

PHYTOCHEMICALS, ANTI-NUTRITIONAL FACTORS AND PROXIMATE ANALYSIS OF SIMAROUBA GLAUCA SEEDS

**Haleshappa R^{*1}, Sajeeda Niketh², Rajeev R. Kolgi³, Sharangouda J. Patil^{*4},
K. R. Siddalinga Murthy⁵**

^{1,2,3}Assistant Professor, Department of Chemistry and Biochemistry, Government Science College (Autonomous),
Nrupathunga University, Bengaluru, Karnataka, India

⁴Associate Professor, Department of Zoology, NMKRV College for Women,
Bengaluru, (Autonomous), Karnataka, India

^{1,5}Professor, Department of P.G. Studies and Research in Biochemistry,
Bangalore University, Bengaluru, Karnataka, India

Abstract: Medicinal plants are rich treasure for nutrition and are considered as bio-medicine in day-to-day life. Simaroubaglauca is an Indian traditional medicinal plant commonly called as “Paradise Tree or Lakshmi Taru” worldwide due to its potent medicinal stuff. The goal of this study was to determine several phytochemicals, antinutritional factors and proximate analysis in the *S. glauca* seeds. Ethyl acetate extract of seeds of *S. glauca* was analyzed for the qualitative and quantitative phytochemicals by various validated methods. To determine the anti-nutritional factor used the different physical processes to identify oxalate, phytate, saponins and tannins following standard methods. In the qualitative method of phytochemicals exhibited positive Steroids and Triterpenoids, Alkaloids, Tannins, Flavonoids, Glycosides and Carbohydrates and in the quantitative method of phytochemicals found total flavonoid content highest at 80 μ g concentration of extract exhibited 2.152 μ g/ml, total proanthocyanidin content highest at 80 μ g concentration of extract exhibited 2.246 μ g/ml and in total phenol content highest at 100 μ g concentration extract of exhibited 2.450 μ g/ml. The seeds of *S. glauca* were subjected to processing treatments like roasting and Soaking to reduce or inactivate the anti-nutritional factors and the results of roasted seeds exhibit moderate concentrations of oxalates and saponins, lower concentrations of tannins and phytates. Similarly, in soaked seeds also exhibited higher concentrations of tannins and oxalates, moderate concentration of saponins, whereas the lower concentrations in phytates. The seeds of *S. glauca* are subjected to proximate analysis to determine the crude composition and were found to have 8.6 % moisture, 1.5 % ash, 8.1 % fibers, 40.2 % fats, 25.5 % proteins and 16.1 % carbohydrates.

Keywords: Phytochemical, Anti-nutrition, Proximate, Simaroubaglauca, Seeds

I. INTRODUCTION

Medicinal plants have been mentioned and protocol as important healthcare remedy from ancient times. Plants which furnish medicinal attributes are known to have an advantage of possessing a range of chemical diversities of secondary metabolites. At this point view around 30% of the vast range of species has been discovered, meaning the potential for many more effective cures are there, just waiting to be cured and utilized [1]. Even now they are economically important, being used in the agriculture, pharmaceutical, cosmetic, perfumery and nutraceutical industries [2,3]. Often these are of superior, and if not, of homogeneous attributes as contemporary synthetic drugs [4]. A vast range of phytochemicals that includes glycosides, flavonoids, anthocyanin, alkaloids and fatty acids among others, have been discovered. Medicinal ideas which have been passed onto generations, other than that few are very commonly seen and applied in day to day life, should be brought into scientific insight as an effective and alternative solution. Plant chemistry has been developed over the years as a definite discipline between natural product chemistry and plant organic chemistry. It is related to the wide range of organic substances that are produced and accumulated by plants, deals with the chemical components, structures of these substances, their biosynthesis, metabolism and biological function[5, 6].

Antinutritional factors are produced in plants as a secondary metabolites serve as important agents in defense mechanism against to herbivores and insects. They are produced in plants by normal metabolism. The anti-nutritional factors range from high molecular weight proteins, such as lectins, protease inhibitors, amylase inhibitors and toxins, such as ricin, to simple amino acids, such as β -diamino propionic acid and mimosine to oligosaccharides, such as α -galactosides. These act to counter optimum nourishment of nutrients resulting in nutritional deficiency disorders due to malnutrition [7]. Proximate analysis also support for determining the crude nature of plant products. In that context crude protein, crude fibre, total ash, moisture content, dry matter as a proximate composition of food samples are influenced by various factors such as variety, climate, cultivar, maturity and production conditions, handling, processing and storage [8]. Proximate analysis for foods helps in nutrient labeling and data validation for food processing.

Simarouba glauca is Indian traditional medicinal plant commonly called as “Paradise Tree or Lakshmi Taru” in worldwide due to its potent medicinal stuffs. Simarouba is a unique tree that all its parts are useful in one way or other. Seeds are economically important, as they contain 55–65% edible oil, which can be used in manufacture of vegetable fat or margarine. Simarouba oil is also used in industrial manufacture of soap, lubricant, paint, polishes and pharmaceuticals etc. Shells (endocarp) are used in hard board industry. Semi sweet fruit pulp, containing 11–12% sugars is eaten and is well suited for fermentation/beverage industry [9]. Leaf litter makes good manure. Bark and leaf of simarouba contain triterpenes useful in curing amoebiasis, diarrhoea and malaria. Simarouba is subject of one US patent, whereby its water extract was found to increase skin keratinocyte differentiation and to improve skin hydration and moisturization. Scanty scientific literature available on Simarouba, mainly deals with composition and characteristics of its fat. The odorless, greenish yellow fat melts at 26.4 C and has an iodine value of 52.6 and saponification value 190.5 [10]. Fatty acid composition of Simarouba fat has been investigated by several researchers [11] and major components are oleic (52–54%), stearic (27–33%) and palmitic acid (11–12%). Jeyarani and Reddy [12] reported that characteristics of Simarouba fat and its fatty acid composition of Indian origin do not significantly differ from those reported from seeds of other countries.

In our current study determined to identify the phytochemicals, anti-nutritional factors and proximate analysis of Simarouba glauca seeds, which act as potential natural drug, toxic and adherent for the nutritional properties and also to know the crude nature of drugs.

II. MATERIAL AND METHODS

Collection of seeds

The fresh seeds of Simarouba glauca were collected from Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bengaluru, Karnataka, India., during fruiting season i.e., in the month of April to August and authenticated by Dr. Shiddamallayya Mathpathi, Research Officer (Botany), at Regional Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH and Govt. of India. Collected seed material was immediately sprayed with ethanol to cease the enzymatic degradation of secondary metabolites. The seeds were shade dried, chopped into small fragments and powdered inside the laboratory within 10-15 days at room temperature (28-30°C).

Soxhlet extraction of seed constituents

The shade dried, powdered 100gm seed material was subjected to soxhlet extraction successively and separately from non-polar to polar solvents i.e., petroleum ether (b.p. 60–80°C), ethyl acetate and ethanol (95%) in a soxhlet extractor for 48 hours each. The extracts were concentrated to dryness in a flash evaporator (Buchi) under reduced pressure and controlled temperature (50–60°C) to obtain the crude extract. Remaining trace of the solvent if any was further removed by placing the crude extracts in vacuum overnight.

Phytochemical investigation of the crude extracts of Simarouba glauca seeds.

Phytochemical screening of the ethyl acetate extract of the plant was carried out in order to know the class of organic compounds present in the different extracts of the seeds selected for the study, which further facilitates for the identification of active constituents and their isolation.

The petroleum ether, ethyl acetate and ethanol extracts of Simarouba glauca seeds were subjected to standard phytochemical tests as described by Sharangouda and Patil [13], Harnborne, [14] and Fransworth, [15] to qualitative determination of steroids & triterpenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates, proteins and amino acids.

Quantitative phytochemical assay of crude extract of Simarouba glauca seeds

The ethyl acetate extract was used for the quantitative phytochemical analysis in triplicate to obtain concurrent values for statistical analysis while maintaining positive and negative control in particular wavelength in spectrophotometer to determine the $\mu\text{g/ml}$ concentration [14].

Determination of total flavonoid content

Ethyl acetate extract of *S. glauca* seeds were carried out to determine the total flavonoid content (TFC) using AlCl_3 method with standard quercetin [16]. The 510 nm wavelength used for the absorbance to read the sample. The TFC was expressed as μg of quercetin equivalents/mg of ethanol extract.

Determination of total proanthocyanidin content

Ethyl acetate extract of *S. glauca* seeds were carried out to determine the total proanthocyanidin content (TPAC) using vanillin–hydrochloride method as described by Kamala et al., [17]. The 500 nm wavelength used for absorbance to read the sample along with the blank vanillin–hydrochloride. The prepared catechin solution was used at different concentrations of 5–25 $\mu\text{g/mL}$ in methanol to detect the standard curve. The TPAC content was expressed as μg catechin equivalents/mg of ethanol extract.

Determination of total phenolic content

Ethyl acetate extract of *S. glauca* seeds were carried out to determine total phenolic content (TPC) using quantified method of Folin–Ciocalteu [18] and as a standard used gallic acid. The 765 nm wavelength used for absorbance for test sample. To determine the standard curve of TPC of the resulting extract and it was expressed as μg gallic acid equivalent (GAE)/mg ethanol extract by following formula.

$T = (C \times V)/M$ formula.

Where T is the TPCs in $\mu\text{g}/\text{mg}$ of the extracts as GAE,

C is the concentration of gallic acid in $\mu\text{g/mL}$,

V is the volume of the extracts in mL,

M is the weight in mg of the extract.

Assessment of anti-nutritional factors of the Simarouba glauca seeds**Determination of Tannin Content**

Tannin was analysed using the method of Schanderi [19]. Powder sample (0.25 g) was extracted with 37.5 ml distilled water and heated the flask gently and boiled for 30min. The sample mixer was centrifuged at 2000 rpm for 20min and the volume of the supernatant was finally made up to mark with distilled water in a 100 ml flask. An aliquot of 500 μl of the sample was treated with 1 ml of Folin–Denis reagent followed by 2 ml of sodium carbonate and allowed to stand for color development. The absorbance of the reaction mixture was measured at 700 nm in a spectrophotometer (Specord 2000, Analytik Jena, Germany). Tannic acid used as standard. Tannin content was expressed as Tannic acid equivalents (TAE) in gram per gram dry wt.

Determination of Phytic acid

An indirect colorimetric method of Wheeler and Ferrel [20] was used for phytate determination. This method depends on an iron to phosphorus ratio of 4:6. 5g of the test samples were extracted with 3% trichloroacetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2N HNO_3 and the colour read immediately at 480 nm. The standard solution was prepared from $\text{Fe}(\text{NO}_3)_3$ standard curve. The phytate concentration was calculated from the iron result assuming a 4:6 iron: phosphorous molecular ratio.

Determination of Saponin Content

Saponin was determined using the method of Obadoni and Ochuko[21]. The powder sample (3 g) was dispersed in 30 ml of 20% aqueous ethanol. The suspension was stirred for 12hrs with constant stirring at about 55°C on a hot plate (Spinot, Tarson make). The mixture was filtered and the residue was re-extracted with another 30 ml of 20% aqueous ethanol. The combined extracts (filtrates) were reduced to 15 ml over water bath at about 90°C. The concentrated sample extract was transferred into 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated twice. To the combined aqueous, 20 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the concentrated sample was dried in dry bath to a constant weight and saponin content was calculated as detailed below.

$\% \text{ Saponin} = \frac{W_2 - W_1}{\text{Wt. of Sample}} \times 100$

Where, W_1 = Weight of evaporating disc

W_2 = Weight of disc + Sample

Determination of Oxalate Content

Oxalate was determined according to the guideline of Franco and Krinitz, [22] AOAC method. 1g of the sample was weighed in 100 mL conical flask. 75 ml of 3 mol/L H_2SO_4 was added and the solution was stirred intermittently with a

magnetic stirrer for about 1 hour and then filtered using Whatman No. 1 filter paper. 25 mL of the sample filtrate was collected and titrated against hot 0.1N KMnO₄ solution to the point when a faint pink colour appeared. The concentration of oxalate in each sample was obtained from the calculation:

1 mL 0.1 permanganate = 0.006303g oxalate.

Processing of seeds

The *S. glauca* seeds were subjected to various processing treatments described by Mubarak [23].

Soaking: The whole *S. glauca* seeds were soaked in distilled water (1:10, w/v) for 24 hrs at room temperature.

Roasting: The seeds were rinsed with water, dried and roasted on a sand bath at around 180°C for 20 min.

The processed and control seeds were dried, ground to fine powder, sieved (20 mesh) and stored in plastic sample bottles. The powder was used to analyze the anti-nutritional factors as described above.

Proximate analysis of the crude extracts of Simarouba glauca seeds

The fine powder of the seed sample was analyzed for ash, fat, fibre, protein and carbohydrate.

Determination of Moisture

The moisture content of the samples was determined by the methods described by AOAC (Association of Official Agricultural Chemists, 10th edition, Washington DC, 1965) [24]. The crucibles were washed and dried at 105°C, placed in desiccators for cooling and weighed. The sample (2.0 g) was weighed into the crucibles and heated overnight at 105°C, placed in desiccators, cooled and weighed. The per cent of moisture content was calculated by multiplying the fraction of weight loss on drying and initial weight by 100.

Determination of Lipid

The crude lipid was determined by the soxhlet extraction method as described by AOAC [24]. The sample (2.0 g) was extracted with petroleum ether for 4 hrs, and the miscella obtained was evaporated on a hot water bath, dried at 105°C for 30 min, cooled in a desiccator and weighed. The percentage of crude lipid was calculated by multiplying the fraction of mass of extract and mass of sample by 100.

Determination of Fibre

The crude fiber was determined by the method of Maynard [25]. The sample (2.0 g) was digested with 200 ml of 0.2 N H₂SO₄ for 30 min, filtered through a muslin cloth and washed with boiling water until acid-free. The residue was further digested with 200 ml of 0.3 N NaOH for 30 min, filtered through a muslin cloth and washed successively with 25 ml of hot 0.46 N H₂SO₄, 50 ml of water three times and 25 ml of alcohol. The residue obtained was dried for 2 hours at 100°C, cooled in a desiccator and weighed. The percentage of crude fiber was calculated by multiplying the fraction of extract weight and weight of the sample by 100.

Determination of total Protein

The protein content of the sample was determined by the Kjeldahl technique. The sample (100 mg) was weighed and transferred to a 30 ml digestion flask. 0.5 ml of 14 % of mercuric sulphate solution in 1 N Sulphuric acid and a pinch of potassium sulphate is added along with 2.5 ml of concentrated Sulphuric acid. The sample is digested till the solution becomes colourless. After cooling, the digest is diluted with a small amount of ammonia-free distilled water and transferred to the distillation apparatus, and 10 ml of 60 % sodium hydroxide - 10 % sodium thiosulphate solution is added. The sample is steam distilled, and the liberated ammonia is collected in 10 ml of 2 % boric acid solution containing mixed indicator (0.2 % methyl red and 0.2 % of methylene blue in 90 % ethanol mixed in the 2:1 ratio) and titrated against standard 0.1 N H₂SO₄ (1 ml of 0.1 N H₂SO₄ is equivalent to 1.401 mg of N). The total protein content is calculated using a factor of 6.25 [26].

Determination of Ash

The ash content of the sample was determined by the method described by AOAC [24]. Ash contains inorganic matter - the total of the incombustible sample left after ignition at 600°C (ash). The sample (2.0 g) is ignited at 600°C for 3 hrs to burn all organic matter. The inorganic matter which does not burn or volatilize at that temperature is called ash. The percentage of ash is calculated by multiplying the fraction of mass of ash and mass of sample by 100.

Determination of Carbohydrates

The nitrogen-free extract (NFE) of a feed is determined by the difference method after the analysis has been complete for ash, fibre, fat, and protein using the arithmetical difference method [27]. The carbohydrate content was calculated and expressed as the nitrogen-free extract. The NFE represents the difference in dry weight of the fraction obtained by subtracting the total dry weight of the sample from the sum of the fractions of ash, fiber, fat and protein on a dry weight basis. The chief components of NFE are sugars and starch, whereas fiber is chiefly of cellulose-like components.

III. RESULTS

Quantitative Phytochemical Assay of Ethyl Acetate Extract of *S. glauca* Seeds (Table 1)

The qualitative analysis of phytochemicals of *Simaroubaglauca* seeds resulted for ethyl acetate extract positive for steroids & triterpenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates and fatty acids and negative for proteins and amino acids (Table 1).

Quantitative phytochemical assay of ethanol extract of *S. glauca* seeds (Table 1)

The ethyl acetate extract of *S. glauca* seeds showed positive for flavonoids and carbohydrates in qualitatively, further to clear the concept of phytochemicals which are rich in the concentration of novel metabolites studied for total content of flavonoids, proanthocyanidin and phenol by the quantitative standard methods.

Table 1. Phytochemical investigations of the ethyl acetate extract of *Simaroubaglauca* seeds

Sl.No.	Qualitative phytochemical assay		Quantitative phytochemical assay			
	Test	Ethyl Acetate Extract	Concentration	Total Flavonoid Content	Total Proanthocyanidin Content	Total Phenol Content
1	Steroids and Triterpenoids	+ve	20 μ g	1.164	0.102	0.705
2	Alkaloids	+ve	40 μ g	0.950	1.028	1.257
3	Tannins	+ve				
4	Flavonoids	+ve	60 μ g	0.942	1.626	1.799
5	Glycosides	+ve				
6	Carbohydrates	+ve	80 μ g	2.152	2.246	2.345
7	Proteins	-ve				
8	Amino acids	+ve	100 μ g	1.977	1.946	2.450
9	Fatty Acids	+ve				

Determination of total Flavonoid Content (TFC)

To determine the TFC in quantitative method with a graded concentration of the ethyl acetate extract at 20 μ g, 40 μ g, 60 μ g, 80 μ g and 100 μ g exhibited 1.164, 0.950, 0.942, 2.152 and 1.977 using standard quercetin as blank (Table 1).

Determination of total Proanthocyanidin Content (TPAC)

To determine the TPAC in quantitative method with a graded concentration of the various extracts at 20 μ g, 40 μ g, 60 μ g, 80 μ g and 100 μ g in ethyl acetate extract exhibited 0.102, 1.028, 1.626, 2.246 and 1.946 using standard vanillin-hydrochloride as blank (Table 1).

Determination of total Phenol Content (TPC)

To determine the TPC in quantitative method with a graded concentration of the various extracts at 20 μ g, 40 μ g, 60 μ g, 80 μ g and 100 μ g in ethyl acetate extract exhibited 0.705, 1.257, 1.799, 2.345 and 2.450 using standard gallic acid as blank (Table 1).

Screening of Anti-nutritional Factors

Analysis of anti-nutritional factors of the seeds of *S. glauca* indicates the presence of alkaloids, oxalates, lignin, saponins and tannins. The results were represented in Table 2 to 3. tannin and oxalates were present in high concentrations, saponins and phytates were present in lower concentrations in the unprocessed raw (control) seeds of *S. glauca*.

In the studied anti-nutritional factors oxalates and tannin exhibited highest in raw and soaked seeds, low in roasted seeds compared to other factors. Following tables are the results of the studied anti-nutritional factors and their concentration (Table 2).

Table 2. Concentration of anti-nutritional factors of *Simaroubaglauca* seeds

Sl. No.	Anti-nutritional Factors	Raw seeds	Roasted seeds	Soaked seeds
		Concentration (mg/g)	Concentration(mg/g)	Concentration(mg/g)
1	Tannin	5.3	1.6	4.0
2	Oxalate	6.5	2.2	6.0
3	Saponin	3.8	2.6	2.8
4	Phytate	3.10	1.55	1.05

Various Processing Treatments of the Seeds of *S. glauca*

The seeds of *S. glauca* were subjected to processing treatments like, roasting, and soaking to reduce or inactivate the anti-nutritional factors, and the results are presented in Table 2. Raw seeds exhibit high concentrations of tannins and oxalates, whereas moderate concentrations of saponins and phytate. Roasted seeds exhibit moderate concentrations of oxalates and saponins, lower concentrations of tannins and phytates. Soaked seeds also exhibited higher concentrations of tannins and oxalates, moderate concentration of saponins, the lower concentrations in phytates.

Proximate Analysis

The seeds of *Simarouba glauca* are subjected to proximate composition analysis, and the data is presented in Table 5. The *S. glauca* seeds were found to have 8.6 % moisture, 1.5 % ash, 8.1 % fibres, 40.2 % fats, 25.5 % proteins and 16.1 % carbohydrates.

Table 3. Proximate analysis (%) of the seeds of *Simarouba glauca*

Sl. No.	Composition	Proximates (%)
1	Moisture	8.6
2	Ash	1.5
3	Fibre	8.1
4	Oil/Fat	40.2
5	Proteins	25.5
6	Carbohydrates	16.1

IV. DISCUSSION

Phytochemical profile of *Simarouba glauca* seeds extract were exhibited remarkable results and evidenced by the positive phytochemicals for steroids & triterpenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates and fatty acids qualitatively whereas, total flavonol and flavonoid content exhibited optimum at the quercetine equivalent/mg/ml concentration, compare to total phenol, proanthocyanidic content exhibited maximum when compared standard catechin and gallic acid equivalent/mg/ml concentration. These contents were determined using aluminium chloride method to form stable complex with the group of carbonyl at C4, hydroxyl at C3 and C5 to represent as flavonols and flavones, these flavonoids bound with ortho position in B rings of hydroxyl group to act as labile acid complexes. Similar results were observed in the findings of Goyal et al., in the quantitative phytochemicals were shown in the methanolic leaf extract of *Bambusa vulgaris* "Vittata" [28]. Qualitative phytochemical analysis of *Simarouba glauca* seeds extract contain positive for flavonoid and carbohydrate and in quantitative analysis exhibited TFC highest at 80 µg concentration of extract exhibited 2.152 µg/ml, TFC was determined using aluminium chloride method and it will form stable complex with the group of carbonyl at C4, hydroxyl at C3 and C5 to represent as flavonols and flavones, these flavonoids bound with ortho position in B rings of hydroxyl group and act as labile acid complexes. These results were evidenced with the findings of Kamala et al., [29] in the quantitative phytochemicals were shown in the rhizomes of *Cyperus rotundus* L. These findings were agreed with the recent research reports of Umesh [30] and Osagie-Eweka [31]. Proanthocyanidins are spread over the plant kingdom, including fruits, seeds of some plants, flower, nuts or barks. Total proanthocyanidin content highest at 80 µg concentration of extract exhibited 2.246 µg/ml, Proanthocyanidins, a subclass of the most complex flavonoids, are the nonpolar, condensed tannins and polymer of flavan-3-ols and constitute an important group of polyphenols because of their bioactivities, like anti-inflammatory, antioxidant and anticancer activities [32]. The total phenols contents in extract was evaluated in the present study and found TPC content highest at 100 µg concentration extract of exhibited 2.450 µg/ml. Puranik et al., [33] reported similarly with ethanolic extract of *S. glauca* leaf contain secondary metabolites as phenolic compounds and revealed

their anticancer activity against bladder cancer. Whereas, Jose et al., [34, 35] addressed the presence of complex phytochemical agents in *S. glauca* leaves and act as potential anticancer principle.

Non-conventional plant seeds contain nutrients like protein, carbohydrates, fats and minerals or types of secondary metabolites which can be exploited in place of conventional nutrient-rich legumes as animal or human feeds. The disadvantages are the anti-nutritional factors of the seeds, which prevent the proper utilization and conversion as balanced feed. Various processing methodologies have been developed to reduce or neutralize anti-nutritional factors in such seeds as a feeds. Anti-nutritional factors play a negative role in plants physiological mechanism to interfere in the normal nutritional parameters. These factors are inhibiting the nutritive quality and quantity of the foodstuffs. These factors are significantly effect in the normal digestion, absorption and metabolism processes of any higher organisms and cause nutrition-related dysfunctions and diseases. Legumes and cereals contain rich source of macronutrients but also exhibit lower level of anti-nutritional factors. Major anti-nutritional factors found in edible crops include alkaloids, amylase inhibitors, goitrogens, gossypol, lectins, oxalic acids, phytate, protease inhibitors, saponins and tannins. Anti-nutritional factors reduce the bioavailability of nutrients and also functional properties of the food [36]. In the current study, the anti-nutritional factors, such as oxalates, phytate, saponins and tannins, were found in the seeds of *S. glauca*. Similar findings were reported in seeds of *Pithecellobium dulce* [37]. The phytochemical analysis of the seeds of *S. glauca* reported for the presence of alkaloids, saponins and tannins [38, 39] and also in various leaf extracts by Jose et al., [35].

The saponins and oxalates were found to be concentrated in the endosperm. Oxalate binds to dietary calcium and decreases its absorption in the intestine resulting in bone and teeth deformities. Saponins adversely affect the feed/food palatability and lead to foam formation in different solutions and also affect the immune system [40]. Oxalate salts are also reported to cause renal stones and development of idiopathic calcium oxalate nephrolithiasis [41]. Saponins were found in seed may bind to dietary cholesterol, making it unavailable for absorption and causing hypocholesterolemia and they also cause hemolysis of RBC [42]. Tannins are concentrated in the seed and bind to dietary proteins and digestive enzymes, minimising the digestion process, resulting in decreased bioavailability of feed to animals [43] and causing reduced palatability and growth rate [44]. It is necessary for elimination of anti-nutrient problem related to phytate from the food and as a phytic acid chelates nutrients such as zinc, iron and calcium resulting availability of minerals absorption in body decrease that and also responsible for disorder like Osteoporosis due to calcium reduction [45]. The analysis of anti-nutritional factors of the untreated seed extract (control) showed the presence of anti-nutritional factors in varying amounts. Very high concentrations of saponins and oxalates, moderate concentrations of tannins and lower concentrations of phytate were found in the seed extracts.

In the present study, the seeds were subjected to processing treatments like roasting and fermenting instead of raw material usage. In general, all the processing treatments were found to be effective in reducing the anti-nutritional factors present in the seed sample. Roasting, fermenting, autoclaving and germination processing treatments are very effective in reducing the saponins and oxalates load compared to other methods. Similar findings were reported while carrying out different processing treatments on pea seeds and kidney beans to reduce the anti-nutritional factors [46, 47]. The reduction of tannin and lignin after the processing treatments is due to their predominance in the seed coat [48] and water solubility [49]. Consequently, they are leached out of the seeds by the physical process. The decrease in amount is also because they are heat-labile [49] and degrade upon heat treatment. The soaking and germination of seeds are also found to activate the endogenous enzymes that can degrade the anti-nutritional factors, and an increase in temperature can denature the anti-nutritional factors. The cooking of presoaked winged beans effectively reduces anti-nutritional factors [50]. Several studies reported autoclaving is the best method for reducing most of the anti-nutritional factors compared to other processing treatments [51].

The proximate analysis gives the nutritive value of food and corresponds to a proportion of groups of substances present in 100 g of food. The percentage of fat in seeds ranged from 0.64 % (Lychee) to 50.2 % (Cashew). The oilseeds revealed approximately 20 % of fats to consider better impact on health [52]. However, in the seeds of *S. glauca* contain more than 20 % of fat (40.2 %). The protein content in the seeds ranges from 4.9 % to 25.7 %. In general, the protein values are higher for cereals, pulses and legumes [53]. The protein content of seeds of *S. glauca* was exhibited to be 25.5 %, indicating a significant level of protein. The total carbohydrate content in seeds ranges from 23.1 % to 81.7 %. The *S. glauca* seeds contain 16.1 % of carbohydrates, which serve as a significant source of calories for the seed. The lowest ash was reported in Cashew (1.2 %) and highest in *Pliniacauliflora* seeds of 3.2 % [54]. The values represented in our study were within the range of standards. Ash content represents the inorganic part of the plant. These inorganic elements play a significant role in the physiological process in metabolism, which were influenced in human health. Dietary fibers play a significant role in health by lowering the risk of diabetes, cardiovascular diseases, gastrointestinal disorders and obesity [55]. The fiber content of *S. glauca* seeds was found to be 8.1 g which is relatively high compared to the fiber content in the seeds of *Pithecellobium dulce* (7.1), *Tamarindus indica* (5.1 g), *Millettiagriffonianus* (4.0 g) and *Albizia saman* (2.1 g) [56, 57]. The moisture content of the dry legume seeds ranges from 8.1 g to 16.0 g [58]. The value for the *S. glauca* seeds lies within this range. The minimal moisture content is required for the prolonged and proper storage of seeds. The low moisture content reduces the spoilage by microbial

infection. The *S. glauca* seeds would set up potent energy source if included in the diet because of their higher proteins, carbohydrate and fat contents. Seeds also serve as an excellent fiber source as they contain higher compared to other non-conventional seeds. The proximate composition of food samples are influenced by various factors such as variety, climate, cultivar, maturity and production conditions, handling, processing and storage [59,60]. Proximate analysis for foods helps in nutrient labeling and data validation for food processing. Nutrient labeling increased the interest among the consumers and resulted in significant interest in the composition of foods. This resulted in the labeling of nutrients based on amounts per serving. Legume seeds contain moderately high amounts of protein, fats, carbohydrates, vitamins and minerals. This makes them the most valuable plant source for human and animal nutrition. The human population depends on legumes as a major nutrient source, particularly in combination with cereals [61].

V. CONCLUSION

The present study can be concluded that the ethyl acetate extract of *Simarouba glauca* seeds possess substantial amounts of phytochemicals as flavonoid, proanthocyanidin and phenolic compounds. It is also identified processing method to reduce the level of anti-nutritional factor which act as adherent or toxic element in the non-conventional seeds. It is further studied for their proximate analysis and found all the composition which act as potent nutraceutical component and can be used for the animal as well as human consumption. Further studies on in vivo model to determine toxicity and pharmacological action needs to be carried out and considered for future therapeutic agent.

REFERENCES

1. S.Sreedharan, A.Gothe,K.Aier, K.Shivasharanappa, K.Praveen Kumar and S.J.Patil, "Bioactive molecules and antimicrobial studies of Indian traditional medicinal plant *Rhus semialata* seed," *Research Journal of Medicinal Plants*, vol. 13, pp.10-17, 2019.
2. V. Subhose, P. Srinivas and A. Narayana, "Basic principles of pharmaceutical science in Ayurveda", *Bulletin of Indian Institute of Historical Medicine*, vol. 35, pp. 83-92. 2005.
3. B. Ballabh, and O.P. Chaurasia, "Traditional medicinal plants of cold desert Ladakh-used in treatment of cold, cough and fever" *Journal of Ethnopharmacology*, vol. 112, pp. 341-349, 2007.
4. M. M., Pandey, S. Rastogi and A. K. S. Rawat, "Indian herbal drug for general healthcare: An overview" *International Journal of Alternative Medicine*, vol. 6, 2008.
5. B. Patwardhan, D. Warude, P. Pushpangadan and N. Bhatt, "Ayurveda and traditional chinese medicine: A comparative overview" *Evidence-Based Complementary Alternative Medicine*, vol. 2, pp. 465-473, 2005.
6. R. E. Dimayuga and S.K. Garcia, Antimicrobial screening of medicinal plants from Baja California Sur, Mexico. *Journal of Ethnopharmacology*, vol.31, pp. 181-192, 1991.
7. S. Khokhar and B. M. Chauhan, "Antinutritional factors in moth bean (*Vigna acutifolia*): Varietal differences and effects of methods of domestic processing and cooking", *Journal of Food Science*, vol. 51, pp.591-594, 1986.
8. P. S. Prasanthi, N. Naveena, V. M. Rao and K. Bhaskarachary, "Compositional variability of nutrients and phytochemicals in corn after processing" *Journal of Food Science and Technology*, vol. 54, pp. 1080-1090, 2017.
9. S. P. Rath, C. Srinivasulu and S. N. Mahapatra, "Investigations on *Simarouba glauca* – a new oilseed of Indian origin" *Journal of Oil Technologists Association of India*, vol. 9, pp. 64-65, 1987.
10. K. V. S. Rao and A. G. Lakshminarayana, "Characteristics and composition of *Simarouba glauca* D. C. seed and fat" *Journal of Food Science and Technology*, vol. 20, pp. 176-177, 1983.
11. J. Chikara, B. D. Shethia, M. Rathod and J. B. Pandya, "*Simarouba glauca* – a rich source of oleic acid for rehabilitation of marginal land of the country" *Journal Oil Technologists Association of India*, vol. 30, pp. 177-179, 1998.
12. T. Jeyarani and S. Y. Reddy, "Cocoa butter extender from *Simarouba glauca* fat" *Journal of American Oil Chemists Society*, 78: 271-276, 2001.
13. Sharangouda and S. B. Patil, "Phytochemical screening and antifertility activity of various extracts of *Citrus medica* (Lemon) seeds in albino rats" *Advances in Pharmacology and Toxicology*, vol. 8, pp. 71-74, 2007.
14. J. B. Harborne, *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edn., Chapman & Hall, London, UK., pp: 60-63.
15. N. R. Farnsworth, O. Akerele, A. S. Bingel, D. D. Soejarto and Z. Guo, "Medicinal plants in therapy". *Bulletin of World Health Organization*, vol. 63, pp. 965, 1985.
16. A. Patel, A. Pate and N. M. Patel, "Estimation of flavonoid, polyphenolic content and in vitro antioxidant capacity of leaves of *Tephrosia purpurea* Linn (Leguminosae)" *International Journal of Pharmaceutical Sciences and Research*, vol. 1, pp. 66-77, 2010.

17. A. Kamala, S. K. Middha, C. Gopinath, H. S. Sindhura, C. S. Karigar, "In vitro antioxidant potentials of *Cyperus rotundus* L. rhizome extracts and their phytochemical analysis". *Pharmacognosy Magazine*, vol. 14, pp. 261–267, 2018.
18. V. L. Singleton and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents" *American Journal of Enology and Viticulture*, vol. 16, pp. 144–158, 1965.
19. S. H. Schanderi, *Methods in food analysis*. Academic Press, New York, USA: 1970
20. E. L. Wheeler, and R. E. Ferrel, "A method for phytic acid determination in wheat and wheat fractions" *Cereal Chemistry*, vol. 48, pp. 312–320, 1971.
21. B. O. Obadoni and P. O. Ochuko, "Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria" *Global Journal of Pure and Applied Sciences*, vol. 8, pp. 203–208, 2002.
22. V. Franco and B. Krinitz, "Determination of oxalic acid in foods" *Journal of Association of Official Analytical Chemists*, 173; 56(1):164–166, 1965.
23. A. E. Mubarak, "Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes" *Food Chemistry*, vol. 89, pp. 489–495.
24. D. Firestone, "Report on Oils, Fats, and Waxes" *Journal of Association of Official Agricultural Chemists*, vol. 48, pp. 127–134, 1965.
25. A. J. Maynard, *Methods in Food Analysis* Academic Press New York pp. 176, 1970.
26. D. S. Fabricant and N. R. Farnsworth, "The value of plants used in traditional medicine for drug discovery" *Environment and Health Perspectives*, vol. 109, pp. 69–75, 2001.
27. L. E. Lloyd, B. E. McDonald and E. W. Crampton, *Fundamentals of Nutrition* (2nd Ed.). W. H. Freeman and Company, San Francisco, CA, 1978.
28. A. K. Goyal, S. K. Middha and A. Sen, "Evaluation of the DPPH radical scavenging activity total phenols and antioxidant activities in indian wild *Bambusa vulgaris* "Vittata" methanolic leaf extract" *Journal of Natural Pharmaceutics*, vol. 1, pp. 40–45, 2010.
29. A. Kamala, S. K. Middha, C. Gopinath, H. S. Sindhura and C. S. Karigar, "In vitro antioxidant potentials of *Cyperus rotundus* L. rhizome extracts and their phytochemical analysis" *Pharmacognosy Magazine*, vol. 14, pp. 261–267, 2018.
30. T. G. Umesh, "In vitro antioxidant potential, free radical scavenging and cytotoxic activity of *Simarouba glauca* leaves". *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 7, pp. 411–416, 2014.
31. S. D. E. Osagie-Eweka, "Phytochemical analyses and comparative in vitro antioxidant studies of aqueous, methanol and ethanol stem bark extracts of *Simarouba glauca* DC. (Paradise tree)" *African Journal of Plant Sciences*, vol. 12, pp. 7–16, 2018.
32. K. Zhou, J. J. Raffoul, "Potential anticancer properties of grape antioxidants" *Journal of Oncology*, pp. 803–294, 2012.
33. S. I. Puranik, S. C. Ghagane, R. B. Nerli, S. S. Jalalpure and M. B. Hiremath, "Evaluation of in vitro antioxidant and anticancer activity of *Simarouba glauca* leaf extracts on T-24 bladder cancer cell line" *Pharmacognosy Journal*, vol. 9, pp. 906–912, 2017.
34. A. Jose, E. Kannan and S. V. Madhunapantula, "Anti-proliferative potential of phytochemical fractions isolated from *Simarouba glauca* DC leaf" *Heliyon*, vol. 6, pp. e03836, 2020.
35. A. Jose, E. Kannan, A. R. P. Vijay-Kumar and S. V. Madhunapantula, "Therapeutic Potential of Phytochemicals Isolated from *Simarouba glauca* DC for Inhibiting Cancers: A Review" *Systemic Reviews in Pharmacy*, vol. 10, pp. 73–80, 2019.
36. H. T. Duguma, S. F. Forsido, T. Belachew and O. Hensel, "Changes in anti-nutritional factors and functional properties of extruded composite flour" *Frontiers in Sustainable Food Systematics*, vol. 5, pp. 713–701, 2021.
37. A. S. Surendra and K. R. Siddalinga Murthy, "Preliminary study on antinutritional factors and evaluation of antioxidant and anticancer activities of seeds extracts of *Pithecellobium dulce*" *Journal of Emerging Technologies and Innovative Research*, vol. 6, 2019.
38. R. Haleshappa, S. J. Patil, T. Usha and K. R. Siddalinga Murthy, "Phytochemicals, antioxidant profile and GCMS analysis of ethanol extract of *Simarouba glauca* seeds" *Asian Journal of Biological and Life Sciences*, vol. 9, pp. 379–85, 2020.
39. R. Haleshappa, S. J. Patil, K. R. Siddalinga Murthy, "Phytochemical analysis, in vitro evaluation of antioxidant and free radical scavenging activity of *Simarouba glauca* Seeds. *Advances in Pharmacology and Pharmacy*, vol. 9, pp. 1–8, 2021.
40. B. Singh, J. P. Singh, N. Singh and A. Kaur, "Saponins in pulses and their health promoting activities: A review", *Food Chemistry*, vol. 233, pp. 540–549, 2017,

41. R. P. Holmes and M. Kennedy, "Estimation of the oxalate content of foods and daily oxalate intake", *Kidney International*, vol. 57, pp. 1662-1667, 2000.
42. T. K. Das, D. Banerjee, D. Chakraborty, M. C. Pakhira, B. Shrivastava and R. C. Kuhad, "Saponin: role in animal system", *Veterinary World*, vol. 5, pp. 248-254, 2012.
43. V. A. Aleator, "Allelochemicals in plant foods and feeding stuffs. Part I. Nutritional, biochemical and physiopathological aspects in animal production" *Veterinary and Human Toxicology*, vol.35, pp. 57-67, 1993.
44. M. H. M. Yacout, "Anti-nutritional factors & its roles in animal nutrition: *Journal of Dairy, Veterinary and Animal Research*, vol. 4, pp. 237-239, 2016.
45. M. Yadav, P. Singh, R. Kaur, R. Gupta, S. S. Gangliya and N. K. Sing, "Impact of food phytic acid on nutrition, health and environment" *Plant Archives*, vol. 13, pp. 605-611, 2013.
46. B. Kumar, N. Tirkey and S. Kumar, "Anti-nutrient in fodders: A review," *Chemical Science Review Letters*, vol. 6, pp. 2513-2519, 2017.
47. R. Y. Khattab and S. D. Arntfield, "Nutritional quality of legume seeds as affected by some physical treatments. 2. Antinutritional Factors," *LWT—Food Science and Technology*, vol. 42, pp. 1113-1118, 2009.
48. N. R. Reddy and M. D. Pierson, "Reduction in antinutritional and toxic components in plant foods by fermentation," *Food Research International*, vol. 27, pp. 281-290, 1994.
49. N. R. Kumar, A. N. Reddy and K. N. Rao, "Levels of phenolic substances on the leached in cicer seed", *Journal of Experimental Biology*, vol. 17, pp. 114-116, 1979.
50. S. Rakic and P. Silvana, K. Jelena, J. Milka, T. Vele, P. Dragan, S. Marinkovic and Slavica, "Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorn from Serbia," *Food Chemistry*, vol. 104, pp. 830-834, 2007.
51. V. R. Devaraj and N. H. Manjunath, "Effects of cooking on proteinase inhibitors of dolichos lablab bean (*Dolichos lablab* prepureus)," *Plant Food, Human Nutrition*, vol. 48, pp. 107-112, 1995.
52. V. Vadivel, M. Pugalenti and M. Megha, "Biological evaluation of protein quality of raw and processed seeds of gila bean (*Entadascandensbenth.*)," *Journal of Tropical and Sub Tropical Agroecosystems*, vol. 8, pp. 125 - 133, 2008.
53. F. T. Orthoefer, "Ice bran oil: healthy lipid source," *Food Technology*, vol. 50, pp. 61-64, 2008
54. R. Lásztity, *The chemistry of cereal proteins*, 2nd Edn., 10.1201/9780203737057, 2017
55. D. M. M. Luzia and N. Jorge, "Study of antioxidant activity of non-conventional Brazilian fruits," *Journal of Food Science and Technology*, vol. 51, pp. 1167-1172, 2014.
56. O. Semih and O. Selin, Health effects of dietary fiber. *ActaScientiarumPolonorum. TechnologiaAlimentaria*, vol. 13, pp. 191-202, 2014.
57. A.S. Surendra, K. R. Siddalinga Murthy and M. R. Malini, "Review on pharmacological properties of *Pithecellobium dulce* tree," *Research Journal of Life Science, Bioinformatics, Pharmacy and Chemical Sciences*, pp. 263-271, 2019.
58. A. Adewuyi, R. Oderinde, B. Rao, R. B. N. Prasad and M. Nalla, "Proximate analysis of the seeds and chemical composition of the oils of *Albizia Saman*, *Millettiagriffonianus* and *Tamarindus indica* from Nigeria. *Annals of Food Science and Technology*, vol. 12, 2011.
59. R. Kamboj and V. Nanda, "Proximate composition, nutritional profile and health benefits of legumes – A review," *Legume Research - An International Journal*, 10.18805/LR-3748., 2017.
60. X. Yu, F. Voort, L. Zhixi and T. Yue, "Proximate composition of the apple seed and characterization of its oil. *International Journal of Food Engineering*, vol. 3, 10.2202/1556-3758.1283, 2007.
61. Y. Maphosa and V. A. Jideani, *The Role of Legumes in Human Nutrition, Functional Food - Improve Health through Adequate Food*, Maria ChavarriHueda, IntechOpen, 2017, doi: 10.5772/intechopen.69127.