Karnataka University Journal of Science 54(4), (2023), 73–78

Karnataka University Journal of Science

ISSN: 0075-5168

Original Article

GC-MS Analysis of Phytoconstituents from Methanolic Leaf Extract of *Simarouba glauca*

Priyadarshini S Shettar[1], Murigendra B Hiremath[1,*]

[1] *Department of Biotechnology and Microbiology, Karnatak University, Dharwad, Karnataka, India*

ARTICLE INFO

Article history:
Received 29.09.2023
Accepted 15.11.2023
Published 27.12.2023

* *Corresponding author.*
Murigendra B Hiremath
mbhiremath.lab@gmail.com

https://doi.org/
10.61649/kujos/v54i4.23.4

ABSTRACT

Simarouba glauca (SG) is known for exhibiting anti-inflammatory, anti-neoplastic, anti-diabetic and analgesic properties. The aim of this study is to analyze the phytochemical composition, presence of total phenols and flavonoids, anti-oxidant activity and Fourier Transform Infrared spectroscopy (FT-IR) and Gas Chromatography- Mass Spectroscopy (GC-MS) analysis of *Simarouba glauca* leaf extracts. The total phenolic content of chloroform and acetone extracts was 136.71 ± 0.06 and 98.35 ± 0.02 mg/g GAE respectively. The total flavonoid content of chloroform and acetone extracts was 311.06 ± 0.03 and 358 ± 0.05 mg/g QE respectively, which are higher than that of other extracts. Both acetone and methanol extracts showed higher anti-oxidant capacities in contrast to other solvent extracts. The Gas Chromatography- Mass Spectroscopy (GC-MS) analysis of methanol extract exhibited the presence of twenty-two major peaks. The biological activities of these compounds are discussed in the present study. The overall results of this work provide remarkable evidence for the use of the methanol fraction of *Simarouba glauca* as a competent source of phytochemicals that are effective against various diseases.

Keywords: GC-MS Analysis; FR-IR Analysis; *Simarouba glauca*; Anti-oxidant Activity

1 INTRODUCTION

Simarouba glauca, commonly called '*Laxmitaru* or Paradise Tree,' belongs to the *Simaroubaceae* family. It is a native tree of Central and South America and is found in countries like South Florida, Caribbean islands, Cuba, Costa Rica, Mexico, Bahamas, Jamaica, and so on. This plant was introduced to India in 1960 [1]. The leaf, bark, pulp, fruit and seeds of this plant are widely used in the treatment of various diseases. *Simarouba glauca* (SG) is known for exhibiting anti-neoplastic, anti-diabetic and analgesic properties. Many genera of this family are employed in the treatment of malaria, gastritis, inflammation, diarrhoea and diabetes. In addition to their ethnopharmacological uses, plants from the *Simaroubaceae* family can also be emphasized for their chemical diversity. The presence of quassinoids, alkaloids, terpenes, steroids, flavonoids, anthraquinones, coumarins, saponins, etc. has been determined [2].

The main constituents that promote the medicinal properties of this plant are quassinoids. The quassinoids and alkaloids extracted from the plant possess cytotoxic activity and anti-proliferative properties [3]. The quassinoids found in *Simarouba glauca* are glaucarubin, glaucarubol and glaucarubolone [4]. The pharmacological activities of the isolated compounds reported are cytotoxic, insecticidal, anti-tumour, hypoglycaemic and anti-ulcer activities [5]. However, no systematic work has been carried out on Gas Chromatography- Mass Spectroscopy (GC-MS) profiling and Fourier Transform Infrared spectroscopy (FT-IR) analysis of phytochemical constituents and biological activities of leaf extracts of *Simarouba glauca*. Therefore, this study intends to evaluate the anti-oxidant capacity by DPPH assay, presence of total phenolic and flavonoid contents. The GC-MS analysis of the methanol extract of *Simarouba glauca* exhibited the presence of phytoconstituents that are known to have certain medicinal properties. In this paper, some industrially important compounds are focused on their properties such as: production of various cosmetics, fragrances, biosurfactants and pesticides.

2 METHODOLOGY

2.1 Collection of plant material

The fresh leaves of *Simarouba glauca* (Voucher specimen no.: KUD/BT/PS/MH/01) were collected, identified and authenticated from the Department of Botany, Karnatak University Dharwad, Karnataka, India (Figure 1). The leaves were washed, shade dried and homogenized to a coarse powder with a mechanical grinder. The dried samples were pulverized into powder and preserved in an air-tight container for further study.

Figure 1: *Simarouba glauca*

2.2 Preparation of plant extracts

The extraction of dried leaf powder (50 g) of *Simarouba glauca* (SG) was carried out with solvents (500 ml) of increasing polarity, starting with Petroleum ether (SGP), Chloroform (SGC), Acetone (SGA), Methanol (SGM) and Distilled water (SGW) in the Soxhlet apparatus for 8-10 hours. The solvents from each fraction were evaporated in a rotary evaporator under reduced pressure and the concentrated fraction was dried. The resultant crude yield is weighed. The extracts obtained were kept in moisture-free conditions.

2.3 Phytochemical screening

Phytochemical screening was carried out for all five solvent extracts of *Simarouba glauca* by following the protocol of Deepti *et al.,* [6]. The crude extracts were tested for the presence of phytochemicals such as alkaloids, phenols, flavonoids, glycosides, lignins, saponins, sterols, tannins, terpenoids and anthraquinone.

2.4 Determination of Total Phenolic Content

The Folin- Ciocalteau method was used to determine the total phenol content as previously described by Singleton *et al.,* with slight modifications [7]. Various concentrations of gallic acid solution were incubated with 0.5 ml of 50% FCR and 1.5 ml of 7% Sodium carbonate for 30 min at room temperature. The absorbance was measured at 760 nm. The leaf extracts were diluted and processed in the same way as described for gallic acid. The absorbance of leaf extract was extrapolated into a standard curve to determine the concentration of total phenols.

2.5 Determination of Total Flavonoid Content

The total flavonoid content of leaf extracts of SG was determined by following the method described by Phuyal *et al.,* with minor modifications [8]. Quercetin was used as a standard. A known amount of extract was taken and the volume was made up to 3 ml with methanol, followed by the addition of 0.1 ml of Aluminium chloride (10 %), 0.1 ml of Sodium acetate (1.0 M) and distilled water (2.8 ml). After incubation (30 min) at room temperature, the absorbance was measured at 415 nm. Distilled water was used as a blank. The concentration of total flavonoids in leaf extract was determined by extrapolating the absorption of unknown samples into a standard calibration curve obtained with quercetin.

2.6 Free radical scavenging assay

Radical scavenging activities of leaf extracts of *Simarouba glauca* were determined using the DPPH radical as a reagent, according to the methods of Rice-Evans *et al.,* [9]. DPPH radical solution (1 ml) in methanol (60 μM) was mixed with 50-250 μl of sample solution in methanol. The mixture was incubated for half an hour in the dark at room temperature and then absorbance was measured at 517 nm using a UV-visible spectrophotometer. The DPPH scavenging activity was calculated using the following equation:

Percentage of inhibition = Ac- At/Ac x 100

where, Ac is the absorbance of the control reaction (100 μl of methanol with 100 μl of DPPH solution) and At is the absorbance of the test sample.

2.7 FT-IR analysis

The FT-IR analysis (Model: Nicolet iZ 10) was carried out at the University Scientific Instrumentation Center (USIC), Karnatak University, Dharwad, Karnataka, India.

2.8 Identification of bioactive compounds by GC-MS analysis

It was performed on a combined GC-MS instrument (Agilent 5977 MSD) at the Sophisticated Analytical Instrument Facility (SAIF), IIT Madras. Around 1 μl aliquot of sample was injected into the column using a Split mode in which the temperature was set at 260° C. The GC program was initiated by a column temperature set at 75° C for 0.5 min, increased to 180° C at a rate of 5° C/ min and held for 5 min. The mass spectrometer was operated with the mass source at 250° C. The Helium was used as the carrier gas (1.00 ml/min).

2.9 Statistical analysis

All experiments were performed in triplicate (n=3) and the data is represented as the mean ± standard error, which is calculated using Microsoft Excel.

3 RESULTS

3.1 Phytochemical screening

Phytochemical analysis of crude extracts revealed the presence of secondary metabolites synthesized in the plant,

Table 1: Phytochemical screening of *Simarouba glauca* leaf extracts

Constituent	Test	Petroleum ether	Chloroform	Acetone	Methanol	Aqueous
Alkaloids	Dragendroff 's	-	+	+	+	+
	Wagner's	+	+	+	+	+
Flavonoids	Shinoda	+	-	-	+	+
	NaOH	-	+	+	-	-
Glycosides	Keller-Killani	-	+	-	+	-
	Bromine water	+	-	+	+	+
Phenols	Lead acetate	-	+	+	+	-
	Ellagic acid	+	-	+	+	+
Lignins	Labat	-	+	-	+	-
Saponins	Foam test	-	-	-	+	+
Sterols	Salkowski's	-	+	+	+	+
Tannins	FeCl3 test	-	+	+	-	+
	Gelatine	-	-	+	-	+
Anthraquinone	Bomtrager's	-	-	-	-	+
Terpenoids	Salkowski's	-	-	-	-	+

'+' indicates the presence and '-' indicates the absence of phytoconstituents

which include alkaloids, phenols, flavonoids, saponins, anthraquinones, glycosides, lignins and tannins (Table 1).

3.2 Total Phenolic Content

The total phenolic content of chloroform extract, calculated from the calibration curve (R^2 = 0.9601) was the highest (136.71 ± 0.06 mg/g GAE). The T.P.C. of acetone, petroleum ether, aqueous and methanol extracts are 98.35 ± 0.02 mg/g, 90.30 ± 0.02 mg/g, 72.62 ± 0.07 mg/g and 67.74 ± 0.02 mg/g GAE respectively.

3.3 Total Flavonoid Content

The total flavonoid content of chloroform extract was higher (884.99 ± 0.03 mg/g QE) than other extracts. The T.F.C. of acetone, petroleum ether, aqueous and methanol extracts are 358.77 ± 0.05 mg/g, 311.06 ± 0.08 mg/g, 21.72 ± 0.02 mg/g and 14.95 ± 0.06 mg/g QE respectively, as calculated from the calibration curve (R^2 = 0.9954).

3.4 Free radical scavenging activity

Various concentrations of petroleum ether, chloroform, acetone, methanol and aqueous extracts of SG leaf extracts were subjected to DPPH free radical scavenging assay. The results are presented in Figure 2 . Ascorbic acid was used as a standard.

3.5 FT-IR analysis

The FT-IR interpretation of the methanolic extract of *Simarouba glauca* showed the presence of ten functional groups (Figure 3). The various functional groups indicate the presence of aliphatic secondary amine (N-H stretch), alkane (C-H stretch), methyl ether (C-H stretch), methyl group (C-H asym. /sym. bend), amine (C-N stretch), aliphatic iodo compound (C-I stretch), polysulfides (S-S stretch) and aryl disulfides (S-S stretch) at 3329.30, 2942.46, 2831.69, 1449.79, 1020.88, 586.24, 499.57, 467.21, 441.50 and 423.35 respectively (Table 2). The various functional groups identified in this plant have diverse medicinal properties, such as anti-oxidant, anti-microbial and anti-proliferative potentials.

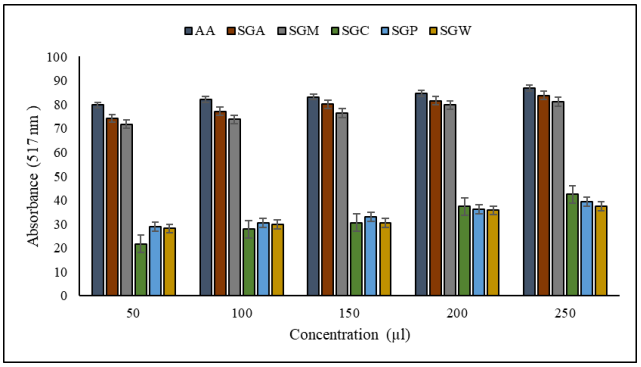

Figure 2: Determination of percentage inhibition by DPPH activity of *Simarouba glauca* extracts

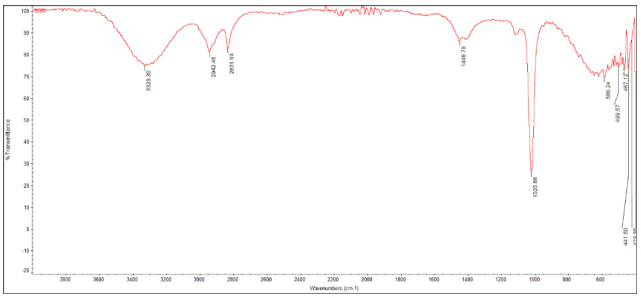

Figure 3: FT-IR Spectra of methanol extract of *Simarouba glauca*

3.6 Identification of active compounds by GC-MS analysis

GC-MS analysis of the methanol extract of SG is shown in Table 3. The GC-MS chromatogram of 22 peaks of the compounds detected is shown in Figure 4. The phytoconstituents were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the National Institute of Standard and Technology (NIST) library database.

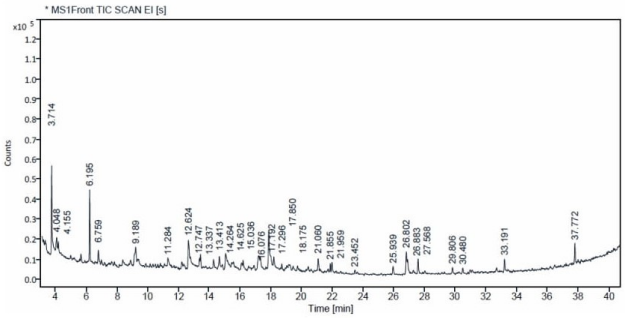

Figure 4: GC-MS Chromatogram of methanol fraction of *Simarouba glauca*

4 DISCUSSION

The plant kingdom consists of an enormous variety of compounds with a wide range of bioactive properties, such as anti-aging, moisturizing, fragrances and skin-whitening properties. Most of the plant-based products exhibit anti-inflammatory, anti-proliferative, anti-apoptotic, protection against harmful radiations and anti-oxidant activities. It may be due to the adaptability of plants, which makes them able to survive in highly oxidative environments [10].

The quassinoids and alkaloids extracted from this plant possess cytotoxic activity and anti-proliferative properties [3]. Many genera of the *Simaroubaceae* family are employed in the treatment of malaria, gastritis, inflammation, diarrhoea and diabetes. Along with ethnopharmacological uses, plants of this family can be emphasized for their chemical diversity. The presence of quassinoids, alkaloids, terpenes, steroids, flavonoids, coumarins, anthraquinones, saponins, etc. has been determined. The pharmacological activities of the isolated compounds reported are cytotoxic, insecticidal, anti-tumour, hypoglycaemic and anti-ulcer activities [5].

Earlier studies suggest that phenolic compounds present in the plants have redox properties that allow them to act as potential anti-oxidants. The results obtained from this study showed a significant level of phenolic compounds in various leaf extracts of *Simarouba glauca*. The chloroform and methanol extracts showed the highest phenolic content. Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities [11]. Chloroform and acetone extracts exhibited higher flavonoid contents than other extracts.

The anti-oxidant assay of crude extracts in this study are comparable to the anti-oxidant values of earlier studies [12, 13]. Interestingly, in acetone and methanol extracts, the highest anti-oxidant capacity was observed. In the DPPH assay (Figure 2), maximum inhibition was observed in the acetone and methanol fractions and minimum inhibition was observed in the aqueous fraction. The dose-dependent increase in the percentage of inhibition for all samples was observed. Based on the percentage of inhibition, the order of scavenging activity of various fractions is as follows: SGA> SGM> SGC> SGP> SGW.

The GC-MS analysis of the methanol extract exhibited the presence of 32 major peaks. Among all the solvent extracts, the methanol extract showed the highest number of compounds. Out of 32 compounds, 22 are found to exhibit various biological activities. Hence, a detailed account of these compounds is given here. The first peak obtained was glycerine. The following compounds are believed to possess anti-cancer properties: peak 2 and 18 [14, 15]. Compounds such as Undecane, peak 13 [16], 15 and 18 possess anti-oxidant and anti-inflammatory properties [15].

Many of the compounds have anti-microbial potential, such as peak 6, 9 [17], 15, cis-Sinapyl alcohol and Umckalin [18]. These compounds have been proven to have biological activities that induce anti-allergic, anti-inflammatory, anti-microbial, anti-neoplastic effects (Figure 5).

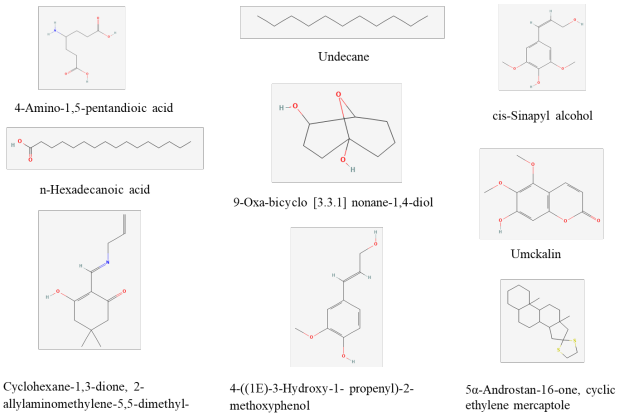

Figure 5: Phytoconstituents identified in methanol fraction of *Simarouba glauca*

A few of the industrially important compounds were identified that are used in the production of various cosmetics, fragrances and flavouring agents. Dodecanoic acid, 2- methyl- has biosurfactant properties [19]. 4-Vinylcyclohexene diepoxide is used as a rodenticide [20]. Ethyl α-d-glucopyranoside is a flavour component of fermented foods such as refined sake, wine and sweet sake (Japanese rice wine). It has skin conditioning and moisturizing effects [21]. Our findings are in accordance

Table 2: FT-IR analysis of methanol extract of *Simarouba glauca*

Sl. no.	Wavelength range (cm-1)	Bond	Functional group
1	3329.30	N-H stretch	Aliphatic secondary amine
2	2942.46	C-H stretch	Alkane
3	2831.69	C-H stretch	Methyl ether
4	1449.79	C-H asym. /sym. bend	Methyl group
5	1020.88	C-N stretch	Amine
6	586.24	C-I stretch	Aliphatic iodo compounds
7	499.57	S-S stretch	Polysulfides
8	467.21	S-S stretch	Aryl disulfides
9	441.50	S-S stretch	Aryl disulfides
10	423.35	S-S stretch	Aryl disulfides

Table 3: C-MS analysis of methanol extract of *Simarouba glauca*

Peak	Name of the compound	Retention Time	Area %
1	Glycerine	3.714	11.89
2	4-Amino-1,5-pentandioic acid	4.048	2.85
3	9-Hydroxy-7-nonanal	4.155	0.85
4	Undecane	6.195	8.52
5	3-(Prop-2- enoyloxy) dodecane	6.759	1.81
6	5α-Androstan-16-one, cyclic ethylene mercaptole	9.189	6.66
7	1,2,3-Benzenetriol	12.624	6.42
8	α-Pyrrolidinopentiophenone metabolite 1	12.747	4.01
9	2,3-Trimethylene-4- pyrone	14.264	1.79
10	Tricyclo[2.2.1.0(2,6)]heptane-3-methanol, 2,3- dimethyl	14.625	1.57
11	2-Acetylamino-3-hydroxypropionic acid	15.036	6.57
12	13-Oxatetracyclo [4.4.1.1(7, 10).1(9,11)] trideca-2,4- diene	16.076	0.82
13	9-Oxa-bicyclo [3.3.1] nonane-1,4-diol	17.850	10.86
14	Ethyl α-d-glucopyranoside	18.175	2.22
15	4-((1E)-3-Hydroxy-1- propenyl)-2- methoxyphenol	21.060	2.49
16	4-Vinylcyclohexene diepoxide (isomer 2)	23.452	0.63
17	Dodecanoic acid, 2- methyl-	25.939	1.17
18	n-Hexadecanoic acid	26.802	2.80
19	l-(+)-Ascorbic acid 2,6-dihexadecanoate	26.804	2.8
20	cis-Sinapyl alcohol	27.568	2.33
21	Umckalin	29.806	1.19
22	Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl-	30.480	0.82

with a study by Patil *et al.,* (2020) [22], which reported the GC-MS analysis of SG seeds (Table 3).

The FT-IR interpretation is a spectroscopic technique that is used for the characterization of chemical constituents [23]. It confirmed the presence of ten functional groups in the methanol extract. Previous studies have reported the FT-IR absorbance values in the purified fractions [13, 24].

5 CONCLUSION

The present study on phytochemical analysis, FT-IR and GC-MS profiling of the methanol extract of *Simarouba glauca* leaves revealed the presence of various phytoconstituents. The methanol fraction exhibited excellent anti-oxidant activity; hence, this extract was continued with further study. The GC-MS analysis of the methanol fraction indicated the presence of twenty-two major compounds. Based on these observations, it is necessary to emphasize on the detailed mechanism of action of the identified compounds, which are of pharmaceutical importance.

6 ACKNOWLEGEMENT

The authors express their sincere gratitude to Dr. Jayaraj M., Department of Botany, Karnatak University, Dharwad, for identifying the plant. The authors are grateful to IIT- Madras, (SAIF) for the GC-MS facility and to the Department of Biotechnology, Karnatak University, Dharwad for providing

DST- FIST funding and laboratory facilities.

REFERENCES

1) D. P. Patel, V. D. Verma, T. R. Loknathan, K. C. Bhatt, and J. Mishra, Simarouba glauca - A non-traditional oil source, (1997).
2) 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*, 9, *6*, 906 (2017)URL https://doi.org/10.5530/pj.2017.6.142.
3) F. A. Valeriote, T. H. Corbett, P. A. Grieco, E. D. Moher, J. L. Collins, and T. J. Fleck, Anticancer activity of glaucarubinone analogues, *Oncology research*, 10, *4*, 201 (1998)URL https://pubmed.ncbi.nlm.nih.gov/9778691/.
4) S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler, and C. W. Sigel, Tumor inhibitors. 100. Isolation and structural elucidation of bruceantin and bruceantinol, new potent antileukemic quassinoids from Brucea antidysenterica, *The Journal of Organic Chemistry*, 40, *5*, 648 (1975)URL https://doi.org/10.1021/jo00893a023.
5) I. A. B. S. Alves, H. M. Miranda, L. A. L. Soares, and K. P. Randau, Simaroubaceae family: botany, chemical composition and biological activities, *Revista Brasileira de Farmacognosia*, 24, *4*, 481 (2014)URL https://doi.org/10.1016/j.bjp.2014.07.021.
6) K. Deepti, P. Umadevi, G. Vijayalakshmi, and B. V. Polarao, Antimicrobial Activity and Phytochemical Analysis of Morinda tinctoria Roxb. Leaf Extracts, *Asian Pacific Journal of Tropical Biomedicine*, 2, *3*, 1440 (2012)URL https://doi.org/10.1016/S2221-1691(12)60433-X.
7) V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventós, [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, *Oxidants and Antioxidants Part A*, 299, 152 (1999)URL https://doi.org/10.1016/S0076-6879(99)99017-1.
8) N. Phuyal, P. K. Jha, P. P. Raturi, and S. Rajbhandary, Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts ofZanthoxylum armatumDC, *The Scientific World Journal*, 1–7 (2020)URL https://doi.org/10.1155/2020/8780704.
9) C. Rice-Evans, N. Miller, and G. Paganga, Antioxidant properties of phenolic compounds, *Trends in Plant Science*, 2, *4*, 152 (1997)URL https://doi.org/10.1016/S1360-1385(97)01018-2.
10) J. K. Liu, Natural products in cosmetics, *Natural Products and Bioprospecting*, 12, *1*, 40 (2022)URL https://link.springer.com/article/10.1007/s13659-022-00363-y.
11) A. Crozier, M. N. Clifford, and H. Ashihara, Plant secondary metabolites: occurrence, structure and role in the human diet, 1–24, John Wiley & Sons (2006)URL https://www.wiley.com/en-au/Plant+Secondary+Metabolites:+Occurrence,+Structure+and+Role+in+the+Human+Diet-p-9781405125093.
12) K. P. Mugaranja and A. Kulal, Alpha glucosidase inhibition activity of phenolic fraction from Simarouba glauca: An in-vitro, in-silico and kinetic study, *Heliyon*, 6, *7*, e04392 (2020)URL https://doi.org/10.1016/j.heliyon.2020.e04392.
13) S. P. Ramasamy, A. Rajendran, M. Pallikondaperumal, P. Sundararajan, F. M. Husain, A. Khan, M. J. Hakeem, A. A. Alyousef, T. Albalawi, P. Alam, H. M. Ali, and A. Alqasim, Broad-Spectrum Antimicrobial, Antioxidant, and Anticancer Studies of Leaf Extract of Simarouba glauca DC In Vitro, *Antibiotics*, 11, *1*, 59 (2022)URL https://doi.org/10.3390/antibiotics11010059.
14) V. Aparna, K. V. Dileep, P. K. Mandal, P. Karthe, C. Sadasivan, and M. Haridas, Anti-Inflammatory Property of n -Hexadecanoic Acid: Structural Evidence and Kinetic Assessment, *Chemical Biology & Drug Design*, 80, *3*, 434 (2012)URL https://doi.org/10.1111/j.1747-0285.2012.01418.x.
15) G. E. Trease and W. C. Evans, Phenols and phenolic glycosides, *Pharmacognosy*, 14, 218 (1996)URL https://doi.org/10.12691/ajfst-4-4-3.
16) M. J. Kadhim, In Vitro antifungal potential of Acinetobacter baumannii and determination of its chemical composition by gas chromatography-mass spectrometry, *Der Pharma Chemica*, 8, *19*, 657 (2016)URL https://www.derpharmachemica.com/pharma-chemica/in-vitro-antifungal-potential-of-acinetobacter-baumannii-and-determination-of-its-chemical-composition-by-gas-chromatogr.pdf.
17) S. D. Cook, An Historical Review of Phenylacetic Acid, *Plant and Cell Physiology*, 60, *2*, 243 (2019)URL https://doi.org/10.1093/pcp/pcz004.
18) L. Franco and B. H. De Oliveira, Determination of umckalin in commercial tincture and phytopreparations containing Pelargonium sidoides by HPLC: Comparison of sample preparation procedures, *Talanta*, 81, *4-5*, 1368 (2010)URL https://doi.org/10.1016/j.talanta.2010.02.036.
19) M. Ray, V. Kumar, C. Banerjee, P. Gupta, S. Singh, and A. Singh, Investigation of biosurfactants produced by three indigenous bacterial strains, their growth kinetics and their anthracene and fluorene tolerance, *Ecotoxicology and Environmental Safety*, 208, 111621 (2021)URL https://doi.org/10.1016/j.ecoenv.2020.111621.
20) C. A. Dyer, S. Raymond-Whish, S. Schmuki, T. Fisher, B. Pyzyna, A. Bennett, and L. P. Mayer, Accelerated follicle depletion in vitro and in vivo in Sprague-Dawley rats using the combination of 4-vinylcyclohexene diepoxide and triptolide, *Journal of Zoo and Wildlife Medicine*, 44, *4s*, 9 (2013)URL https://doi.org/10.1638/1042-7260-44.4S.S9.
21) K. Kirimura, W. Cao, Y. Onda, I. Yoshioka, and Y. Ishii, Selective and high-yield production of ethyl α-d-glucopyranoside by the α-glucosyl transfer enzyme of Xanthomonas campestris WU-9701 and glucose isomerase, *Journal of Bioscience and Bioengineering*, 134, *3*, 220 (2022)URL https://doi.org/10.1016/j.jbiosc.2022.06.012.
22) S. Patil and K. R. Murthy, Antioxidant Profile and GCMS Analysis of Ethanol Extract of Simarouba glauca Seeds, *Asian J Biol Life Sci*, 9, *3*, 379 (2020).
23) C. Karpagasundari and S. Kulothungan, Analysis of bioactive compounds in Physalis minima leaves using GC MS, HPLC, UV-VIS and FTIR techniques, *Journal of Pharmacognosy and Phytochemistry*, 3, *4*, 196 (2014)URL https://www.phytojournal.com/vol3Issue4/Issue_nov_2014/47.1.pdf.
24) A. Jose, M. V. N. L. Chaitanya, E. Kannan, and S. V. Madhunapantula, Tricaproin Isolated From Simarouba glauca Inhibits the Growth of Human Colorectal Carcinoma Cell Lines by Targeting Class-1 Histone Deacetylases, *Frontiers in Pharmacology*, 9 (2018)URL https://doi.org/10.3389/fphar.2018.00127.