*ISSN 2583– 0260 (online)* *ISSN 2394 -7837 (print)*

# PHYOCHEMICAL SCREENING OF *SPILANTHES ACMELLA* (L.) L. AN ETHNO MEDICINAL PLANT OF KERALA

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**Received: 21/7/2021** **Revised: 23/11/2021** **Accepted:28/11/2021**

***Abstract***

Plants have been important role in human health because its value in medicine is also understood. *Spilanthes acmella* (L.) L. is one of the valuable plants so it is considered as the most important medicinal plant in the Ayurvedic medicine. The aim of the present study is to estimate nutritional, medicinal and anticancer properties of *S. acmella* in fresh sample and crude methanolic leaf extract. Different phytochemicals such as reducing sugar, glycosides, flavonoids, alkaloids, tannins, steroids, terpenoids, saponins, coumarins, anthraquinones, phlobatannins, iridoids were qualitatively estimated. Various nutritional factors reducing sugars, total carbohydrate, total proteins, pigments and starch were analyzed by standard estimation methods and found to be higher. Non-enzymatic antioxidants like total proline, lycopene, total polyphenol, and enzymatic antioxidants like superoxide dismutase (SOD), polyphenol oxidase (PPO) were also quantitatively estimated and found to be higher in leaves of *S. acmella*. *In vitro* anticancer activity of crude methanolic leaf extract of *S. acmella* were analyzed by using different cell lines like DLA (Dalton's Lymphoma Ascites) and EAC (Ehrlich Ascites Carcinoma) and showed very promising result. This result is applicable for further pharmacological analysis of *S. acmella* and isolation of new drugs from the plant. So the plant can be used as a potent nutraceutical agent for the future generation.

***Keywords****: Spilanthes acmella, DLA, EAC*, SOD, PPO

## Introduction

Among ancient civilization, India has one of the oldest and richest repository of medicinal plants, which are larger collected as raw materials for manufacture of drugs. About 8000 herbal remedies have been codified in AYUSH system in India. According to World Health Organization (WHO) in 2008 more than 80% of the world population believes on traditional medicine for their primary health care needs (Pradeesh and Praveena, 2020).

Medicinal plants are the local heritage with universal importance. The term medicinal plants include various types of plants used in herbalism. It is the use of plants for medicinal purposes and the study of such uses. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases lead to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments (Pradeesh and Praveena, 2020).

*Spilanthes acmella* belongs to the family Asteraceae consumed by the common peoples of Southern Western Ghats of Kerala in India. It is important to analyze phytochemical, nutritional, medicinal, antioxidant and anticancer properties for effective utilization of the plant. In the present work different preliminary phytochemicals such as reducing sugar, glycosides, flavonoids, alkaloids, tannins, steroids, terpenoids, coumarins, saponins, anthraquinones, phlobatannins and iridoids were qualitatively analyzed. Nutritional evaluation of plant possess high amount of reducing sugar, total carbohydrate, total protein, pigments and starch. Non-enzymatic antioxidants like proline, lycopene, total polyphenol and enzymatic antioxidants like superoxide dismutase (SOD) and polyphenol oxidase (PPO) were quantitatively estimated and found to be higher in *S. acmella.* In future *S. acmella* is important natural source of developing new drugs. So the study of *S. acmella* is relevant to the -

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modern society.

## Materials and methods

### Collection and Preparation of Sample

*S. acmella* were collected as fresh from Neyyattinkara, Thiruvanathapuram district of Kerala. Aerial parts were separated, shade dried, grind well using mechanical blender to fine powder and transferred it to airtight containers for further analysis.

### Extraction from plant parts

The fine powder was used for extraction by methanol solvent. Fifty grams of sample powder kept into the soxhlet apparatus for distillation. Methanol was taken in the round bottom flask. The apparatus was kept over heating mantle and heated for 8 hours at 70°C. After completing the process, extract was collected in a beaker and was kept in oven at 37°- 40°. The crude concentrated extract was again weighted and used for further biochemical analysis and *in vitro* anticancer studies using *DLA* and *EAC* cell lines.

### 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, coumarins, saponins, anthraquinones, phlobatannins and iridoids were tested.

### Biochemical Analysis

The fresh samples of the *S. acmella* 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, superoxide dismutases (SOD) and polyphenol oxidase (PPO).

### 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 absorbency 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 methanolic leaf extract of *S. acmella* was studied by using *DLA* and *EAC* cells. The crude methanolic leaf extracts from *S. acmella* 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).

### Dalton's lymphoma Ascites cells (*DLA*) and Ehrlich Ascites Carcinoma (*EAC*)

Varying concentrations (100, 500 and 1000 µg/ml) of crude methanolic leaf extract of -

of *S. acmella* were prepared. The cancer cells were aspirated from peritoneal cavity of cancer bearing mice and were washed thrice with normal saline. The cell suspensions ($1\times10^6$ *DLA*/ *EAC* cells in 0.1 ml) were added to tubes containing various concentrations of test extracts (100, 500 and 1000 µg/ml) and volume was made up to 1 ml using phosphate buffer saline (PBS). Control tube contained only cell suspension. The mixtures were incubated for 3 hours at 37ºC and were added with two drops of Trypan blue dye. Dead cells take up the blue colour of Trypan blue while live cells do not. Further percentages of dead cells were evaluated by Trypan Blue Exclusion method. The numbers of stained and unstained cells were counted separately.

**% Dead cells** = [Number of Dead cells/Number of viable cells + Number of Dead cells] × 100.

## Results and Discussion

### Phytochemical Screening

The methanolic extract of *S. acmella* were qualitatively analyzed for the presence of different phytochemicals such as reducing sugar, glycosides, flavonoids, alkaloids, tannins, terpenoids, steroids, coumarins, saponins, anthraquinones, phlobatannins and iridoids. Phytochemical screening of *S. acmella* revealed the presence of reducing sugar, glycosides, flavonoids, tannins, steroids and saponins. But the presence of alkaloids, terpenoids, coumarins, anthraquinones, phlobatannins and iridoids were not detected (Table 1). The presence of these phytochemicals explains the use of this wild leafy plant in ethno medicine for the treatment of various diseases.

### Nutritionl Analysis

To understand the chemical composition of the genus, major primary and secondary metabolites from the plant *S. acmella* were selected and quantified. Sugars plays a vital role in plants both nutrient and central signaling or regulatory molecules that modulate gene expression related to plant growth, development, metabolism, stress response and disease resistance. Sugars are the major product of photosynthesis in plants. Reducing sugar plays an important role in the central metabolic pathways and help in the production of secondary metabolites that enhance the medicinal properties of plants (Deepa and Sumit, 2020). Reducing sugar from *S. acmella* was extracted in distilled water. The result showed high sugar content in the leaves of *S. acmella* (5.123 mg $g^{-1}$) as expressed in Fig. 1. Carbohydrates produced by photosynthesis are well known for their essential role as vital source of energy and carbon skeletons for organic compounds and storage components. Carbohydrates play a major role in plant immunity (Pradeesh and Praveena, 2020). The maximum amount of carbohydrates was present in the leaves of *S. acmella* (10.54 mg $g^{-1}$) as shown in Figure 1.

Proteins are used for the body building; all the major structural and functional aspects of the body are carried out by protein molecules. All proteins are the polymers of amino acids. Plant proteins are obtained from various sources. Plants can readily produce ton quantities of proteins. Proteins are highly complex substance that is present in all living organisms. Proteins are of great nutritional value and are directly involved in the chemical processes essential for life (Pradeesh and Praveena, 2020). 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 leaves of wild plant *S. acmella* (11.92 mg $g^{-1}$) as expressed in Fig. 1. Starch is an insoluble, non-structural carbohydrate composed of α-glucose polymers. It is synthesized by plants and algae to store energy in a dense, osmotically inert form. Starch has significant value for humans and it serves as the main carbohydrate source in an equilibrated diet (Barbara and Samuel, 2016). The amount of starch in the leaves of *S. acmella* is 0.981 mg $g^{-1}$ (Fig. 2).

Chlorophylls are unique pigments with green colour and are found in diverse plants, algae and cyanobacteria. It converts solar energy into chemical energy that is used to build essential carbohydrate molecules (glucose) which are used as food source for the whole plant.

Chlorophyll is an extremely important bio-molecule found in green plants. (Liu *et al*., 2007). The amount of chlorophyll was measured in *S. acmella* and it was found that high contents of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were present (0.778, 0.693, 1.025, 0.583 mg $g^{-1}$) in the plant (Fig. 3). The nutritional analysis of *S. acmella* showed the presence of high amount of reducing sugar, carbohydrate, protein, starch and pigments.

**Antioxidant Estimation**

Proline, an amino acid plays an important role in plants. Proline has been known to be involved in the response to a number of environmental stresses, particularly salt and drought stress. Proline is involved in flowering and development. (Maurizio *et al*., 2008). The amount of proline present in the leaves of *S. acmella* is 0.829 mg $g^{-1}$ (Fig. 4). Lycopene is considered to be one of the most effective carotenoid antioxidants, possessing free-radical scavenging activity superior to that of β-carotene. The health benefits of lycopene can be attributed to its ability to protect cells against oxidative stress. It has a preventive role towards cancer, HIV infection and other chronic diseases (Pradeesh and Praveena, 2020). The amount of lycopene in the methanolic leaf extract of *S. acmella* is 0.713 mg $g^{-1}$ (Fig. 4). Polyphenols are plant non-nutrient natural products so called plant secondary metabolites found in fruits, vegetables and seeds that consumed daily. Polyphenols are a large family of compounds derived from secondary metabolism that are wide spread in the plant kingdom. Polyphenols have two general classes, one is flavonoids and other is phenolic acids. These compounds have wide range of complex structures. Polyphenols attained the prominent position due to their wide distribution in plant-based foods and significant evidences of negative correlation of their consumption with cancers, diabetics and cardiovascular diseases (Munawar *et al*., 2017). The result revealed that total polyphenol content in the leaves of *S. acmella* is 0.792 mg $g^{-1}$ (Fig. 4).

Superoxide dismutase (SOD) is one of the most effective components of the antioxidant defense system in plant cells against reactive oxygen species (ROS) toxicity. SOD was recognized as a group of metallo-proteins available in most cells and is categorized into three main groups on the basis of the metal cofactor at the active sites. This enzyme act as a good therapeutic agent against reactive oxygen species mediated diseases, serve as an anti-inflammatory agent and can also prevent pre-cancerous cell changes (Walid and Faical, 2018). The amount of SOD in the leaves of *S. acmella* is 0.989 mg $g^{-1}$ (Fig. 5) and found to be high. Plant polyphenol oxidases (PPOs) are widely distributed and well studied oxidative enzymes and their effects on discoloration in damaged and diseased plant tissues. Polyphenol oxidase has been purified and characterized from a wide range of plant species and a variety of tissues. These enzymes are broadly distributed among animals, fungi and plants though many plant PPOs appear to lack tyrosinase activity. PPOs have also been implicated in the biosynthesis of some specialized pigments and other secondary metabolites. PPO plays a major role in the development of brown pigments in plants. It is also responsible for the functions including defense, cell differentiation and somatic embryogenesis (Constabel and Barbehenn, 2008). The result of the present analysis revealed that the leaves of *S. acmella* have sufficient amount of PPO (0.847 mg $g^{-1}$) as shown in Fig. 5.

***In vitro* anticancer studies**

Plants have been used for medicinal purposes since the beginning of human history and are the basis of modern medicine. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives. Many compounds isolated from plants are being vigorously tested for their anticancer properties and that showed specificity towards cancer cells. They can induce cell death and inhibit the growth of tumors (Michal *et al*, 2014). The *in vitro* anticancer activity of *S. acmella* was assessed by the Trypan blue exclusion method (Pradeesh and Praveena 2020). 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 (Prasanth *et al*., 2010). Cyclophosphamide is used as standard anticancer compound. The results obtained from anticancer study revealed that methanol extract of *S. acmella* showed remarkable (dose dependent cytotoxicity) anticancer activity against both the test cell lines (*DLA* and *EAC*). Methanol extract of *S. acmella* showed 50.9, 82.35, 90.71% cytotoxicity in *EAC* compared to *DLA* which showed 40.09, 74.31, 85.36% 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 *S. acmella* (Fig. 6). This *in vitro* anticancer activity of methanol extract in both *DLA* and *EAC* cell lines is higher compared to the standard anticancer drug cyclophosphamide (98.17%). *S. acmella* showed higher anticancer activity compared to the anticancer plant reported like *Bidens biternata* (*DLA* -87.19% and *EAC*-92.26%) of *Asteraceae* (Walid and Faical, 2018).

## Conclusion

Present study revealed that *S. acmella* has high amount of nutritional factors like reducing sugar, carbohydrates, protein, pigments and starch. The non-enzymatic and enzymatic antioxidants like proline, lycopene, total polyphenol, superoxide dismutase and polyphenol oxidase were also found to be higher. The plant extract was found to be effective against *DLA* induced solid tumor and *EAC* induced ascites tumor. This may be used to the development of effective therapeutic approaches towards the prevention or treatments of various types of cancer in human beings. The wild green leafy plant *S. acmella* has sufficient nutrients, antioxidants and *in vitro* anticancer activity also. So the plant can be used as a potent nutraceutical agent against a wide variety of diseases of the modern world.

## Acknowledgments

The author is grateful to Dr. T. S. Swapna, Associate Professor and Head, Department of Botany, University of Kerala, Karyavattom, Thiruvananthapuram for necessary support and the Amala Cancer Hospital and Research Centre, Amalanagar, Thrissur for laboratory analysis.

**Table 1.** Preliminary phytochemical screening in *S. acmella* leaves

| Sl. No. | Phytochemicals | Methanol extract of *S. acmella* leaves |
|---|---|---|
| 1 | Reducing sugar | +++ |
| 2 | Glycosides | ++ |
| 3 | Flavonoids | +++ |
| 4 | Alkaloids | _ |
| 5 | Tannins | ++ |
| 6 | Terpenoids | _ |
| 7 | Coumarins | _ |
| 8 | Steroids | ++ |
| 9 | Saponins | ++ |
| 10 | Anthraquinones | _ |
| 11 | Phlobatannins | _ |
| 12 | Iridoids | _ |

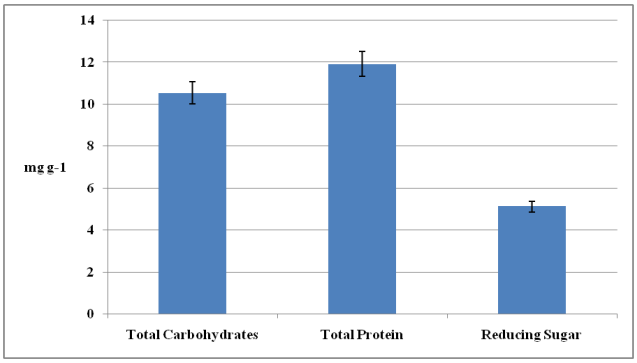


**Figure 1.** Nutritional factors in leaves of *S. acmella*

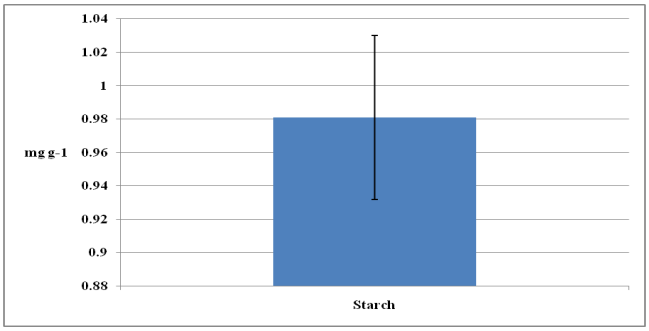


**Figure 2.** Starch in leaves of *S. acmella*

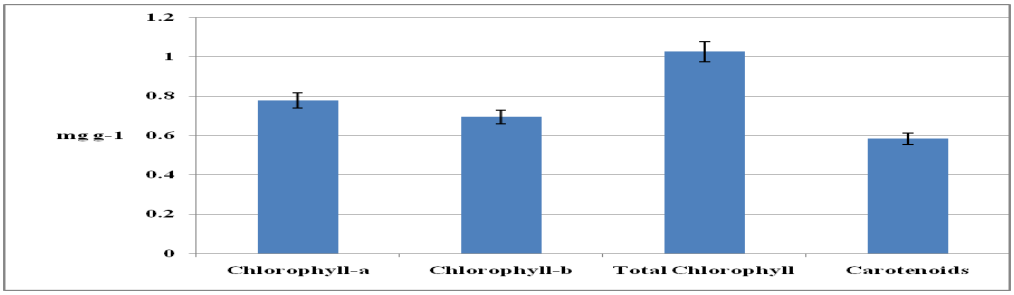


**Figure 3.** Pigments in leaves of *S. acmella*

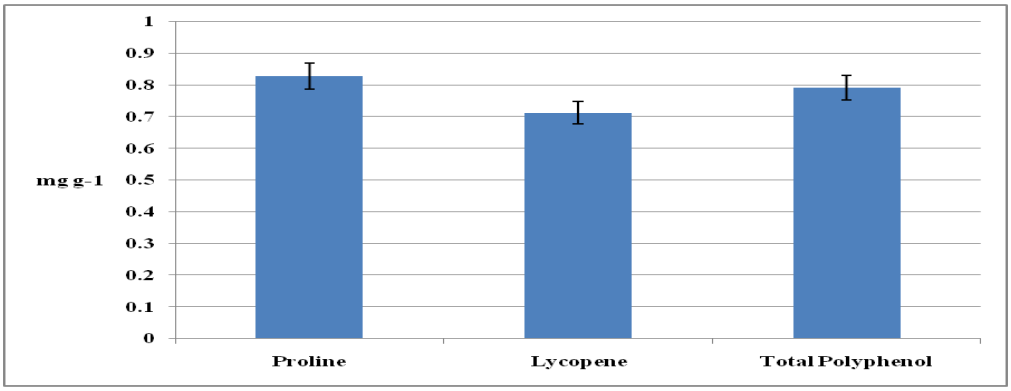


**Figure 4.** Non-Enzymatic Antioxidants in leaves of *S. acmella*

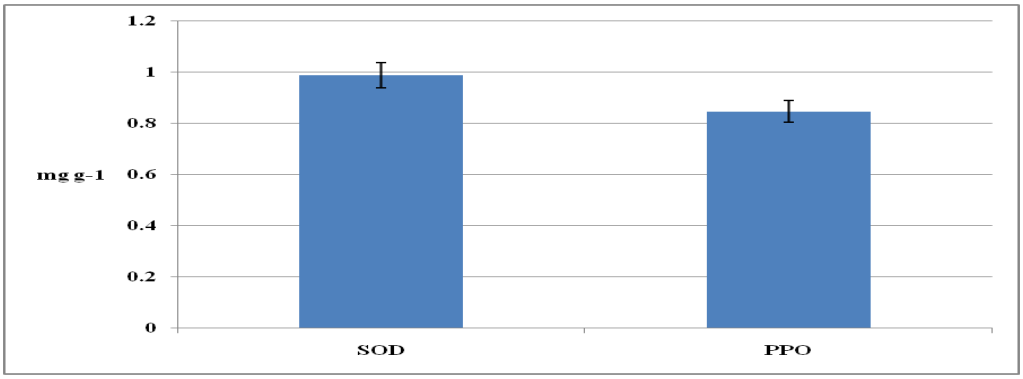


**Figure 5.** Enzymatic Antioxidants in leaves of *S. acmella*

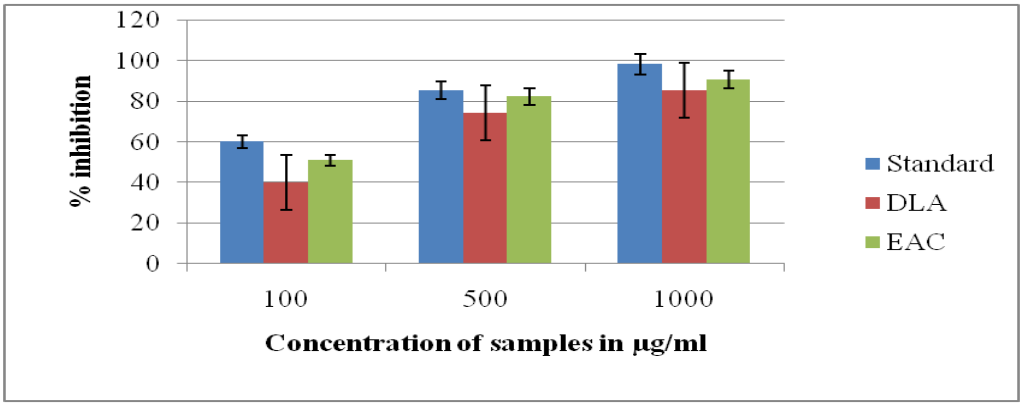


**Figure 6.** *In vitro* anticancer activity in leaves of *S. acmella*

## References

Barbara Pfister and Samuel C.Z. (2016). Cellular and molecular life sciences. Journal of Bio Medical Science. 73 (2): 2781-2807.

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

Constabel C.P. and Barbehenn R. (2008). Defective roles of polyphenol oxidase in plants. J. Intro Plant Resist Herbi. 8(12):253-270.

Deepa Khatri and Sumit B.B. (2020). Reducing sugar, total phenolic content and antioxidant potential of Nepalese plants. Int J Biomed Res. 10(55): 1-10.

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. Sci and Biotechnol. 17: 408-412.

Esterbauer H., Dieber R.M., Striegl G., Waeg G. (1991). Role of Vitamin-E in preventing the oxidation of low density lipoprotein. American J Clinic. 53: 314-321.

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

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

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

Liu Y.T., Perera C.O., Suresh V. (2007). Comparison of three chosen vegetables with others from South East Asia for their lutein and zeaxanthin content. Food Chem. 101: 1533-1539.

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951). Protein measurement with the folin phenol reagent. J Biol Chem. 193: 265-275.

Maurizio T., Roberto M., Paolo C. (2008). Multiple roles of proline in plant stress tolerance anddevelopment. J. Sci Direct. 19(1): 325-346.

Michal L., Elisha S., Sarah S., Helena P., Elaine S. and Haya L.G. (2014). Evaluating medicinal plants for anticancer activity. Scienti World Journal. 31(1): 100-110.

Miller G.L. (1972). Use of dinitrosalycylic acid reagent for determination of reducing sugars. Anal Chem. 31: 426 - 428.

Munawar A., Faqir M.A., Farhan S. and Muhammad A. (2017). Natural polyphenols: an over view. Inter J. Food Proper. 20(8): 1689-1699.

Pradeesh S. and Praveena P. (2020). Phytochemical investigation and *in vitro* anticancer activity of *Sesbania grandiflora* (L.) Pers. – A wild leafy vegetable of Southern Western Ghats. J. Advances in Biol Sci. 7(1&2): 49-53.

Prasanth N.V., Dilip C., Sanal D. (2010). Evaluation of *in -vitro* cytotoxic and antioxidant activities of *Ipomoea batatos*. Inter J Pharma and Pharmaceu Sci. 2(3): 12-17.

Walid S. and Faical B. (2018). Superoxide Dismutase (SOD) and abiotic stress tolerance in plants: an over view. 5(80): 3-4.

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

Zakaria H., Simpson K., Brown P.R., Krotulovic A. (1979).Use of reversed phase HPLC analysis for the determination of provitamin-A, carotenes in tomatoes. J Chromato. 176: 109-117.