Comprehensive nutritional profile analysis of *Simarouba glauca* (DC). leaves extract

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Abstract

Simarouba glauca (DC). [S. glauca (DC).] is an essential medicinal plant known to possess good pharmacological and therapeutic potential. Despite the medicinal importance of S. glauca (DC), there is no documented information on its nutritional composition. Thus, the present work is focused on analysing the proximate composition (carbohydrates, protein, energy, moisture, ash, fat), minerals content (Zn, Cu, Mn, Mg, Fe, Ca) and reducing sugar content. We also looked into the presence of water-soluble vitamins and amino acid contents present in the lyophilized S. glauca (DC) and leaves aqueous extract (SGALp). Analysis of SGALp revealed the presence of 74.69% carbohydrates, 8.86% ash, 12.33% moisture and 4.12% protein and the total energy was calculated to be 315.45 kcal/100g. The leaves contain a significant amount of calcium (120mg/100g) and a lower amount of copper (0.79mg/100g). The reducing sugar content was found to be 0.03g/100g.

Further, the Ultra Pressure Liquid Chromatography-Mass spectroscopy (UPLC–MS) analysis showed the presence of water-soluble vitamins such as pantothenic acid (68.17mg/100g) and riboflavin (0.012mg/100g). Lastly, amino acids analysis revealed the presence of all essential and nonessential amino acids with a high amount of glutamic acid (2.84mg/100g). In conclusion, the current investigation shows that the S. glauca (DC). leaves extract could serve as an excellent source of minerals, reducing sugar, vitamins and amino acids which could be used for therapeutic purposes.

Keywords: *S. glauca* (DC), water-soluble vitamins, amino acids, proximate analysis, mineral content.

Introduction

Plants serve as an excellent source of various nutrients to meet the metabolic requirements of animals and humans for their well-being, growth and productivity. There is, however, a lack of systematic study of some of the multipurpose medicinal plants that are effective at combating malnutrition and meeting dietary needs[1]. The plants are known to contribute towards optimal health and development as well as to assist in reducing the risk of or delaying the onset of various diseases and disorders[10]. Thus, plants are a necessary and integral component of complementary and alternative medicine[26]. These are the primary source of thousands of drugs as well as biologically important minerals, vitamins, amino acids and phytochemicals that play a vital role in living organisms. Plant sources are always known to be risk-free, affordable and easily accessible[24].

In addition, herbal drugs are believed to enhance the body's natural resistance against infection and their immunomodulatory activities have been reported in numerous plants. Among several important medicinal plants, *Simarouba glauca* (DC). [*S. glauca* (DC).] is a rainfed evergreen tree, also called Laxmitaru or paradise tree, which belongs to the family Simaroubaceae. It is universally present due to its wide adaptability to various climatic and agro-climatic conditions such as drought land and marginal wasteland. The tree typically reaches a height of 12-15 meters and has a circular crown. The leaves are bright green and pinnate compound, measuring about 20-50cm long [31]. The bark and leaves have a long history of being used as herbal drugs by traditional practitioners to treat malaria, dysentery and fever.

The *S. glauca* (DC) plant extracts can be used as homeostatic, anti-helminthic, antiparasitic, anti-dysenteric, antipyretic, anti-viral and anti-cancerous agents[20]. It also helps in reducing patchy skin pigmentation. The aqueous extract of *S. glauca* (DC). is known to increase keratinocyte differentiation and facilitate skin hydration and moisturization. The bioactive compounds present in *S. glauca* (DC). plant include glaucarubine, quassinoids, ailanthinone, benzoquinone, holacanthone, melamine, simaroubidin, macrolide, simarubin, simarubolide and sitosterol, which possess magnificent pharmacological and therapeutic properties[31]. Quassinoids, alkaloids present in the Simaroubaceae family, exhibit antimalarial and cytotoxic properties, mainly inhibiting protein synthesis[31].

S. amara belongs to the family Simaroubaceae and exhibits antibacterial properties against pathogenic enterotoxigenic *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by *in vitro* method[14]. The secondary metabolites present in *S. amara* are also responsible for the anti-inflammatory, immunostimulatory, cell-mediated and humoral immune response[15]. Previously, the studies on the *S. glauca* (DC) dried leaves powder showed the presence of important minerals and vitamins[19]. In addition, the phytochemical contents of *S. glauca* (DC) seed and meal have been studied which are known to possess phenolics, flavonoids, saponins, phytic acid, proteins and minerals[11].

According to previous studies, considerable qualitative and quantitative variations in the nutritional composition of the *S. glauca* (DC) have been observed and these variations might have been influenced by various biotic and abiotic factors[6]. The present study aims to investigate the presence of various components and to understand their role in imparting the pharmacological activity of *S. glauca* (DC) leaves.

Material and Methods

Collection of plant leaf extract: The fresh leaves of *Simarouba glauca* (DC) [*S. glauca* (DC).] were collected from the botanical garden of Kuvempu University, Shankaraghatta, Shivamogga during September 2023 (Fig. 1). The leaves were washed with tap water followed by distilled water and dried in the shade for one week and stored in air-tight polythene bags.

Figure 1: Young *Simarouba glauca* (DC). plant

Preparation of *S. glauca* (DC). aqueous leaf extract lyophilized powder (SGALp): The aqueous extract of *S. glauca* (DC) leaves was prepared according to the method described by Ennaifer et al[8]. Briefly, 10 grams of leaves were extracted with 100 ml of distilled water (1:10 w/v ratio) by boiling for 15-20 min at 121°C. After cooling, the mixture was filtered through Whatmann filter paper no.1 to obtain the aqueous extract. The extract was then concentrated using a lyophilizer and stored in airtight vials at 4°C. The percentage yield was obtained to be 1.3g/10g of dried leaves. The fresh lyophilized sample was used for nutritional analysis of the nutritional composition of *S. glauca* (DC).

Proximate composition: The proximate composition such as moisture, total ash, total fat, total protein, total carbohydrates and calorific values of SGALp were determined by following standard protocols[2]. For testing the moisture content, 1g of SGALp was weighed into a crucible and dried for 24 h at 60°C. Based on the weight lost after drying, the moisture content was calculated. The dry-ashing method was used to estimate the ash content by heating 1 g of SGALp at 500° C for 5-6 h in a muffle furnace. The ash content was calculated by comparing the measured weight before and after ignition. The Soxhlet method was used to extract fat and estimate crude fat in SGALp. The total protein was estimated by the micro Kjeldahl method. The total carbohydrates present in SGALp were estimated by calculating the difference as follows:

Carbohydrate (%) =100-[Crude protein (%) +Crude fat (%) +Moisture (%) +Ash (%)]

The calorific value was calculated by using the formula:

Energy value = (% Crude protein) × 4+ (% Carbohydrates) × 4+ (% Crude fat × 9)

Determination of mineral composition: The mineral composition of SGALp was analyzed by atomic absorption spectroscopy (AAS) as described earlier [42] with slight modifications [38]. Initially, the SGALp was kept at 80°C in a porcelain crucible and dried to remove the moisture. Subsequently, 1g of the sample was treated with 12 ml of concentrated nitric acid for 24 h at 200°C. Further, 5 ml of a mixed acid solution containing nitric acid and perchloric acid (3:1 ratio) was added and heated up to 120-130°C for 5-6 h until there were no fumes and the resulting mixture was clear. This was allowed to cool to room temperature and filtered through Whatmann filter paper. The filtrate was made up to 50 ml with Milli-Q water and the solution was used to estimate the micro and macro mineral content by AAS (model GBC-932 AA). The quantitative analysis was made for each element by using calibration standards.

Estimation of reducing sugars: The reducing sugars present in SGALp were estimated by the dinitro salicylic acid (DNS) method with some modifications[23]. The measurement was performed according to the procedure described earlier [41]. For the measurement, 2 ml of DNS reagent was pipetted into a test tube containing 1 ml of SGALp (1mg/ml) and kept at 95°C for 5 min. After cooling, 7 ml of distilled water was added to the solution and the absorbance was measured at 540 nm. The reducing sugar content was calculated from the calibration curve of standard D-glucose (200-1000mg/L) and the results were expressed as mg D-glucose equivalent (GE) per g dry extract.

Quantification of water-soluble vitamins by UPLC-MS/MS

Extraction of water-soluble vitamins: The water-soluble vitamins were extracted by following the procedure as described earlier[37]. 0.5 g of sample was extracted with 5 mL of 10 mM ammonium formate and methanol (50:50, v/v) containing 0.1% butylated hydroxytoluene by vigorously shaking in a micro grinder to obtain the uniform mixing for 5 min, which was then subjected to sonication for 15-20 min at 25ºC in an ultrasonicator. The sample was then centrifuged at 14,000xg for 15 min. The supernatant was withdrawn, filtered through a 0.45 µm nylon filter and the volume was made up to 2 ml. Finally, the supernatant was dried in a nitrogen stream and reconstituted in 1 mL of the mobile phase, filtered through a 0.2 µm nylon filter and injected into a UPLC-MS/MS system to determine the

water-soluble vitamin content. At the time of the extraction process, the sample was kept on ice in dark conditions to minimize vitamin degradation.

LC and MS-MS conditions: The water-soluble vitamins were analyzed using Acquity Ultra Pressure Liquid Chromatography-Mass spectroscopy (UPLC-MS) coupled with a Tandem (Triple) Quadrupole Detector (TQD)-MS/MS system (Waters). For analysis, a 2.1 x 50 mm UPLC BEH C18 column with 1.7µm particle size, protected by a vanguard BEH C-18 guard column with 1.7µm particle size was used (Waters, USA). The column temperature was maintained at 25°C and Mass lynx™ was used for data acquisition and control of the mass spectrophotometer. The mobile phases, A and B used were 0.1% formic acid in water and acetonitrile respectively. The initial gradient composed of 60% A and 40% B at a flow rate of 0.3mL/min, brought down to 30% of A and 70% of B using a linear gradient in 14 min and then brought back to initial conditions after 15 min. The injection volume used was 10 µl. For identification and quantification, the MS was operated at positive ESI mode. The capillary voltage and temperature used were 3.5 kV and 200°C respectively and the collision gas flow was 50 L/h and 0.01ml/min. Subsequent identification and estimation of the eluted compounds were performed using MS with ESI+ ionization followed by multiple reaction monitoring (MRM) methods[32].

Quantification of amino acids by High-Performance Liquid Chromatography (HPLC)

Sample preparation: The extraction of amino acids was performed by a method described earlier[21] with slight modifications[13,29]. Briefly, 0.2g of the sample was homogenized with 6N HCl and kept at 110^0C in a closed vial. After 24h, the sample was centrifuged at 3,000 rpm. The supernatant was derivatized through automated pre-column OPA and it was filtered through a 0.2µm nylon filter membrane and injected into the HPLC column.

HPLC conditions: The amino acid composition was determined by using HPLC (Agilent G6550) equipped with a binary pump, a hip sampler and a diode array detector. Samples were analyzed using a column of Agilent Infinity Lab Poroshell HPH-C16 (4.6 X 100mm) with a pore size of 2.7 microns. The mobile phase solvent A used was water: Acn: MeOH (45:45:10) and the solvent B was phosphate buffer (10mM, pH 7.4). The initial gradient was composed of 98% (A) and 2% (B) with a flow rate of 1000ml/min brought down to 43% (A) and 57% (B) in 13.40 min. The system was brought to its initial conditions after 18 min and the flow rate was 0.1 mL/m. The sample injection volume was 20µL and the column temperature was 37°C. The amino acid content was calculated based on the amino acid calibration standard run at 90, 225 and 900 nmol/ml.

Statistical analysis: The obtained data of proximate composition, mineral composition, reducing sugar, vitamins and amino acids were expressed as mean ± standard deviation (SD). All the tests were carried out in triplicate. Statistical analysis was performed using Microsoft Office Excel 2016.

Results and Discussion

Proximate composition: Proximate analysis was used to identify the approximate composition of various substances present in the plant extract to ensure that it is safe for human and animal consumption (Fig. 2). The proximate composition of SGALp shows that *S. glauca* (DC). plant is primarily composed of carbohydrates, which make up 74.69 % of its overall content. In contrast, the shade-dried leaves of *S. glauca* (DC). were found to contain 40.5 % carbohydrates[27]. As the recommended dietary allowance (RDA) value of carbohydrates for children and adolescents is 130g, this plant preparation could serve as a potential source of energy.

The moisture content, which measures the quantity of water and volatile substances present in the plant, was found to be 12.33 %, which is higher than the value of 9.95% reported in an earlier study on the dried leaves of *S. glauca* (DC)[27]. The ash content of the plant, which refers to the inorganic constituents present in the plant, was also determined. In our study, the ash content was found to be 8.86%, but it was reported to be absent in fresh and dried leaves as per the earlier study[27]. The protein content was found to be 4.12%. Finally, the SGALp lacked total fat but had 315.45 kcal/100 g of energy content. Overall, the data on the proximate composition indicates the variations depending on the plant parts used and the geographical location.

Determination of mineral composition: The mineral content analysis of SGALp shows the presence of six minerals, of which four were microminerals (Fe, Mn, Zn and Cu) and two were macrominerals (Ca and Mg). The macrominerals Ca (120mg/100g) and Mg (4.3mg/100g) were present at high concentrations (Fig. 3). Among microminerals, the extract was found to contain different concentrations of Fe (22.6mg/100g), Mn (6.24 mg/100g), Zn (5.58mg/100g) and Cu (0.79mg/100g). Previously, it has been reported that *S. amara* leaves contain higher amounts of Mg (3240.6 µg/g), Fe (318.4 µg/g), Cu (315.3 µg/g) and low content of Zn (10.1 µg/g) as compared with the values obtained for *S. glauca* (DC). in this study[11].

Calcium is one of the major macronutrients that is mainly helpful in bone and teeth development, muscle contraction and normal blood clots. The RDA for Ca is 1000 mg/day and the lack of calcium leads to rickets in children and osteoporosis in adults[43].

In our present study, the SGALp was found to contain 120 mg/100g, which is lower as compared with *S. glauca* (DC) oilseed meal, having 143mg/100g[11]. Magnesium is an essential and abundant biological element that plays various roles including enzyme activation, as well as facilitating DNA and RNA replication.

The RDA value for magnesium is 400 mg/day[34]. Zinc is an essential trace element that performs many physiological functions such as the formation of hormones acting as a coenzyme. It has a regulatory effect on the production of cytokines, activation of the complement system and antibody formation. It also helps to maintain the integrity of gut mucosa to reduce fluid loss during diarrhea and the RDA value for zinc is 10 mg/day[39].

Iron is one of the most important minerals that performs vital functions in the body such as erythropoiesis, cellular energy metabolism, immune cell development and oxygen transport. Our data has shown that the SGALp is rich in iron to the extent of 22.60 mg/100 g and the RDA value of iron is 8 mg/day [7]. Copper is a micronutrient that mainly helps in the production of RBCs and WBCs and also triggers the release of iron to form haemoglobin. It is also important for infant growth and brain development and the RDA value of copper is 1.35 mg/day [40]. Manganese is one of the important minerals acting as a cofactor for many enzymatic reactions such as phosphoenol pyruvate, carboxyl kinase and glutamine synthetase. The RDA value of manganese is 420 mg/day[17].

Our present study of analysis of minerals in SGALp has indicated the presence of Ca, Fe, Mn, Zn, Mg and Cu which are very essential for proper biological functioning in humans and other higher organisms. The SGALp extract containing a sufficient amount of these essential nutrients could serve as an important source to maintain proper health by coordinating various physiological functions[44].

Estimation of reducing sugar content: The reducing sugar content (RSC) was calculated from the calibration D-glucose standard curve and was expressed as GE/g dry extract weight. The RSC in SGALp was found to be 0.03 mg/g dry weight. These sugars play a prominent role in plant growth, development and metabolism. The secondary metabolites are mainly synthesized by the sugars through the primary carbon metabolic process which enhances the medicinal properties of plants[9].

Quantification of water-soluble vitamins by UPLC-MS/MS method: The water-soluble vitamin content of SGALp was determined as shown in table 1. The major vitamins such as pantothenic acid (vitamin B5), biotin (vitamin B7), niacin (vitamin B3) and pyridoxine (vitamin B6) were found to be present in the extract representing 68.17, 11.10, 5.48 and 4.77mg/100g of lyophilized plant sample respectively.

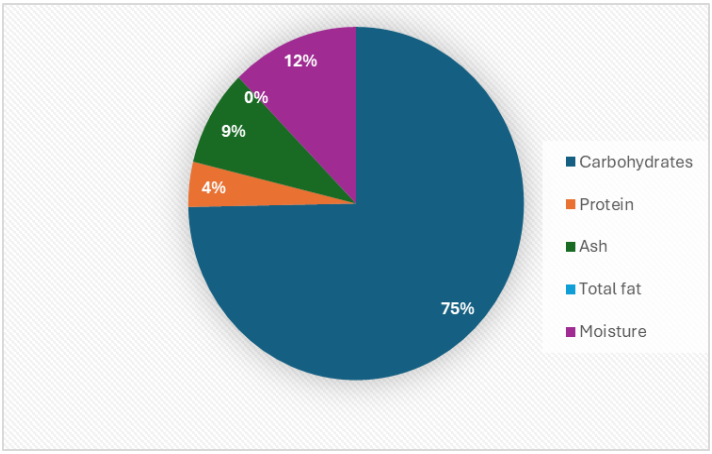

Figure 2: Proximate composition of *Simarouba glauca* (DC).

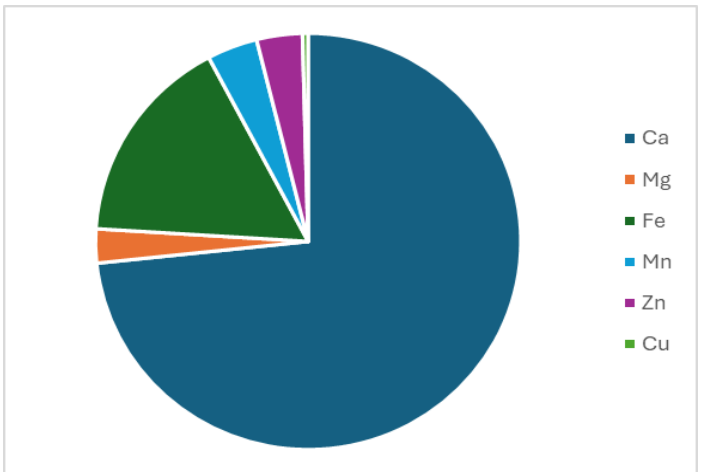

Figure 3: The mineral content of Simarouba *glauca* (DC).

The other vitamins such as folic acid (vitamin B9), thiamine (vitamin B1) and riboflavin (vitamin B2) are present in lower amounts to the extent of 0.056, 0.047 and 0.01mg/100g of dry weight respectively. Previously, it has been reported that *S. glauca* (DC). leaves ethanolic extract has 0.64mg of thiamine (vitamin B1) and 1.65mg of riboflavin (vitamin B2) per 100g, which is higher than what is reported in the present study[33].

Vitamins are the vital nutrients required in our diet in smaller quantities that perform specific biological functions for the organism's normal growth and health. The B vitamins are water-soluble and are helpful in body metabolic functions [18]. Thiamine is an anti-neuritic vitamin that acts as a specific coenzyme, thiamine pyrophosphate, which is associated with carbohydrate metabolism and is helpful in the transmission of nerve impulses[35]. The RDA value of thiamine is 1.1-1.2 mg. Riboflavin is an essential coenzyme that participates in many redox reactions and is responsible for energy production[36]. The daily requirement of riboflavin is 1.1-1.3 mg. Pyridoxine functions as a coenzyme in amino acid metabolism and plays a crucial part in the production of neurotransmitters, DNA and haemoglobin. The RDA value of pyridoxine is 1.3-1.7 mg. The function of biotin is to help in carboxylation, lipid metabolism and the regeneration of hair, skin and nails whose recommended daily intake is about 30 µg[5].

Pantothenic acid acts as a co-enzyme A involved in all carbohydrate, lipid and protein metabolism whose RDA value is about 5 mg. Folic acid is mainly helpful in red blood cell production and neural tube formation. It also acts as a coenzyme in single-carbon metabolism. The recommended daily intake of folic acid is 400 µg[30]. The present study on the analysis of vitamins in SGALp indicates the presence of important vitamins such as thiamine, riboflavin, niacin, pyridoxine, biotin and pantothenic acid which play a vital role in important physiological functions. The consumption of this could serve as an important health supplement to prevent hypovitaminosis which could lead to severe pathological conditions[3].

Quantification of amino acids by High-Performance Liquid Chromatography (HPLC): A total of 5 essential amino acids and 5 non-essential amino acids were found to be present in SGALp (Table 2 and fig. 4). In general, essential and non-essential amino acids need to be supplemented in the optimal ratio to perform healthy physiological functions of living organisms [25,45].

Table 1
LC-MS/MS analysis of water-soluble vitamins in *Simarouba glauca* (DC). leaves

S.N.	Water soluble Vitamin	RT	Quantity (mg/100gDW)
1	Thiamine(B1)	1.60	0.047 ± 0.002
2	Riboflavin (B2)	8.62	0.012 ± 0.001
3	Niacin (B3)	2.15	5.486 ± 0.2
4	Pantothenic acid (B5)	7.20	68.17 ± 0.2
5	Pyridoxine (B6)	3.02	4.772 ± 0.02
6	Biotin (B7)	8.58	11.10 ± 0.08
7	Folic acid (B9)	10.36	0.056 ± 0.02

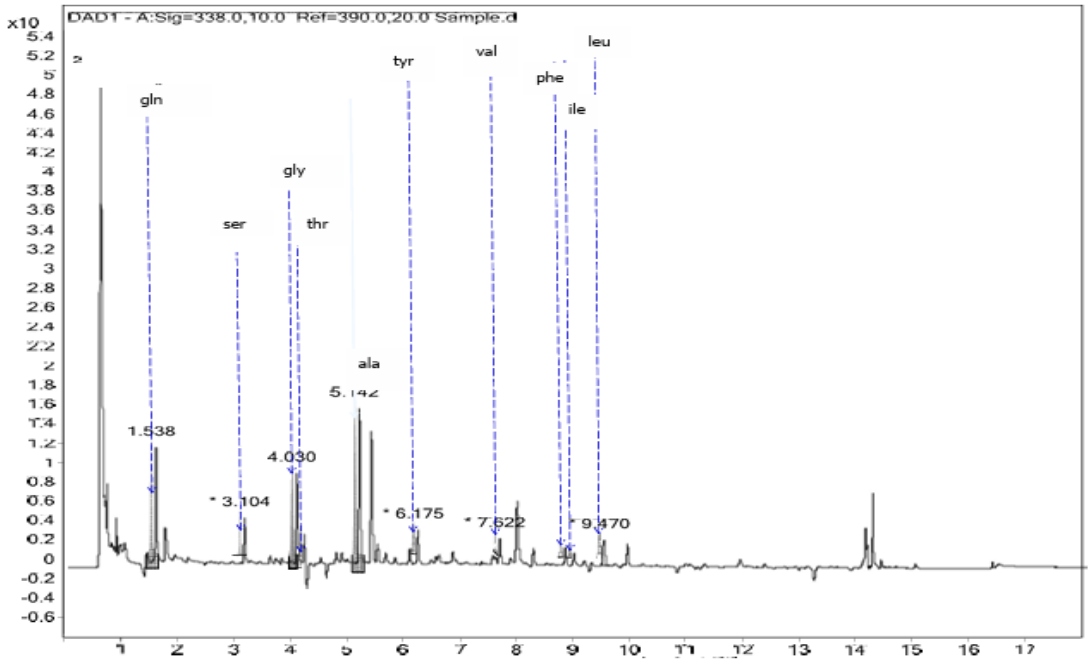

Figure 4: Analysis of *S. glauca* (D C). leaves amino acid composition by HPLC

Table 2
Amino acids content in the *Simarouba glauca* (DC).
leaves extract (n=3), [a] Essential amino acids, [b] nessential amino acids.

Amino acids	Quantity (mg/100g DW)
Phenylalanine	1.46 ± 0.08
Leucine[a]	1.39 ± 0.16
Threonine	1.30 ± 0.14
Isoleucine[a]	0.98 ± 0.30
Valine[a]	0.91 ± 0.05
Glutamic acid[b]	2.84 ± 0.1
Alanine[b]	2.45± 0.08
Tyrosine[b]	1.79 ± 0.04
Glycine[b]	1.32 ± 0.08
Serine[b]	1.15 ± 0.10

Essential amino acids are not synthesized in the body and they are obtained through an exogenous diet[16]. Among essential amino acids, phenylalanine was found to be present at the highest concentration (1.456 mg/100g). Phenylalanine is mainly helpful in the synthesis of protein, catecholamine, melanin and thyroid hormone [22]. Other amino acids such as leucine, threonine, isoleucine and valine are present to an extent of 1.388, 1.297, 0.9836 and 0.905mg/ 100g extract respectively. The non-essential amino acids are mainly synthesized in the body and hence, they are not dependent on exogenous sources. The most abundant non-essential amino acid was found to be glutamic acid followed by alanine, tyrosine, glycine and serine which are present to an extent of 2.841, 2.451, 1.790, 1.319 and 1.149mg/ 100g extract respectively.

In a previous study, *S. glauca* (DC) meal was found to contain all the essential amino acids among which, threonine and leucine were in higher concentrations and tryptophan was in lower amounts[14]. The presence of both essential and non-essential amino acids in the SGALP helps in the efficient synthesis of proteins, neurotransmitters, hormones and enzymes, which are necessary to ensure the optimal functioning and health of the organism.

Conclusion

S. glauca (DC). plant is being used by traditional healers for curing various ailments effectively without understanding the type of active components present in the extract. Previous studies conducted in our laboratory showed antimicrobial, antidiarrheal, immunostimulatory and immunomodulatory properties of this plant extract in mice models[15,28]. Numerous studies have revealed that medicinal plants are also rich in several nutritionally rich components, minerals, vitamins and amino acids. The SGALp is a rich source of essential nutrients such as carbohydrates (74.69%) and proteins (4.12%) without the presence of fats. In addition, it contains a sufficient amount of essential amino acids (phe, leu, thr, iso and val) that are mainly helpful in the synthesis of biologically important molecules and minerals (Ca, Fe, Mn, Zn and Cu) that could meet daily requirements. SGALp also possesses a good amount of components of the vitamin B complex (B1, B2, B3, B5, B6, B7 and B9) which could be a promising supplement to prevent malnutrition and these supplements are also helpful in the recovery from diseases.

The limitation of this study is that we used only water extract for all the analyses and thus, the components that are soluble in different solvents, have not been isolated and studied. However, the use of water extract in our studies is well justified as it was the only solvent used by the traditional healers in treating various ailments. The present data is critical in designing further studies using different solvents and comparisons of different extract compositions. Further, the aqueous extract of *S. glauca* (DC) leaves is safe for human consumption. The obtained data thus indicates that the significant amount of these nutrients in SGALp leaves should help in the development of a potential nutritional supplement for proper human health and growth.

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