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PHYTOCHEMICAL INVESTIGATION AND *IN VITRO* ANTICANCER ACTIVITY OF *SESBANIA GRANDIFLORA* (L.) PERS. – A WILD LEAFY VEGETABLE OF SOUTHERN WESTERN GHATS

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Abstract

Sesbania grandiflora (L.) Pers., commonly known as agasthi, is a fast growing tree belongs to the family Leguminosae. The plant has great medicinal importance and found abundantly in tropical regions. In the present study phytochemical, nutritional, antioxidant and anticancer activities of the plant were carried out. In the phytochemical analysis reducing sugar, alkaloids, tannins, steroids, saponins, flavonoids, terpenoids and anthraquinones were qualitatively estimated. From the result it was found that major phytochemicals are present in the crude methanolic leaf extract of *Sesbania grandiflora*. The different nutritional factors like reducing sugar, total carbohydrate, total protein, pigments and starch were analysed by standard estimation method and it was found to be higher. Antioxidants like proline, lycopene, total polyphenol, polyphenol oxidase (PPO) and super oxide dismutase (SOD) were also estimated quantitatively and found to be high in the leaves of *S. grandiflora*. Anticancer activity of crude methanolic leaf extract were analysed using DLA (Dalton's Lymphoma Ascites) and EAC (Ehrlich Ascites Carcinoma) cell lines and found very promising result. The result is applicable for further pharmacological analysis of the plant. Nutritional and phytochemical information on the plant will be useful for the nutritional education of the public as a means to improve the nutritional status of the population.

Key words: , *Sesbania grandiflora*, DLA, EAC, SOD, PPO.

Introduction

Plants have been used for medicinal purposes long before pre-historic period. Ancient Unani manuscripts, Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical system such as Unani, Ayurveda and Chinese medicine in which herbal therapies were used systematically. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatment is independent of any age groups and sexes (Aja *et al*., 2010).

Sesbania grandiflora is a loosely branching tree up to 15 m tall. Its leaves are pinnately compound up to 30 cm long with 20 to 50 leaflets in pairs. Flowers were large, white yellowish, rose, pink or red with a calyx 15.22 mm long. It is well adapted to hot, humid environment and does not grow well in the subtropics particularly in areas with temperature below about 10^0 C. The dried leaves of *S. grandiflora* are used in some countries as tea which is considered to have antibiotic, anthelmintic, anti-tumer and contraceptive properties. Based on these medicinal properties, it is selected for the present study for the experimentation of using bioactive compounds obtained from the extracts of plant leaves.

Materials and Methods

Collection and Preparation of Sample

S. grandiflora were collected as fresh from Attingal, Thiruvananthapuram district of Kerala. Leaves were separated, shade dried, grind well using mechanical blender to fine powder and transferred into airtight containers for further analysis.

Extraction from plant parts

The fine powder was used for extraction by using methanol. Fifty gram of sample powder was covered with cotton cloth and kept in to the soxhlet apparatus for distillation. Three hundred ml of methanol was

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taken in to the round bottom flask and heated in a mantle for 8 hours at 70° C. After completing the process, extract was collected in a beaker and was kept it in an oven at 37° C – 40° C for evaporation. The crude concentrated extract was again weighed and used for further phytohemical investigation and anticancer studies.

Phytochemical Screening

Phytochemical analysis of plant extract was done as described by Harborne (Harborne, 1977). The different phytochemicals like reducing sugar, glycosides, flavonoids, alkaloids, tannins, steroids, terpenoids, saponins and anthraquinones were tested.

Biochemical Analysis

The fresh leaves of *S. grandiflora* were used for the nutritional and antioxidant analysis and the experiment was repeated three times to confirm the result. The analysis were performed following standard methods for the estimation of reducing sugar, total carbohydrate, total protein, pigments, starch and antioxidants like proline, lycopene, total polyphenol, polyphenol oxidase (PPO) and superoxide dismutases (SOD).

Nutritional evaluation

For the estimation of reducing sugar fresh samples were estimated with distilled water. Total reducing sugar present in the sample was estimated using the dinitrosalycylic acid method and the absorbancy read at 540 nm against a blank (Miller, 1972). The amount of total carbohydrate present in the sample was estimated using the anthrone method (Hedge and Hofreiter, 1962). Total protein was estimated using Lowry's method (Lowry *et al*., 1951) and the chlorophyll content using Arnon's method (Witham *et al*., 1971). For chlorophyll estimation, fresh tissue was homogenized in 80% acetone and the absorbance read at 645, 663 and 652 nm; the chlorophyll present was calculated using Arnon's formula. The amount of starch present in the samples was estimated by Anthrone reagent (Hedge and Hofreiter, 1962).

Antioxidant Estimation

Proline present in the sample was estimated by the method of Bates *et al*. (1973), the level of lycopene was estimated by Zakaria *et al*. (1979) using petroleum ether as a blank. The total polyphenolic content was determined by the Folin-Ciocalteau assay (Eom *et al*., 2008) and expressed as Gallic Acid Equivalents (GAE) in mg/100 g (d/w) of sample. The enzymatic antioxidants like Superoxide Dismutase (SOD) was estimated by NBT (Nitro Blue Tetrazolium) method as described by Gong *et al*. (2005) and the determination of polyphenol oxidase (PPO) was done by the method of Esterbauer *et al*. (1991).

***In vitro* anticancer studies**

Anticancer effect of crude methanol leaf extract of *S. grandiflora* was studied by using *DLA* and *EAC* cells. The crude methanol extracts from *S. grandiflora* at high concentration damaged the cells and make pores on the membrane through which Trypan blue enters. The damaged cells are stained with Trypan blue stain and can be distinguished from viable cells. Since live cells are excluded from staining, this method is also known as dye exclusion method (Prasanth *et al.,* 2010).

Results and Discussion

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the leaves of *S. grandiflora* revealed the presence of reducing sugar, flavonoids, alkaloids, tannins, terpenoids, steroids and saponins. But the presence of anthraquinones were not detected (Table 1). The presence of these phytochemicals explains the use of this leafy vegetable in ethno medicine for the treatment of various ailments.

Table 1. Preliminary phytochemical evaluation of *S. grandiflora* leaves

Sl. No.	Phytochemicals	Methanol extract of *S.*
1	Reducing sugar	+++
2	Flavonoids	+++
3	Alkaloids	++
4	Tannins	++
5	Terpenoids	++
6	Steroids	++
7	Saponins	++
8	Anthraquinones	–

Nutritional Analysis

To understand the chemical composition of the genus, major primary and secondary metabolites from the plant *S. grandiflora* were selected and quantified. Sugars are the product of photosynthesis and in

plants having high photosynthetic efficiency, the amount of sugar produced will be high (Somogyi, 1952). Reducing sugar from *S. grandiflora* was extracted in distilled water. The result showed high sugar content in the leaves of *S. grandiflora* (8.521 mg g^{-1}) as expressed in Fig. 1. Carbohydrates are widely present in the plant kingdom and the important components of storage and structural materials in plants comprising the mono-, di-, oligo- and polysacchirides. They exist as free sugars and polysaccharides (Hedge and Hofreiter, 1962). The maximum amount of carbohydrates was present in leaves of *S. grandiflora* (17.38 mg g^{-1}) as shown in Fig. 1. Proteins are present in the living world, irespective of the size of the organism, since they form the structural and functional basis of the cell (Lowry *et al*., 1951). Analysis of total protein in the plant was done by extracting the protein with phosphate buffer of p^H 7. The results showed maximum amount of protein in the leves of *S. grandiflora* (16.915 mg g^{-1}) as expressed in Fig. 1. Starch and cellulose are polysaccharides consisting of many monosacharides residues. Starch represents the main plant storage carbohydrate that provides energy during heterotrophic growth. Starch and sucrose are the primary products of photosynthesis (Maria *et al*., 2015). The amount of starch in the leaves of *S. grandiflora* is 5.205 mg g^{-1} (Fig. 1).

The chlorophylls are essential for photosynthesis and occur in chloroplasts as green pigments in all photosynthetic plant tissues. They are bound loosely to proteins but are readily extracted in organic solvents such as acetone or ether. The amount of chlorophyll in a green plant or any crop plant is an index of photosynthetic ability, which in turn is an index of its productivity level, primarily of the primary metabolites. (IUPAC, 1987). The amount of chlorophyll was measured in the leaves of *S. grandiflora* and it was found that high contents of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were present (0.992, 0.791, 1.428, 0.647 mg g^{-1}) in the plant (Fig. 2). The nutritional analysis of the leaves of *S. grandiflora* showed the presence of high amount of reducing sugar, carbohydrate, protein, starch and pigments.

Proline is a basic amino acid and free proline has an important role in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline in plants under stress, one of the hypotheses refers to break down of proteins into amino acids and conversion to proline for storage (Bates *et al*., 1973). The amount of proline present in the leaves of *S. grandiflora* is 0.929 mg g^{-1} (Fig. 3). Lycopene is a dietary carotenoid without provitamin-A activity, found in fruits and vegetables. It is considered to be the most potent oxygen quenching reagent among carotenoids and furthermore, it provides the ability to intervene in reactions initiated by free radicals like OH^- or peroxy radicals (Nina *et al*., 2013). The amout of lycopene in the methanol extract of the leaves of *S. grandiflora* is 0.864 mg g^{-1} (Fig. 3). Polyphenols are the most abundant antioxidants in human diets. Plants having high polyphenolic compounds were proved to be a good source of powerful antioxidants (Xiang *et al*., 2018). The result revealed that total polyphenol content in the leaves of *S. grandiflora* is 0.853 mg g^{-1} (Fig. 3). Superoxide dismutase (SOD) is a group of metalloenzymes that are found in all kingdoms of life. They constitute a very important antioxidant defence against oxidative stress in the body (Duncan *et al*., 2005). The amount of SOD in the leaves of *S. grandiflora* is 0.996 mg g^{-1} (Fig. 3) and found to be high. The polyphenol oxidase (PPO) comprise of catechol oxidase and laccase and are regard to plant defence mechanism against pest and diseases and appearance, palatability and use of plant products. These enzymes are reported to be present in fresh fruits, vegetables, leaves, mushrooms etc. (Esterbauer *et al*., 1991). The result of the present analysis revealed that the leaves of *S. grandiflora* have sufficient amount of PPO (0.901 mg g^{-1}) as shown in Fig. 3.

In vitro anticancer studies

Most of the currently used anticancer drugs are highly toxic, expensive and resistance mechanisms possess a significant problem (Hait and Hambley, 2009). There is a continuing need to identify new drug candidates that are more effective, widely available and less toxic. Plant extracts are an important source of potentially useful compounds for the development of new anticancer drugs (Hait and Hambley, 2009). The *in vitro* anticancer activity of the leaves of *S. grandiflora* was assessed by the Trypan blue exclusion method (Sheeja *et al*., 1997). Reduction in the viable cell count and increased non-viable cancer cell count towards normal in tumor host suggest antitumor effect against *EAC* and *DLA* cells in mice (Bala *et al.,* 2010). Cyclophosphamide

is used as standard anticancer compound. The results obtained from anticancer study revealed that methanol extract of the leaves of *S. grandiflora* showed remarkable (dose dependent cytotoxicity) anticancer activity against both the test cell lines (*DLA* and *EAC*). Methanol extract of *S. grandiflora* leaves showed 51.97, 79.27, 85.24% cytotoxicity in *EAC* compared to *DLA* which showed 30.85, 69.81, 80.39% cytotoxicity at the concentration of 100, 500 and 1000 µg/ml. The result of *in vitro* anticancer study in *DLA* and *EAC* cells lines showed high activity with increasing concentration of the extract such as 100, 500 and 1000 µg/ml of the leaves of *S. grandiflora* (Fig. 4). This *in vitro* anticancer activity of methanol extract in both *DLA* and *EAC* cell lines is higher compared to the standard anticancer drug cyclophosphamide (97.13%). *S. grandiflora* leaves showed higher anticancer activity compared to the anticancer plant reported like *Bidens biternata* (*DLA* -87.19% and *EAC*-92.26%) of *Asteraceae* (Pradeesh and Swapna, 2018).

Conclusion

Present study showed that *S. grandiflora* leaves had sufficient quantities of nutrients along with a wide range of phytochemicals and antioxidants. The plant extract was found to be effective against *DLA* induced solid tumor and *EAC* induced ascites tumor. In general, *S. grandiflora* is an excellent candidate which could be used to improve the health benefits and nutrition of people. Also nutraceutical bioactive products could be developed from this green leafy vegetable of Southern Western Ghats of Kerala.

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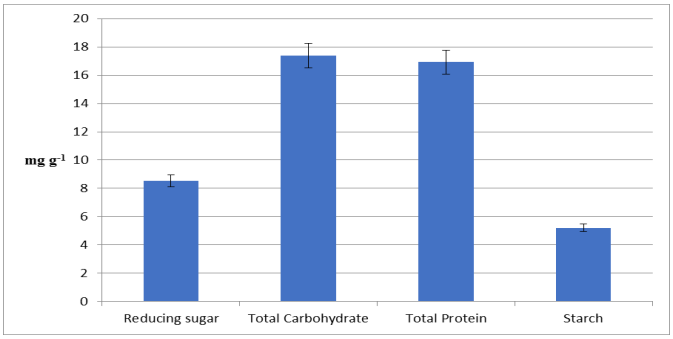

Figure 1. Nutritional factors in leaves of *S. grandiflora*

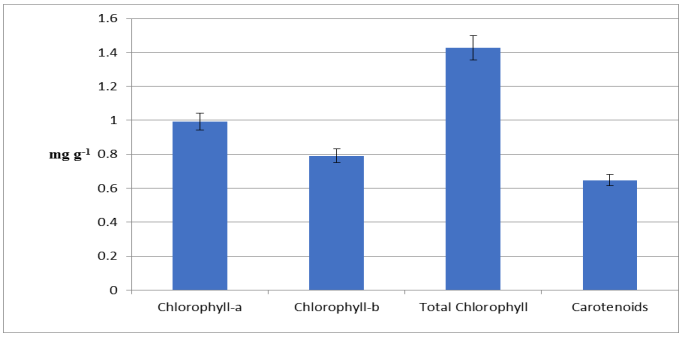

Figure 2. Pigments in leaves of *S. grandiflora*

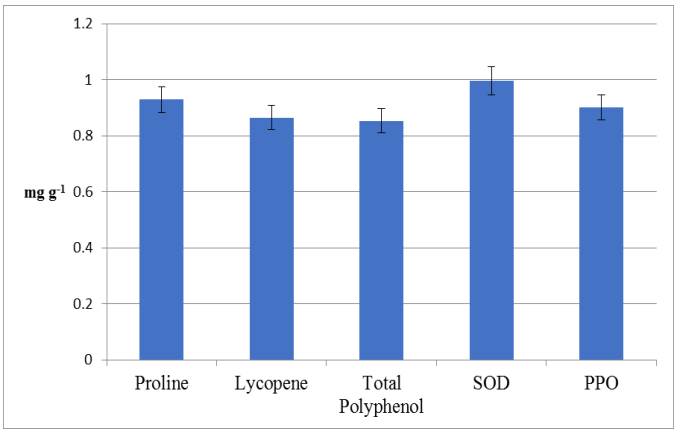

Figure 3. Antioxidants in leaves of *S. grandiflora*

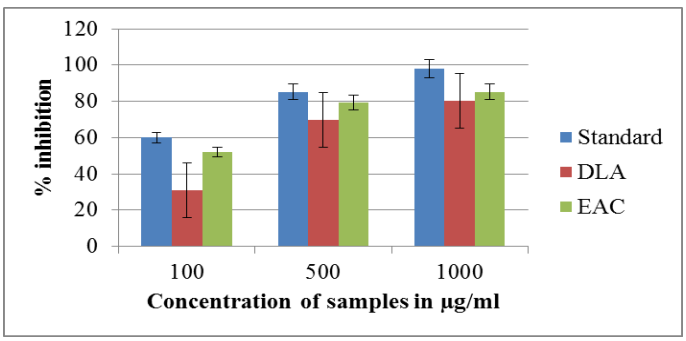

Figure 4. *In vitro* anticancer activity in leaves of *S. grandiflora*

References

Aja M P, Okaka A N, Ibiam U A, Uraku A J and Onu P N. 2010. Proximate analysis of *Talinum triangulare* (water leaf) leaves and its softening principle. *Journal of Nutrition*. 9(6): 524-526.

Harborne J B. 1977. Phenolic glycosides and their natural distribution in the biochemistry of phenolic compounds. Academic Press, New York, London.152-162.

Miller G L. 1972. Use of dinitrosalycylic acid reagent for determination of reducing sugars. Analytical Chemistry. 31: 426-428.

Hedge J E and Hofreiter B T. 1962. *In: Methods in Carbohydrate Chemistry*. (Eds. Whistler R L and Be Miller J N). Academic Press, New York. 17: 420.

Lowry O H, Rosebrough N J, Farr A L and Randall R J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*. 193: 265-275.

Witham F H, Blaydes D F and Devlin R M. 1971. Experiments in plant physiology, Van-Nostrand Reinhold Company, New York, USA. 245.

Bates L S, Waldren R P and Teare I D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*. 39: 205-207.

Zakaria H, Simpson K, Brown P R and Krotulovic A. 1979. Use of reversed phase HPLC analysis for the determination of provitamin-A, carotenes in tomatoes. *Journal of Chromatography*. 176: 109-117.

Eom S H, Park H J, Jin C W, Kim D O, Seo D W and Jeong Y H. 2008. Changes in antioxidant activity with temperature and time in *Chrysanthemum indicum* L. teas during elution processes in hot water. *Food Science and Biotechnology*. 17: 408-412.

Gong H, Zhu X, Chen K, Wang S and Zhang C. 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Science*. 169:313-321.

Esterbauer H, Dieber R M, Striegl G and Waeg G. 1991. Role of Vitamin-E in preventing the oxidation of low density lipoprotein. *American Journal of Clinical Nutrition*. 53: 314-321.

Prasanth NV, Dilip C. and Sanal D. 2010. Evaluation of *in-vitro* cytotoxic and antioxidant activities of *Ipomoea batatos*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2(3): 12-17.

Somogyi M. 1952. Notes on sugar determination. *Journal of Biological Chemistry*. 195: 19-23.

Maria V B, Mariana M and Julieta B. 2015. Starch metabolism in green plants. *Journal of Biotechnology*. 10(78): 329-376.

IUPAC (International Union of Pure and Applied Chemistry). 1987. Standard methods for the analysis of oils, fats and derivatives. 7th edn. Blackwell Scientific Publication, Oxford, UK. 216-219.

Nina P, Holzaptel, Boris M and Dietmar W H. 2013. The potential role of lycopene for the prevention and therapy of prostate cancer from molecular mechanisms to clinical evidence. *International Journal of Molecular Science*. 14(7): 14620-14646.

Xiang P L, Jia J, Xuemin J and Guoliang L. 2018. Antioxidant activities of extracts from sarcocarp of Coton easter multiflorus. *Journal of Chemistry*. 77: 1-17.

Duncan, Stanley K, Farnden J F and Elspeth A M. 2005. Plant amylases: functions and roles in carbohydrate metabolism. *Journal of Biological Science*. 16(16): 65-71.

Hait WN. and Hambley TW. 2009. Targeted cancer therapeutics. *Cancer Research*. 69: 1263-1267.

Sheeja KR, Kuttan G. and Kuttan R. 1997. Cytotoxic and antitumor activity of berberin. *Amala Research Bulletin*. 17: 73-76.

Bala A, Kar B. and Haldar P K. 2010. Evaluation of anticancer activity of *Cleome gynandra* on EAC treated mice. *Journal of Ethnopharmacology*. 129: 131-134.

Pradeesh S. and Swapna TS. 2018. Anticancer potential of *Bidens biternata* (Lour.) Merr. and Sheriff – an ethno medicinal plant of Waynadu District of Kerala. *Trends in Bioscience*. 11 (7): 1394-1397.