25 µg, respectively. Then, 0.5 ml Folin reagent and 2.5 ml 20% sodium carbonate were added. Whole content was mixed properly, and after 40 min, the reading was taken at 725 nm with UV-VIS spectrophotometer.

Estimation of Condensed Tannins

Condensed tannin was estimated as per the method of Porter *et al*.,[15] expressed as leucocyanidin equivalent and calculated as below:

% Condensed tannins = (A_{550nm} × 78.26 × dilution factor)/(% dry matter)

Total Flavonoid Determination

Flavonoids in *S. hyderobadensis* extracts were expressed as quercetin equivalent. Quercetin (Sigma, Germany) was used to prepare the calibration curve [standard solutions of 6.25, 12.5, 25.0, 50.0, 80.0 and 100.0 µg/ml in 80% ethanol (v/v)]. Sample extracts (1 g plant material in 25 ml extract) were all evaporated to dryness and re-dissolved in 80% ethanol for the analytical test.

One milliliter of a sample (ethanolic solution of *S. hyderobadensis*) was mixed with 3 ml 95% ethanol (v/v), 0.2 ml 10% aluminium chloride (m/v), 0.2 ml of 1 mol/l potassium acetate and 5.6 ml water. The same volume of distilled water substituted 10% (m/v) aluminium chloride in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm.[16]

RESULTS AND DISCUSSION

The general assessment of the analytical results for different parts of *S. hyderobadensis* definitely showed individual specificity of each studied part and the rich diverse spectrum of secondary metabolites differing from one another. The highest total flavonoid content was found in leaves [133.95 mg quercetin equivalent (QE)/g] followed by stem (67.65 mg QE/g) and root (53.25 mg QE/g) [Table 1]. Similar results were reported for *Urtica dioica* and *Equisetum maximum*.[11] Flavonoids are secondary metabolites widely distributed in plants and more than 6000 flavonoids have been identified in plants.[17] They are a group of polyphenolic compounds with known properties, which include free radical scavenging inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action.[18] These are vital in combating the free radicals that damage human cells.[9] Numerous epidemiological studies confirm the significant relationship between high dietary intake of flavonoids and reduction of cardiovascular and carcinogenic risk.[19] There have been an increasing number of reports that directly contradict the putative role of flavonoids as antioxidant and anti-cancer agents.[20]

The highest total phenolic content was found in root [160.87 mg tannic acid equivalent/g (TAE)] followed by leaf (73.75 mg TAE/g) and stem (32.62 mg TAE/g). Similar results were reported from *Mellilotus officinalis*.[11] A number of reports have shown that the presence of phenolics in foods is particularly important for their oxidative stability and antimicrobial protection.[21] Phenols are ubiquitous secondary metabolites in plants and comprise a large group of biologically active ingredients. More than 8000 compounds of phenols have been identified so far in plants.[22] Leaves are the best source of non-tannin phenol (30.62 mg TAE/g), followed by stem (23.19 mg TAE/g) and root (14.88 mg TAE/g). These phenolic compounds possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic activities, as well as the ability to modify the gene expression.[23]

The highest total tannin content was found in root (145.99 mg TAE/g), leaf (43.13 mg TAE/g) and stem (09.43 mg TAE/g). Leaf is an excellent source of the condensed tannins (0.318 mg leucocyanidin equivalent/g) and roots serve as the best source for the hydrolysable tannins (145.8 mg TAE/g). Similar results were reported for *Quercus robur* bark[24]

Table 1: Total content of secondary metabolites of *Svensonia hyderobadensis*

Plant part	Flavonoids (mg/gdw)	Total phenols (mg/gdw)	Non-tannin phenols (mg/gdw)	Total tannins (mg/gdw)	Condensed tannins (mg/gdw)	Hydrolysable tannins (mg/gdw)
Root	53.25	160.87	14.88	145.99	0.190	145.8
Stem	67.65	32.62	23.19	09.43	0.181	09.249
Leaf	133.95	73.75	30.62	43.13	0.318	42.812

gdw – Gram dry weight

and galls.[25] Tannins are widely distributed in almost all plant foods.[26] The tannin containing remedies are used as antihelminthic,[27] antioxidants,[28] antimicrobial agents and anti-viral agents.[29] The study reveals that the root is a rich source of phenols and tannins, whereas the leaf can be used as the best source for non-tannin phenols.

CONCLUSION

Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application, and there is a trend to substitute them with naturally occurring antioxidants. The data presented for total phenols, flavonoids and tannins of *S. hyderobadensis* form the basis for assessment of its preventive role against the free radical effect. Among all parts of the plant, root is proved to be an excellent source for the flavonoids, tannins, condensed tannins and hydrolysable tannins. Moreover, the plant parts may be used as an alternative source for flavonoids, phenols and tannins for traditional remedies.

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