Research Article

# Phytochemicals, Antioxidant Profile and GC-MS Analysis of Ethanol Extract of *Simarouba glauca* Seeds

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Submission Date: 13-10-2020; Revision Date: 22-11-2020; Accepted Date: 12-12-2020

## ABSTRACT

Medicinal plants acted as traditional medicine from the ancient time and recognized as scientific medicine in modern days. *Simarouba glauca* is an Indian traditional medicinal plant commonly called as "Paradise Tree or Lakshmi Taru" used for its various medicinal properties. The current study was carried out to know the phytochemicals, antioxidant profile and Gas Chromatography-Mass Spectroscopy (GC-MS) of ethanol extract of the seed *S. glauca*. Qualitative analysis showed the presence of flavonoids and carbohydrates, lacked alkaloids, glycosides, steroids, triterpenoids and tannins. The total flavonoid, proanthocyanidin and phenol content were 25.20 ± 0.15mg quercetin equivalent/g extract, 57.08±1.51mg catechin equivalent/g extract and 41.75 ± 2.31mg gallic acid equivalent/g extract respectively. Antioxidants exhibited maximum 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging of 70% at 100 µg/mL concentrations with an $IC_{50}$ value 50.93µg/mL, decolourization potential of 2,2'-azino-bis - (3-ethyl benzthiazoline-6-sulfonic acid (ABTS) was 65% at 203.87µg/mL concentration and ferric reducing antioxidant potential (FRAP) assay exhibited Ascorbic Acid Equivalents (AAE/ml).The relative contents of the fatty acids were calculated with area normalization by GC-MS. Out of the ten fatty acids, four of them were Ethyl oleate (24.20%), Oleic acid (16.13%), 5-Hydroxymethylfurfural (12.69%) and Hexadecanoic acid ethyl ester (12.22%) and other six fatty acids were present less than 11%.

Key words: Fatty acids, *Simarouba glauca*, Seeds, Chemical component, GC-MS analysis.

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## INTRODUCTION

From the ancient times, searching medicinal plants for various diseases of animals and humans from the mother nature is common and it is proven by literature. The identification and application of the medicinal plants' use were innate and came to know by the findings with animals.[1] In view of the ancient perspectives there was no evidence and proper information for the reason of diseases or ayurvedic applications for the treatments of various diseases were still unclear. Even though, using available literature of traditional practice for the application of medicinal plants to the various diseases were adopted as novel treatment methods, hence, use of medicinal plants taken more importance on research for identify biomolecules as therapeutic action. Awareness of medicinal plants application for the various diseases resulted as many years of effort in identifying drugs in barks, flowers, fruit, seeds, stems, etc. Nevertheless, the unsafe efficacy of synthetic drugs, the increasing risk, side effects were hazardous to health and found alternative as natural drugs in world scenario again by the medicinal plants.

*Simarouba glauca*, is commonly known as 'Lakshmi Taru' or 'paradise tree' belonging to family Simaroubaceae. The specific name glauca means covered with bloom

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DOI: 10.5530/ajbls.2020.9.57

which refers to the bluish green foliage. It is derived from Greek word 'glaukos' (bluish).[2] *S. glauca* has been recognized as Indian traditional medicinal plant due its wide application of medicine as anticancer, antimicrobial, antiviral and antihelminthic agent in all the parts of plants. *S. glauca* is a rich source of phytochemical like quassinoids in that glaucarubin, glaucarubolone and glaucarubinone reported for various application.[3,4] Manasi and Gaikwad revealed the health promoting oil by *S. glauca* extract, analysed the oleic acid and other fatty acid properties.[5] An herbal formulation of Nutrapotent DS prepared by the *S. glauca* extract, explored the anticancer agents in the product and used for the types of cancer treatment.[6] Other studies have reported water extract of *S. glauca* support in the differentiation of skin keratinocytes[5] and also improve hydration and moisturisation of skin.[7] *S. glauca* products are currently in the pharma market for the use of skin disorders in the form of lotion, powder and ointments. As a traditional practice bark of *S. glauca* has been used for the malarial treatment. The tribes of Brazilians used the extract of *S. glauca* as a natural therapy for the management of dysentery issues.[5] Antony *et al*.[8] reported that the bark can be use it for fever, malaria, stomach and bowel disorders, leaces can be use it for haemorrhages and ameobiasis, fruit pulp and seeds can be uses it as analgesic, antimicrobial, antiviral, astringent emmenagogue, stomachic tonic and vermifuse properties. The types of extracts and their active principles reported as glaucarubin, quassinoids, ailanthinone, benzoquinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide and sitosterol also their biological properties. In the present studies, we have thoroughly reviewed the literature on the seeds, there is not much work available on the seeds of *S. glauca* extracts for the phytochemicals (qualitative and quantitative), various antioxidant and GCMS studies.

## MATERIALS AND METHODS

### Plant material

Traditional Indian medicinal plant of *Simarouba glauca* seeds were collected from Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bengaluru, Karnataka, India. The plant samples were authenticated by Dr. Shiddamallayya Mathapathi, Research Officer (Botany), at Regional Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH and Government of India.

### Preparation of plant extracts

The seeds of *Simarouba glauca* were collected, washed cleanly in distilled water and shade dried for complete removal of moisture. The seeds were separated from seed coat, powdered and used for successive Soxhlet extraction with ethanol for 24 hr and dried using Buchi's rotary vacuum evaporator and stored in refrigerated.

### Qualitative phytochemical assay

Qualitative phytochemical screening of the ethanol extract of the seed was carried out in order to analyse the class of organic compounds. The ethanol extract of *Simarouba glauca* seeds were analysed by standard chemical tests as described by Sharangouda and Patil,[9] Harborne[10] and Fransworth[11] to determine steroids and tritepenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates, proteins and amino acids.

### Quantitative phytochemical assay

Quantitative analysis was carried out by standard procedure of Harborne.[12] The ethanol extract was dried and re-dissolved in double distilled water, filtered and used for assay.

### Determination of total flavonoid content

The total flavonoid content (TFC) was determined using $AlCl_3$ method with standard quercetin at 510 nm and was expressed as µg of quercetin equivalents/mg of ethanol extract.[13]

### Determination of total proanthocyanidin content

The total proanthocyanidin content (TPAC) was determined using vanillin–hydrochloride method as described by Kamala *et al*.[14] at 500 nm and was expressed as µg catechin equivalents/mg of ethanol extract.

### Determination of total phenolic content

The total phenolic content (TPC) was determined by the method of Folin-Ciocalteau[15] at 765 nm and expressed as µg gallic acid equivalent (GAE)/mg ethanol extract by following formula.

$$T = [C \times V]/M$$

Where T is the TPCs in µg/mg of the extracts as GAE,
C is the concentration of gallic acid in µg/mL,
V is the volume of the extracts in mL,
M is the weight in mg of the extract.

### Antioxidant profile of ethanol extract of *Simarouba glauca* seeds

The antioxidant profile of the seed extract of *Simarouba glauca* were determined by DPPH assay, ABTS assay and

FRAP assay to estimate free radicals and scavenging activity in *in vitro* condition.

### DPPH free radical scavenging assay

DPPH assay was carried out as described by Blois[16] method. The reaction mixture was well mixed and incubated at room temperature for 30 min and the absorbance was recorded at 517 nm. The control was prepared by adding 2 ml of DPPH solution and 1 ml of methanol.[17] The $IC_{50}$ value was determined by using linear regression equation i.e.

$Y = Mx + C$

where, Y = 50, M and C values were derived from the linear graph trendline.

% Scavenged [DPPH] = [(AC−AS)/AC] ×100

Where AC is the absorbance of the control and

AS is the absorbance in the presence of the sample of extracts or standard.

### ABTS free radical scavenging assay

ABTS assay was carried out as described by Re *et al.*[18] An aliquot of 1 mL of essential oil was mixed with 2 mL of diluted ABTS+ and after 30 min of incubation, ethanol extract sample compared with the standard butylated hydroxytolune (BHT) was added and absorbance was measured at 734 nm. The $IC_{50}$ value was determined by using linear regression equation i.e.

$Y = Mx + C$.

where, Y = 50, M and C values were derived from the linear graph trendline.

The percentage of ABTS+ inhibition was calculated using the following formula:

% Scavenged ABTS = [(Ac−As) / Ac] x 100

Where Ac and As are the absorbance of the control and the sample, respectively.

### FRPF assay

The reducing power was estimated by the method of Benzie and Strain.[19] The mixture was incubated for 30 min in the dark and absorbance was read at 593 nm. Ascorbic acid was used as standard. The increase in absorbance indicated the increased reducing power of the samples. The results were reported as µg of ascorbic acid equivalents (AAE) per mL.

### Gas chromatography–mass spectrometry analysis of *S. glauca* seeds extracts

Gas chromatography–mass spectrometry (GC-MS) for *S. glauca* seeds extract was recorded with Thermo GC-Trace Ultra 5.0, Thermo MS DSQ II (Thermo Fisher Scientific, USA). TR 5-MS capillary standard nonpolar column with 30 m dimension, Id: 0.25 mm, 0.25 mm film was used. Helium gas was used as a carrier gas with flow rate of 1 mL/min.

### Statistical analysis

All the experiments were carried out in triplicates and were expressed as mean ± standard error of the mean. The data were statistically analysed using Microsoft Office Excel 2007.

## RESULTS

The qualitative analysis of phytochemicals of ethanol extract of *Simarouba glauca* seeds was positive for flavonoids and carbohydrates whereas negative for alkaloids, tannins, glycosides, steroids and triterpenoids, proteins and amino acids (Table 1).

### Quantitative analysis of ethanol extract of *S. glauca* seeds

### Determination of total flavonoid content (TFC), total proanthocyanidin content (TPAC) and total phenol content (TPC) of ethanol extract of *S. glauca* seeds

The TFC, TPAC and TPC of ethanol extract of *S. glauca* was found to be 25.20 ± 0.15mg quercetin equivalent (QE/g), 57.08 ± 1.51mg catechin equivalent (CE/g) and 41.75 ± 2.31mg gallic acid equivalent (GAE/g), respectively (Table 2).

The TFC of ethanol extract of *S. glauca* was found to be and the results were calculated using the QE as a standard (Table 2).

The TPAC of ethanol extract of *S. glauca* was found to be and the results were calculated using the CE as a standard (Table 2).

**Table 1: Qualitative phytochemical assay of the ethanol extract of *Simarouba glauca* seeds.**

| | Qualitative phytochemical assay | |
|---|---|---|
| | Test | Ethanol extract |
| 1 | Steroids and Triterpenoids | -ve |
| 2 | Alkaloids | -ve |
| 3 | Tannins | -ve |
| 4 | Flavonoids | +ve |
| 5 | Glycosides | -ve |
| 6 | Carbohydrates | +ve |
| 7 | Proteins | -ve |
| 8 | Amino acids | -ve |

+ = Positive; − = Negative

**Table 2: Quantitative phytochemical assay of ethanol extract of *S. glauca* seeds.**

| Sl. No. | Total Flavonoid (mg QE/g) | Total Proanthocyanidin (mg CE/g) | Total Phenol (mg GAE/g) |
|---|---|---|---|
| 1 | 25.20±0.15 | 57.08±1.51 | 41.75±2.31 |

Values expressed as Mean±SD of triplicate determination

The TPC of ethanol extract of *S. glauca* was found to be and the results were calculated using the GAE as a standard (Table 2).

## DPPH free radical scavenging activity

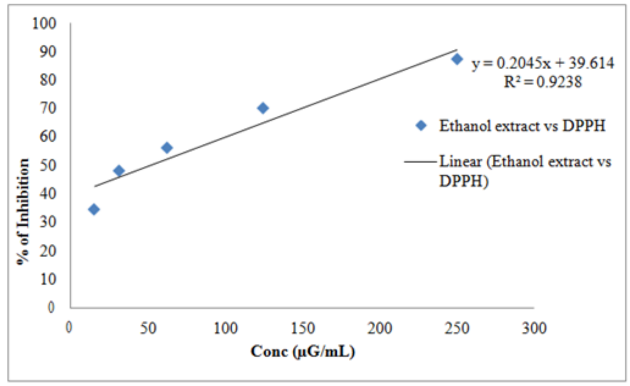
The ethanol extract of *S. glauca* seeds exhibited a significant dose dependent inhibition of DPPH scavenging activity. A concentration-dependent assay was carried out with the extract and the results were presented in Graph 1. Among five graded concentrations were used in the study along with blank, cell control and standard control. Ethanol extract showed scavenging activity as 34.67%, 48.37%, 56.35%, 70.34% and 87.38 % inhibition at 15.62, 31.25, 62.50, 125.00 and 250.00 µg/ml concentrations respectively. On the other hand, standard gallic acid showed 52.80% inhibition. The inhibitory concentration ($IC_{50}$) value of the *S. glauca* seeds extract showed 50.93µG/mL against the DPPH (Graph 1).

## ABTS free radical scavenging activity

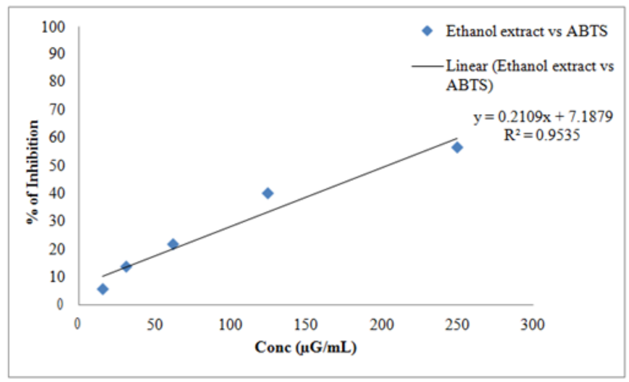
The ethanol extract of *S. glauca* seeds exhibited a significant dose dependent inhibition of ABTS free radical scavenging activity. A concentration-dependent assay was carried out with the extract and the results were presented in Graph 2. Among five graded concentrations were used in the study along with blank, cell control and standard control. Ethanol extract showed scavenging activity as 4.69%, 13.99%, 21.74%, 40.09% and 56.57 % inhibition at 15.62, 31.25, 62.50, 125.00 and 250.00 µg/ml concentrations respectively. On the other hand, standard BHT showed 54.39% inhibition. The inhibitory concentration ($IC_{50}$) value of the *S. glauca* seed extract showed 203.87µG/mL against the ABTS (Graph 2).

## FRAP reducing potential activity

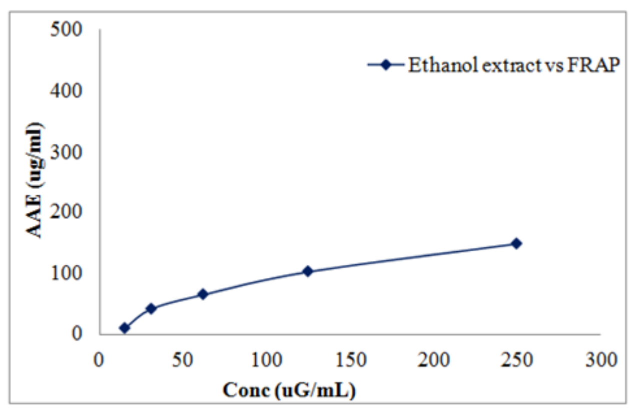
The ethanol extract of *S. glauca* seeds exhibited a significant dose dependent inhibition of FRAP reducing potential activity. A concentration-dependent assay was carried out with the extract and the results were presented in Graph 3. Among five graded concentrations were used in the study along with blank and control. Ethanol extract showed scavenging activity as 11.00 ug/ml, 41.83 ug/ml, 65.16 ug/ml, 102.50 ug/ml and 148.66 ug/ml equivalents at 15.62, 31.25, 62.50, 125.00 and 250.00 µg/ml concentrations respectively. On the other hand, all the values were equivalents to the standard Ascorbic acid (Graph 3).




**Graph 1: Scatter graph showing the % of inhibition of ethanol extract against the DPPH and the inhibitory concentration ($IC_{50}$ Value) observed is 50.93µg/mL.**




**Graph 2: Scatter graph showing the % of inhibition of ethanol extract against the ABTS•+ and the inhibitory concentration ($IC_{50}$ Value) observed is 203.87µg/mL.**




**Graph 3: Scatter graph showing the Ferric reducing antioxidant potential (FRAP) values of the ethanol extract in terms of ascorbic acid equivalents (AAE/ml).**

The correlation coefficients of ethanol extract of *S. glauca* seeds with DPPH and ABTS assays are 0.923 and

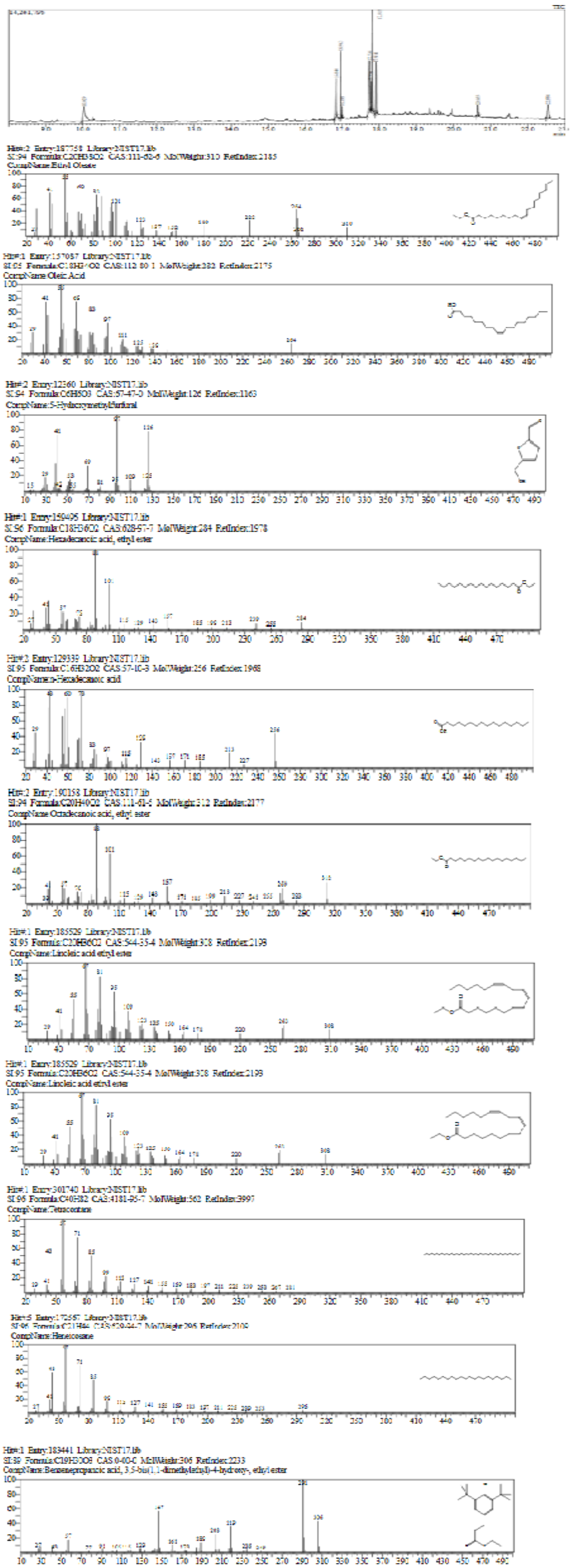


**Figure 1: Gas chromatography–mass spectrometry analysis of ethanol extract of *Simarouba glauca* seeds and their ten bioactive constituents.**

0.953, respectively, confirming that phenolic compounds exhibited 0.961mg/ml at the concentration 100 µg/ml are likely to contribute the free radical scavenging activity and acting as potential antioxidant.

### Gas chromatography–mass spectrometry (GC-MS) analysis

GC-MS analysis of ethanol extract of *S. glauca* seeds exhibited the presence of fatty acid esters such as Ethyl oleate (24.20%), Oleic acid (16.13%), 5-Hydroxy methylfurfural (12.69%) and Hexadecanoic acid ethyl ester (12.22%), n-Hexadecanoic acid (11.45%), Octadecanoic acid, ethy ester (8.27%), Tetraconatne (6.07%), Linoleic acid ethyl ester (4.27%), Heneicosane (3.02%) and Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester (1.68%) (Figure 1).

## DISCUSSION

Qualitative phytochemical analysis of *Simarouba glauca* seeds extract were positive for flavonoids and carbohydrates while quantitative analysis exhibited total flavonoid content exhibited 25.20±0.15mg quercetin equivqlent/g extract, 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.*[14] in the quantitative phytochemicals were shown in the rhizomes of *Cyperus rotundus* L. These findings were agreed with the recent research reports of Umesh[20] and Osagie-Eweka.[21]

Proanthocyanidins are spread over the plant kingdom, including fruits, seeds of some plants, flower, nuts or barks. Total proanthocyanidin content exhibited 57.08±1.51mg catechin equivqlent/g extract, 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 ant-inflammatory, antioxidant and anticancer activities.[22,23]

The total phenol content in extract was exhibited 41.75 ± 2.31mg gallic acid equivalent/g extract. Puranik *et al.*[24] reported similarly in ethanolic extract of *S. glauca* leaf contain secondary metabolites as phenolic compounds and revealed their anticancer activity against bladder cancer. Whereas, Jose *et al.*[25], Iranshahi *et al.*[26] addressed the presence of complex phytochemical agents in *S. glauca* leaves and act exhibited potential anticancer activity.

The effect of ethanol extract of *S. glauca* seeds on free radical scavenging was studied using DPPH assay and found better and higher radical scavenging activity than the controls. The increased scavenging activity is concentration dependent of the extract and it is may be due to its potent hydrogen donating ability.[18] The $IC_{50}$ value 50.93 µG/mL of ethanol extract indicated its high free radical scavenging activity, indeed it's an indication of good antioxidant activity. Such scavenging activity observed similary and inversely proportionate to the $IC_{50}$ value of the studied extracts.[27] These findings are higher $IC_{50}$ value exhibited in the studies was due to the extract crude nature, may be possible compound(s) were reacting as antioxidant potential. Thus, ethanol extract can be exhibit *in vivo* as well as *in vitro* DPPH free radical scavenging activity.[14]

ABTS assay is easy to analyse the anti-free radical activity as hydrophilic and lipophilic antioxidant and can be used it in any solvent system to compare with the rest of the antioxidant assay. This assay depends on the antioxidant abilities to react with the ABTS radical cation and reduce the decolourization property.[28] In the current study, ethanol extract of *S. glauca* seeds was analysed for the ABTS free radical scavenging assay. Butylated hydroxytolune was used as standard and its $IC_{50}$ values was 10.00µG/mL and $IC_{50}$ values of ethanol extract exhibited maximum 203.87 µG/mL. Similar findings as moderate to weak antioxidant activity by ABTS assay was shown in various medicinal plants and their extracts.[29]

The antioxidant metabolites were held responsible for the reduction of ferric ($Fe^{3+}$) to form it as ferrous ($Fe^{2+}$) ion, these addition of $FeCl_3$ to form it as ferrous tripyridyltriazine by blue colored complex formation and can be determined by measuring of reduction of the colored complex at 593 nm.[30] The reducing activities associated with the presence of metabolites involved in their action by breaking the chain of free radicals by donating a hydrogen atom.[31] Ethanol extract of *S. glauca* seeds showed greater FRAP value as 148.66 at the concentration of 250mg/ml (mg equivalent of ascorbic acid /g of extract). The extract has the ability to reduce iron (FRAP) suggests that they contain metabolites that are electron donors to react with free radicals for convert into more stable to terminate the radical chain reaction. Similar findings reported by Labiad *et al.*[32] that redox properties can be present in phenolic compounds and by them specific activity such as reducing agents, hydrogen donators and singlet oxygen quenchers.

GC-MS analysis of ethanol extract of *S. glauca* seeds revealed the presence of fatty acid esters such as Ethyl oleate (24.20%), Oleic acid (16.13%), 5-Hydroxy methylfurfural (12.69%) and Hexadecanoic acid ethyl ester (12.22%), n-Hexadecanoic acid (11.45%), Octadecanoic acid, ethyl ester (8.27%), Tetraconatne (6.07%), Linoleic acid ethyl ester (4.27%), Heneicosane (3.02%) and Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester (1.68%) was detected first time in the seeds. There are no reports found associated with all these compounds previously with the selected plant extract. Patil *et al.*[33] reported similar bioactive constituents in petroleum ether extract in *Citrus medica* seeds. The consumption of these constituents or the use of these as dietary supplements may prevent human aliments due to its antioxidant richness.

## CONCLUSION

The present study based on the experimental findings, it can be concluded that the ethanol extract of *Simarouba glauca* seed exhibited significant antioxidant free radical scavenging activity on all tested assays (DPPH, ABTS and FRAP) and they possess substantial amounts of phytochemicals as flavonoid, proanthocynidin and phenolic compounds. It is confirmed by the GC-MS analysis by exploring 10 novel fatty acid compounds, they are held responsible for these activities and considered as novel source of antioxidants which might be beneficial application for combating reactive oxygen species.

## ACKNOWLEDGEMENT

The authors are thankful to the Principal and HoD, Department of Chemistry, Government Science College (Autonomous), Bangalore for providing all the necessary facilities for the research work and Department of Collegiate Education for pursing Ph.D. in Bangalore University, Bangalore.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**GCMS:** Gas chromatography-mass spectroscopy; **$AlCl_3$:** Aluminium chloride; **TFC:** Total flavonoid content; **TPAC:** Total proanthocyanidin content; **TPC:** Total phenolic content; **GAE:** Gallic acid equivalent; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl; **ABTS:** 2,2'-azino-bis - (3-ethyl benzthiazoline-6-sulfonic acid; **FRAP:** ferric reducing antioxidant potential; **AAE:** ascorbic acid equivalents; **$IC_{50}$:** inhibitory concentration; **BHT:** butylated hydroxytolune; **QE:** quercetin equivalent; **CE:** catechin equivalent; **$FeCl_3$:** ferric chloride; **nm:** nano meter; **μG:** micro gram; **mL:** mili litre; **g:** gram; **mg:** mili gram; **mL/min:** mili litre/minute; **min:** minute.

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**Cite this article:** Haleshappa R, Patil SJ, Usha T, Murthy KRS. Phytochemicals, Antioxidant Profile and GCMS Analysis of Ethanol Extract of *Simarouba glauca* Seeds. Asian J Biol Life Sci. 2020;9(3):379-85.