Phytochemical Analysis, In Vitro Evaluation of Antioxidant and Free Radical Scavenging Activity of Simarouba glauca Seeds

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Received November 17, 2020; Revised February 20, 2021; Accepted March 1, 2021

Abstract

Traditional Indian medicinal plant Simarouba glauca is a highly sacred plant known as “Paradise Tree or Lakshmi Taru” which is recognized for its pharmacological and pharmaceutical properties. The present study was envisaged to know the in vitro phytochemical, antioxidant and free radical scavenging activity of S. glauca seeds. Petroleum ether was used as a solvent to extract plant material using hot extraction method. In our investigation, phytochemical analysis was carried out qualitatively and quantitatively and for the evaluation of antioxidant profile carried out in vitro studies on free radical scavenging potential by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, decolourization potential of 2,2’-azino-bis[3-ethyl benzthiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant potential (FRAP) assay were determined in in vitro studies. The qualitative phytochemical analysis of this plant was positive for flavonoids, fatty acids, proteins, steroids and terpenoids and in quantitative analysis total flavonoid content was 17.32±0.12 mg/μg quercetin equivalent, total proanthocyanidin content exhibited 62.91±0.61 μg catechin equivalent, total phenol content exhibited 17.75±5.82 μg gallic acid equivalent and the total flavonol content was 1.54±0.01 μg quercetin equivalent of the extract. In the antioxidant profile, the maximum DPPH scavenging activity exhibited was 74% with an IC₅₀ value 137.89 μg/mL, the maximum ABTS scavenging activity exhibited was 35% with an IC₅₀ value 337.29 μg/mL and FRAP scavenging activity exhibited 62.5 μg/mL as ascorbic acid equivalents (AAE/ml) concentration. These findings evidenced that, the petroleum ether extract of S. glauca seeds has potential source of natural antioxidants and can be used it as therapeutic medicine.

Keywords Simarouba glauca, Seeds, Phytochemical, Antioxidant, Free radical, Medicine

1. Introduction

The quest for medicinal plants for various ailments of humans from nature is common and it is evidenced by literature. Nature is nurturing us by a rich source of medicinal plants and their products from many centuries, and potential modern drugs have been isolated as natural products, with ayurvedic traditional medicine (ATM) as the base for the common to specific applications [1]. The plant origin according to ATM continues to play an pivotal role in health care and around 80% of the world’s population depends on plant based medicine for primary use for various diseases and disorders [2]. The current focus, on medicinal plants is an emerging field for the researchers to address as a cost effective alternative source for various health issues to cost effective allopathic drugs to manage personal health, in safer bioprospecting for
newer plant products as drugs [3]. The ongoing enthusiasm on medicinal plant research has several reasons to opt in, considering the faith on herbal medicines as ayurvedic traditional medicine (ATM). Therefore, an increasing trend in medicinal plants use is observed for the remedies, as industrial outcome has been identified to the process of extraction, isolation, innovation of newer drugs and its application as pharmacotherapeutics from selected plants as ATM (2). As per the World Health Organization (WHO), medicinal plants can be the best natural source to identify a number of drugs. Hence, a lot of attention is concentrated on such novel plants to understand their, medicinal properties and safety [4].

*Simarouba glauca*, is popularly called as ‘Laxmi taru’ or ‘Paradise Tree’ belongs Simaroubaceae family. The species glauca means covered with bloom which exhibit bluish green foliage and the term derived from Greek word ‘glaukos’ (bluish) [5]. *S. glauca* has been recognized as ATM plant due its huge medicinal application as anticancer, antimicrobial, antiviral and antihelminthic principle in various parts of the plant. *S. glauca* has various phytoactive compounds like quassinooids, glaucarubin, glaucarubolone and glaucarubinone required for pharmacological applications [6, 7]. The health promoting oil, extracted and characterized from *S. glauca* was composed of oleic acid and other fatty acid molecules [8]. Earlier findings report that water extract of *S. glauca* effectively influence and differentiate the skin of keratinocytes [8] and also showed improvement in hydration rate and supported moisturisation of skin [9]. The non polar to polar solvent extraction obtained, wide range of secondary metabolites and reported their active principles such as ailanthinine, benzoquinone, glaucarubin, holacanthone, melianone, quassinooids, simarolide, simaroubidin, simarubin, simarubolide and sitosterol from biological studies [10]. In our present study, we have extensively reviewed the literature on biological properties of *S. glauca* seeds, and research findings available on the seed extract on phytochemical and biochemical profile is scanty. Hence, we are presenting our findings on in vitro studies on the phytochemical and biochemical profile of the *S. glauca* seeds from petroleum ether extract for the development of a natural product for application.

### 2. Materials and Methods

#### 2.1. Plant Material

*Simarouba glauca* seeds were collected from University of Agricultural Sciences, Bengaluru, Karnataka, India in the summer season. The seed samples were authenticated by Dr. Shiddamallayya Mathapathi, Senior Research Officer (Botany), at Regional Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH and Government of India.

#### 2.2. Preparation of Plant Extracts

*Simarouba glauca* seeds were collected, decoated manually, washed cleanly in distilled water and shade dried for complete removal of moisture. The seeds were ground manually, powdered and used for successive Soxhlet extraction with petroleum ether for 18 hours, and dried at Buchi’s rotary vacuum evaporator and stored in refrigerator.

#### 2.3. Analysis of Qualitative Phytochemicals

Qualitative phytochemical analysis of the petroleum ether extract of the seeds was processed to identify the secondary metabolites. The petroleum ether extract of *Simarouba glauca* seeds were analysed by defined phytochemical methods as per Fransworth [11], Harborne [12] and Sharangouda and Patil [13] to explore the metabolites such as steroids, triterpenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates, fatty acids, proteins and amino acids. Further, knowing the presence of possible metabolites analysed quantitatively total flavonol, flavonoid, proanthocynodin and phenol content as per the standard procedure of Harborne [14]. The extract was suspended in 0.2% Tween 80, dissolved in double distilled water and filtered. The filtrate was used for the phytochemical analysis in triplicates to obtain concurrent values for statistical analysis.

#### 2.4. Analysis of Quantitative Phytochemicals

The extract was used for the qualitative 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 μg/ml concentration.

##### 2.4.1. Estimation of Total Flavonol Content

The total flavonol content was estimated using AlCl₃ method with standard quercetin [15] at 510 nm and was expressed as μg of quercetin equivalents/mg of ethanol extract.

##### 2.4.2. Estimation of Total Flavonoid Content

The total flavonoid content was estimated using AlCl₃ method with standard quercetin [15] at 510 nm and was expressed as μg of quercetin equivalents/mg of ethanol extract.

##### 2.4.3. Estimation of Total Proanthocyanidin Content

The total proanthocyanidin content was estimated using vanillin–hydrochloride method as described by Kamala et al., [16] at 500 nm and was expressed as μg catechin equivalents/mg of ethanol extract.

##### 2.4.4. Estimation of Total Phenolic Content

The total phenolic content was estimated by Folin–
Ciocalteau method [17] at 765 nm, expressed as µg gallic acid equivalent (GAE)/mg ethanol extract by following formula.

\[ T = \frac{(C \times V)}{M} \]

Where \( T \) is the total phenolic content 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

2.5. In vitro Biochemical Profile of Petroleum Ether Extract of Simarouba glauca Seeds

The in vitro biochemical profile of the seed extract of Simarouba glauca were estimated by DPPH, ABTS and FRAP assay to know the free radical scavenging activity in triplicate value to minimize the error for the concurrent value by statistical analysis with maintaining positive and negative control in particular wavelength in spectrophotometer to determine the µg/ml concentration.

2.5.1. DPPH Assay

DPPH assay was carried out to determine antioxidant potential as described by Blois [18] method. The reaction mixture was mixed well and incubated at room temperature for 30 minutes and the absorbance was recorded at 517 nm. The control was prepared by adding 2 ml of DPPH solution and 1 ml of methanol [19]. The statistical analysis determined the IC50 value using linear regression equation i.e.

\[ Y = Mx + C \]

Where, \( Y = 50 \), \( M \) and \( C \) values were derived from the linear graph trendline.
\[
\% \text{ Scavenged DPPH} = \left( \frac{(Ac - As)}{Ac} \right) \times 100
\]

\( Ac \) and \( As \) are the absorbance of the control and the sample, respectively.

2.5.2. ABTS Free Radical Scavenging Assay

ABTS assay was carried out to determine antioxidant potential as described by Re et al., [20]. 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 hydroxytoluene (BHT) was added and absorbance was measured at 734 nm. The statistical analysis determined the IC50 value using linear regression equation i.e.

\[ Y = Mx + C \]

Where, \( Y = 50 \), \( M \) and \( C \) values were derived from the linear graph trendline.

\[
\% \text{ Scavenged ABTS} = \left( \frac{(Ac - As)}{Ac} \right) \times 100
\]

\( Ac \) and \( As \) are the absorbance of the control and the sample, respectively.

2.5.3. FRPF Assay

The FRPF assay carried out to determine antioxidant potential as described by the method of Benzie and Strain [21]. 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.

2.6. Statistical Analysis

All the experiments were carried out in triplicates and were expressed as mean difference of standard deviation ± standard error (SD ± SE). The data were statistically analyzed using Microsoft Office Excel 2007.

3. Results

3.1. Qualitative Phytochemical Analysis of Petroleum Ether Extract of S. glauca Seeds

The qualitative analysis of phytochemicals of ethanol extract of Simarouba glauca seeds was positive for flavonoids and carbohydrates whereas negative for steroids & triterpenoids, alkaloids, tannins, glycosides, proteins and amino acids (Table1).

| Qualitative phytochemical analysis |
|-----------------------------------|
| **Test**                          | **Petroleum ether 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                        |
| 9 Fatty acids                      | +ve                        |

+ = Positive; – = Negative
3.2. Quantitative Phytochemical Analysis of Petroleum Ether Extract of *S. glauca* Seeds

3.2.1. Estimation of Total Flavonol Content

The total flavonol content of petroleum ether extract of *S. glauca* was found to be 1.54±0.01 µg quercetin (QE/g) and the results were calculated using the QE as a standard (Table 2).

3.2.2. Estimation of Total Flavonoid Content

The total flavonoid content of petroleum ether extract of *S. glauca* was found to be 17.32±0.12 µg quercetin (QE/g) and the results were calculated using the QE as a standard (Table 2).

3.2.3. Estimation of Total Proanthocyanidin Content

The total proanthocyanidin content of petroleum ether extract of *S. glauca* was found to be 62.91±0.61 µg catechin (CE/g) and the results were calculated using the CE as a standard (Table 2).

3.2.4. Estimation of Total Phenol Content

The total phenol content of petroleum ether extract of *S. glauca* was found to be 17.75±5.82 µg gallic acid equivalent (GAE/g) and the results were calculated using the GAE as a standard (Table 2).

3.3. DPPH Scavenging Activity

The petroleum ether 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 are presented in Graph 1. The five graded concentrations were used in the study along with blank, cell control and standard control. Petroleum ether extract showed free radical scavenging activity as 13.20%, 26.99%, 36.55%, 50.64% and 74.08 % 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 (IC₅₀) value of the *S. glauca* seed extract exhibited 137.89 µG/mL against the DPPH (Graph 1).

### Table 2. Quantitative Phytochemicals assay of petroleum ether extract of *S. glauca* seeds.

| Sl. No. | Total Flavonol Content (µg QE/mg) | Total Flavonoid Content (µg QE/mg) | Total Proanthocyanidin Content (µg CE/mg) | Total Phenol Content (µg GAE/mg) |
|---------|----------------------------------|------------------------------------|------------------------------------------|----------------------------------|
| 1       | 1.54±0.01                        | 17.32±0.12                         | 62.91±0.61                               | 17.75±5.82                      |

Values expressed as SD ± SE of triplicate determination of experiments.
3.4. ABTS Scavenging Activity

The petroleum ether 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. The five graded concentrations were used in the study along with blank, cell control and standard control. Petroleum ether extract showed free radical scavenging activity as 1.69%, 10.69%, 17.98%, 26.22% and 35.91% 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 exhibited 337.29µG/mL against the ABTS (Graph 2).

3.5. FRAP Scavenging Activity

The petroleum ether 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. The five graded concentrations were used in the study along with blank and control. Petroleum ether extract showed scavenging activity as 1.33ug/ml, 15.50ug/ml, 28.16 ug/ml, 42.66ug/ml and 62.50ug/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 (Graph3).
The correlation coefficients of ethanol extract of S. glauca seeds with DPPH and ABTS assays were 0.9515 and 0.8892 respectively, confirming that proanthocyanidin compounds exhibited 62.91±0.61 µg CE/mg at the concentration are likely to contribute the free radical scavenging activity and acting as principle molecule as antioxidant in in vitro condition.

4. Discussion

Oxidative stress by exposure of radiation releases free radicals excessively and damage of the biomolecules which are produced by the cells for the support of biological function [22]. Phytochemical and biochemical profiling of Simarouba glauca seeds extract exhibited remarkable results as evidenced by the positive phytochemicals for fatty acids, proteins, steroids and terpenoids qualitatively whereas, total flavonol and flavonoid content exhibited optimum at the quercetin equivalent/mg/ml concentration, compared to total phenol, proanthocyanidonic content exhibited maximum when compared to standard catechin and gallic acid equivalent/mg/ml concentration. These contents were determined using aluminium chloride method to form stable complex with the carbonyl group at C4 position, 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” [23]. Our findings are in agreement with the recent research reports of Kamala et al.,[16] and Vinson et al., [24]. Proanthocyanidins covered all type of plant species, including fruits, seeds, leaves, flower, nuts or barks of the plants. Proanthocyanidins, are a subclass of the complex flavonoids, metabolite of nonpolar extract, may be condensed with tannins and flavan-3-olsas polymer and bound with the group of polyphenols due to their biological properties, like anticancer, antiinflammatory and antioxidant potential [25]. Puranik et al., reported that phenolic compounds are highly active in the ethanolic extract of S. glauca leaf as key secondary metabolites of the plant to combat bladder cancer [26]. Whereas, presence of complex phytochemical agents in S.glaucya leaves exhibit potential characteristics against cancer cell suppression, proliferation and tissue regeneration [27, 28].

The petroleum ether extract of S. glauca seeds on free radical scavenging activity using DPPH assay showed maximum activity in comparison to Ascorbic acid control. The concentration dependent increase in scavenging activity of the extract may be due to the ability of hydrogen donor during oxidation reaction [29]. It was also observed that no one concentration could achieve total inhibition of the enzymes in the studied extract. The dose inhibition curve and IC_{50} value 137.89 µg/mL of petroleum ether extract, showing maximum free radical scavenging activity due to the crude nature of the extract, considering it as a sign of possessing potential antioxidant property. Such free radical scavenging activity results were observed with similar findings, and also IC_{50} value higher than that of standard when the crude extract of plants for the biochemical studies were used [30].

ABTS assay can be used to determine the anti free radical scavenging activity as well as the hydrophilic and lipophilic biochemical reactions, and apply it for all types of solvent extracts to compare it with other antioxidant assays. ABTS assay depends on the presence of antioxidant reactants with the radical cation and reduction in decolourization potential property [20]. In the present study, petroleum ether extract of S. glauca seeds were assessed for its ABTS free radical scavenging activity. Standard BHT IC_{50} value was 10.00µg/mL and petroleum ether extract exhibited maximum IC_{50} value was 337.29 µg/mL. Similar findings were exhibited with different standards with stronger to weaker antioxidant activity, the ABTS decolourization potential assay were shown by various medicinal plant extracts in crude nature as well as isolated fractions [31].

The biochemical components are responsible for changing ion reaction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) to form a blue-colored ferrous tripyridyltriazine complex (Fe2+-TPTZ) at pH 3.6. The change was monitored spectrophotometrically at 593 nm [32]. The reduction of ferric cyanide complex (Fe3+/(CN)^-) to ferrous cyanide form (Fe2+/(CN)^-) is an indicator that the extract has an electron-donating ability [33]. Petroleum ether extract of S. glauca seeds exhibited maximum FRAP value as 62.5ug/ml at the concentration of 250mg/ml and equivalent to ascorbic acid/g of extract. The extract has the iron reduction potential, thus suggesting that some phytochemicals are electron donors, that react with free iron radicals to convert as stable radical chain reaction to terminate the process. Similar observations were reported by Oka et al., on Sphenocentrum jollyanum leaf extracts having potential ability to reduce as a chelating metal agent, exhibiting scavenging activity. Furthermore, the extract showed flavonoid and phenol content, thus inferring that the biochemical reactions may be due to the extract, which might have a redox potential as novel antioxidants [34].

5. Conclusions

We conclude our research findings to acknowledge that the petroleum ether extract of Simarouba glauca seeds exhibits active phytochemicals, in vitro antioxidants and free radical scavenging potential evidenced by the activity of DPPH, ABTS and FRAP assays and they also possess strong total content of flavonol, flavonoid, proanthocyanidin and phenolic constituents. Our future studies would focus
on isolation and characterization of specific compounds responsible for their potential antioxidant activity, and their mechanism of action may be useful for their application against cardiovascular, cancer, diabetes and neurodegenerative diseases due to the strong antioxidant nature of the plant extract.

REFERENCES

[1] Pandey MM., Rastogi S., AKS. Rawat, "Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation", Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 376327, 12, 2013. doi.org/10.1155/2013/376327

[2] Ekor M, "The Growing use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety". Frontiers in Pharmacology, 4,177.doi: 10.3389/fphar.2013.00177

[3] Mordeniz E, "Introductory Chapter: Traditional and Complementary Medicine, Traditional and Complementary Medicine", Cengiz Mordeniz, IntechOpen, 2019, doi: 10.5772/intechopen.86373.

[4] "WHO Global Report on Traditional and Complementary Medicine 2019". Geneva: World Health Organization; 2019.

[5] Stojanoski N, "Development of Health Culture in Veles and its Region from the Past to the End of the 20th Century". Veles: Society of Science and Art. 1999:13–34.

[6] Joshi S.S., Hiremath, "Simarouba - A Potential Oilseed Tree". Current Science, 78,6,694-697, 2000.

[7] Ham EA, Schafer HM, Denke walter RG, Brink NG, "Structural studies on glaucarubin from Simarouba glauca". Journal of American Chemical Society, 76,23,6066-6068, 1954.

[8] Patil MS., DKA. Gaikwad, "Critical Review on Medicinally Important Oil Yielding Plant Laxmitaru (Simarouba glauca DC.).". Journal of Pharmaceutical Sciences and Research, 3,4, 1195-1213, 2011.

[9] Govindaraju K., Darukeshwara J., SK. Srivastava, "Studies on Protein Characteristics and Toxic Constituents of Simarouba glauca oil Seed Meal". Food and Chemical Toxicology, 47,6,327-332, 2009.

[10] Antony J., Thomas A., Gnanasekaran D.,SH. Elizabeth,"Review Study on Pharmacological Importance of Simarouba glauca". International Journal of New Technology and Research, 2, 10, 59-62, 2016.

[11] Farnsworth NR., Akerele O., Bingel AS., Soejarto DD., Z. Guo, "Medicinal Plants in Therapy". Bulletin of World Health Organization, 63,6, 965, 1985.

[12] Harborne JB, Phytochemical Methods: "A Guide to Modern Techniques of Plant Analysis", Chapman and Hall, London, UK, 1973.

[13] Sharangouda. SB. Patil, "Phytochemical Screening and Antifertility Activity of Various Extracts of Citrus medica (Lemon)Seeds in Albino Rats". Advances in Pharmacology and Toxicology, 8, 2,71-74, 2007.

[14] Harborne JB, "Phytochemistry". London: Academic Press, pp. 89–131, 1983.

[15] Patel A., Patel A., NM. 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,1, 66–77, 2010.

[16] Kamala A., Midhda SK., Gopinath C., Sindhrua HS.,CS. Karigar, "In Vitro Antioxidant Potentials of Cyperus rotundus L. Rhizome Extracts and Their Phytochemical Analysis". Pharmacognosy Magazine, 14,54, 261–267, 2018.

[17] Singleton VL.,JA. Rossi, "Colorimetry of Total Phenolics with Phosphomolybdic-phosphotungstic Acid Reagents". American Journal of Enology and Viticulture, 16, 144–158, 1965.

[18] Blois MS, "Antioxidant Determinations by the Use of a Stable Free Radical". Nature, 181, 1199–1250, 1958.

[19] Haleshappaa R., Keshamnaa E., Girijaa CR., Thanmayii M., Nagesh CG.,Lubna Fahmeena GH., Lavanya M.,SJ. Patil, "Phytochemical Study and Antioxidant Properties of Ethanolic Extracts of Euphorbia milii". Asian Journal of Biological Sciences, 13, 1, 77-82, 2020.

[20] Re R., Pellegrini N., Proteggentee A., Pannala A., Yang M., C. Rice-Evans,"Antioxidant Activity Applying an Improved ABTS Radical Cation DecolorizationAssay". Free Radical Biology and Medicine, 26, 1231-1237, 1999.

[21] Benzie IFF., JJ. Strain, "Ferric Reducing/Antioxidant Power Assay: Direct Measure of Total Antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration". Methods in Enzymology, 299, 15–27, 1999.

[22] Kara A., Gedikli S., Sengul E., Gelen V.,Ozkanlar, "Oxidative Stress and Autophagy, Free Radicals and Diseases", Rizwan Ahmad, IntechOpen, 2019. doi: 10.5772/64569.

[23] Goyal AK., Midhda SK., 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, 1,1, 40-45, 2010.

[24] Vinson YJ., Mamdough AD., MS. Jang, "Plant Flavonoids, Especially Tea Flavonols, Are Powerful Antioxidants Using an in Vitro Oxidation Model for Heart Disease". Journal of Agricultural and Food Chemistry, 43,11, 1995DOI: 10.1021/jf00059a005

[25] Rodriguez-Pérez C.,Garcia-Villanova B., Guerra-Hernández E.V. Verardo, "Grape Seeds Proanthocyanidins: An Overview of In Vivo Bioactivity in Animal Models". Nutrients, 11, 2435, 2019.

[26] Puranik SI., Ghagane SC., NeraliRB., Jalalpure SS., MB. Hiremath, "Evaluation of In Vitro Antioxidant and Anticancer Activity of Simarouba glauca Leaf Extracts on
T-24 Bladder Cancer Cell Line". Pharmacognosy Journal, 9, 6, 906–912, 2017.

[27] Jose A., Kannan E., Vijay-Kumar ARP., SV. Madhunapantula, "Therapeutic Potential of Phytochemicals Isolated from Simarouba glauca DC for Inhibiting Cancers: A Review". Systemic Reviews in Pharmacy, 10,1, 73–80, 2019.

[28] Jose A., Kannan E., SV. Madhunapantula, "Anti-proliferative Potential of Phytochemical Fractions Isolated from Simarouba glauca DC Leaf". Heliyon, 6,e03836, 2020.

[29] Iranshahi M., Askari M., Sahebkar A., D. Adjipavlou-Litina,"Evaluation of Antioxidant, Anti-inflammatory and Lipoxygenase Inhibitory Activities of the Prenylated Coumarin Umbelliprenin". DARU Journal of Pharmaceutical Sciences, 17, 99–103,2009.

[30] Middha SK., Usha T., V. Pande,"HPLC Evaluation of Phenolic Profile, Nutritive Content and Antioxidant Capacity of Extracts Obtained from Punica granatum Fruit Peel". Advances in Pharmacological Sciences, 1, 296236, 2013.

[31] Pisoschi AM., Pop A., Cimpeanu C., G. Predoi, "Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review". Oxidative Medicine and Cellular Longevity, 9130976, 36, 2016.doi.org/10.1155/2016/9130976

[32] Chung YC., Chang CT., Chao WW., Lin CF., ST. Chou, "Antioxidative Activity and Safety of the 50 Ethanolic Extract from Red Bean Fermented by Bacillus subtilis IMR-NK1". Journal of Agriculture and Food Chemistry, 50, 8, 2454–2458, 2002.

[33] Farhan H., Malli F., Rammal H., Hijazi A., Bassal A., Ajouz N., B.Badran, "Phytochemical Screening and Antioxidant Activity of Lebanese Eryngium cicutum L". Asian Pacific Journal of Tropical Biomedicine, 2,3, S1217-S1220, 2012.

[34] Uka E., Etim OE., Effiong AO., IE. Jacobs, "Phytochemicals, Acute Toxicity and In-Vitro Antioxidant Activity of Ethanol Extract of Sphenocentrum jollyanum Leaves". Journal of Drugs and Pharmaceutical Science, 4, 2, 10-20,2020.doi.org/10.31248/JDPS2020.035