Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [1] December 2022 : 66-71

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

GC-MS Analysis of *Sapindus emarginatus* (Sapindaceae) Seed Extract

Dinesh Kumar*, Akil Khan
Department of Chemistry, Bipin Bihari College, Jhansi-284001, UP, India
*Corresponding Author: gautamdinesh_123@yahoo.co.in

ABSTRACT

The seeds of Sapindus emarginatus, were analysed for petroleum ether soluble chemical constituents using gas chromatography-mass spectrometry (GC-MS) technique. Oil was extracted in a soxhlet extraction apparatus using petroleum ether (60-80 °C) and then subjected for GC-MS analysis. Results indicated more than 50 compounds which included thirteen major compounds. These major compounds were as identified based on the available NIST database with a relative abundance of namely cis- Vaccenic acid ($C_{18:1}$), cis-13-Octadecenoic acid ($C_{18:1}$), trans-13-Octadecenoic acid ($C_{18:1}$), Oleic acid ($C_{18:1}$) at RT 22.174 and its peak area 29.121 %. These compounds were present in rich amount, whereas cis-13-Eicosenoic acid ($C_{20:1}$), cis-11-Eicosenoic acid ($C_{20:1}$), cis-10-Heptadecenoic acid ($C_{17:1}$) at RT 23.568 and its peak area 4.711% and n-hexadecanoic acid (C16:0), Pentadecanoic acid (C15:0), Tridecanoic acid (C13:0), Tetradecanoic acid (C14:0) at RT 20.684 and its peak area 0.686% and Sigmasterol, 7, 22-Ergostadienol at RT 30.143 and its peak area 0.778% were present in small amount in seed oil. The results show revealed the potential of the seed oil as anti-hypercholesterolemic, anti-inflammatory, antifungal, antibacterial, anti-tumor, anti-cancer, anti-proliferative activities and therapeutic uses. Further, isolated phytochemical constituents from the seed extract of S. marginatus will give more pharmaceutically valuable results.

Keywords: *Sapindus emarginatus, Seed oil, GC-MS technique, Phytochemical constituents.*

Received 14.10.2022 Revised 16.11.2022 Accepted 20.12.2022

INTRODUCTION

The medicinal plants always play an important role for the better development of human health. According to the world health organisation (WHO) medicinal plants would be the best source to obtain a large variety of Phytochemical compounds and drug molecules. About 80% of population in the world use traditional medicines. The presence of bioactive secondary metabolites in the medicinal plants is primarily responsible for curing a variety of human ailments [1]. Plants are referred to as "nature's chemical factories" because they may contain phytochemical compounds that exert a defined physiological effect on the human body when consumed [2]. Various bioactive compounds have previously been isolated from different parts of medicinal plants [3-5]. The medicinal plants are reliable sources for the treatment of many health problems. People have been dependent on herbs in the past and even in the present time. Several studies have reported elemental contents in plant extracts, which are consumed by human beings, either as herbal heath drink or medicine [6]. Hence a through validation of the herbal drugs has emerged as a new area of science emphasizing and prioritising the standardisation natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Chromatography is the term used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with selectively absorbent stationary phase. It is also crucial and analytical tools for quality control and standardisation of phytotherapeutics [7].

In recent years, Gas Chromatography-Mass Spectrometry studies have been widely used for the analysis of plants. This technique has proved to be a valuable method for the analysis of non polar components and volatile essential oils, fatty acids, lipids [8] and alkaloids [9]. Moreover, GC-MS has been applied unambiguously to identify the structures of different phytoconstituents from plant extracts [10]. GC-MS obtained results can also be showed in fragmentation pattern of mass spectrum [11].

Medicinal plants are gift of nature; many researchers have reported the isolation and deployment of numerous bioactive materials in traditional medicine [12]. On other hand, *S. emarginatus* is small to medium-sized tree that grows to 30 feet tall and found in tropical climates in India. It belongs to the family Sapindaceae and commonly known as Soapnut tree. It is the south Indian species of genus Sapindus. The

S. emarginatus species are abundantly rich in sapo nins, flovonoids, tannins, triterpenoids alkaloids and carbohydrate etc. [13]. Saponins, fatty acids and acyclic sesquiterpene and diterpene oligoglycosides have been isolated as main secondary metabolites of several Sapindaceae species used in traditional oriental medicine [14, 15]. Many species belonging to Sapindaceae family are commonly used commercially as food, detergents, hair tonic, soap production and other purpuses. The various parts of *S. emarginatus* are well known for their medicinal values [16]. Moreover, *S. emarginatus* seed powder was used to removal of phosphate from soil and its fruit extract embedded with gold nanoparticles against MCF7 breast cancer cell line [17, 18]. Therefore, our interest of the present work to analysis the bioactive compounds from seed oil of *S. enarginatus* by using gas chromatography-mass spectrometry.

MATERIAL AND METHODS

a) Sample Collection

Seeds of *S. emarginatus* (250g.) were collected in Sept. 2019 from *www.seedseller.in.* Seeds were identified in the Department of the Chemistry, Bipin Bihari College, Jhansi. For the removal of dust particles from seeds, they were washed with distilled water, kept for air dry for two weeks under sunlight. Air-dried seeds were crushed, kernel was separated from seeds and pulverised in to powder with the help of mortar grinder which was stored in an airtight glass container for further processing (Fig. 1).

Seed kernel

Figure 1: Both sides of seeds of *S. emarginatus*

b) Extraction of oil from Seeds:

73g. of the pulverised powder of kernel was packed with 600mL of petroleum ether and oil was extracted by using soxhlet extractor at 60^0C-80^0C with an electric heating mantle for 12 hours. For condensation, separate water condenser was used to condense Petroleum ether vapours and oil gathered in a round bottom flask. The vapour of steamed oil layer was on top of the water, which was separated with the help of analytical grade ether. Then, oil layer was cooled and anhydrous sodium sulphate was added to remove moisture from the oil and then filtered through 0.22µm membrane. The extracted oil (3.88g.) was preserved in a dry glass vial for GC-MS analysis (Figure-2).

The % yield of oil was calculated by using the following formula [19].

$$\text{Yield (\%)} = \frac{\text{Amount of oil recovered (g)}}{\text{Amount of plant material used((g)}} \text{ X } 100$$

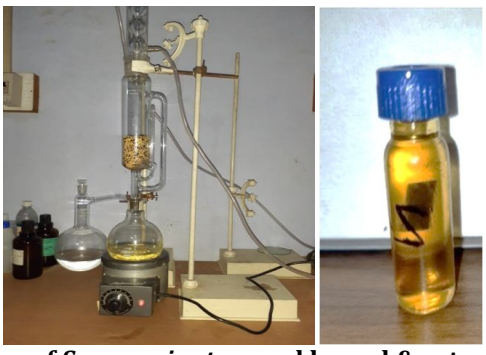

Figure 2: Soxhlet extraction of *S. emarginatus* seed kernel & extracted oil sample for GC-MS Analysis

c) Sample preparation and GC-MS Parameters for analysis:

For GC-MS analysis, we took seed oil and chloroform in ratio of 1:1 into glass vial and then well. Sample was filtered by using 0.45 µm syringe and was used for analysis glass by GC-MS (Perkin Elmer). The column used in GC was Perkin Elmer Elite-5 capillary columns measuring 30 m × 25 µm long. The acquisition parameters conditions were followed such as; the initial oven temperature was programmed at 40˚C for 5 minutes, ramp 12˚C/min to 260˚C, held for 10 minutes, Injector-Bauto=250˚C. The sample injection volume utilized was 1 µL, split ratio = 50:1. The helium gas was used as carrier and delayed for 2 minutes. The MS transfer line was maintained at 180˚C temperature. The source temperature was maintained at 200˚C.

d) Identification of the compounds:

The mass spectrum of *S. emarginatus* was interpretated using the National Institute of Standards and Technology (NIST) 2017 library, database having 574,826 spectra patterns [20]. The unknown component's spectrum was compared to the known component's spectrum in NIST library database, according to their molecular weight, molecular formula, and retention time, peak area% and biological activities reported into different databases such as PubChem, Drug Bank, Chemical book, and Human metabolome databases. Different components spectra of NIST 2017 library is shown in Figure-3.

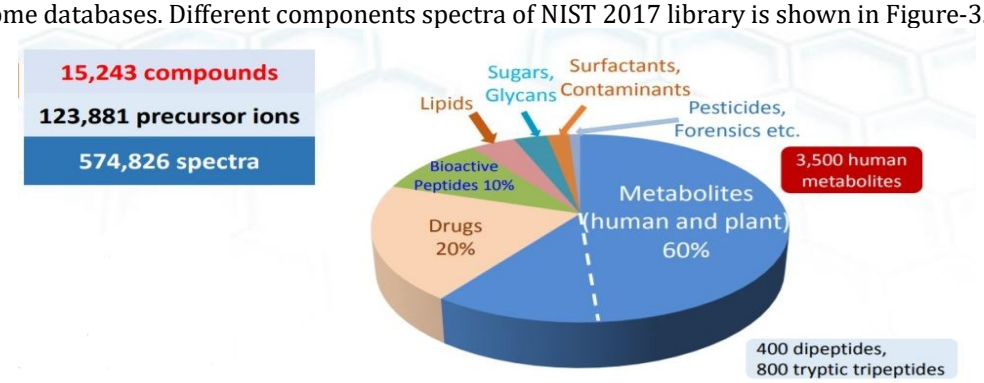

Figure 3: Spectra of NIST 2017 Library

RESULTS AND DISCUSSION

Seed extract of *S. emarginatus* in petroleum ether was subjected to gas chromatography and mass spectrometry examination. A GC-MS investigation was conducted to determine chemical components contained in seed extract. The compounds were identified according to their retention time, peaks area; molecular weight and molecular formula (Table-1). Using the retention time and molecular mass, the isolated compounds were matched with the National Institute Standard Technology Mass Spectrum database. Figure-4 depicts the GC-MS Spectra of the recognised peaks of discovered substances.

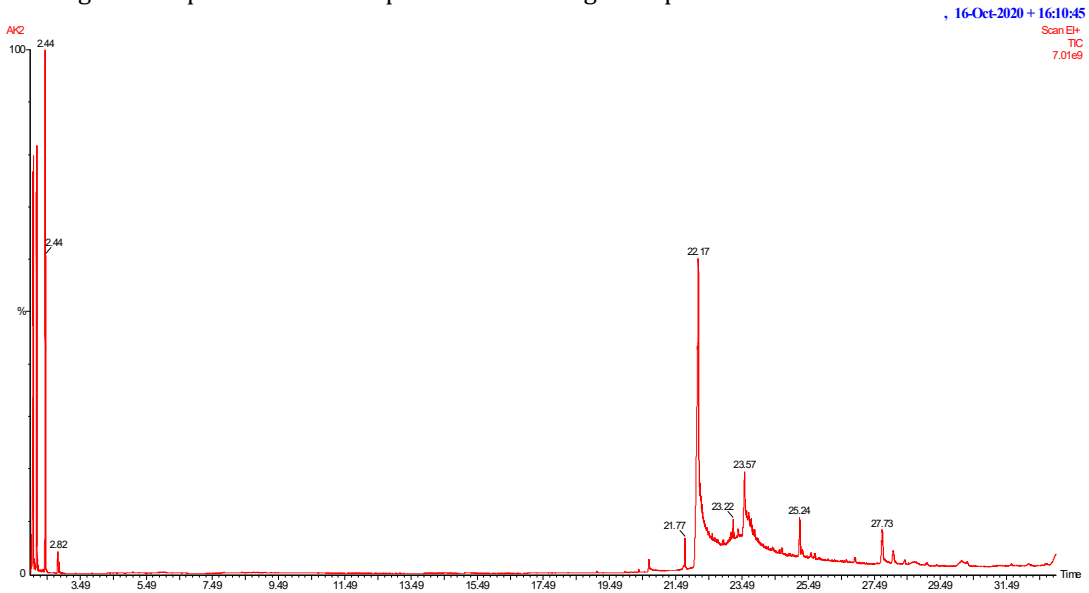

Figure 4: GC-MS Chromatogram of *S. emarginatus*

First separated retention time on 22.174 covered 29.121% of the peak area identified as cis- Vaccenic acid, cis-13 –Octadecenoic acid, trans-13-Octadecenoic acid and Oleic acid which was the highest percentage area amongst all the chemical constituents. Second RT 23.568 covered 4.711% peak area and the identified

compounds as cis-13-Eisosenoic acid, cis-11-Eisosenoic acid and cis-10-Heptadecenoic acid. Third RT 20.684 covered 0.686 peak area and the identified compounds as n-hexadecanoic acid (C16:0), Pentadecanoic acid (C15:0), Tridecanoic acid (C13:0), Tetradecanoic acid (C14:0). Fourth RT 30.143 covered 0.778 peak area and the identified compounds as Sigmasterol and 7, 22-Ergostadienol. Cis–Vaccenic acid is an omega -7 fatty acid which shows antibacterial, anti-hypercholesterolemic and anti-inflammatory activity. Cis-13-Eicosenoic acid also known as Paullinic acid which has double bond at position 13 and functions as metabolite in mice and plants, whereas Trans-13- Octadecenoic acid has a role as a human metabolite. Cis-11- Eicosenoic acid is also called gondoic acid has a role as human and plant metabolite. Cis-10-Heptadecenoic acid is a $C_{17:1}$ monounsaturated fatty acid and it has anti-tumor activity [21]. Cis-13-Octadecenoic acid has therapeutic uses in medicines. The gas chromatogram demonstrates that the relative amounts of individual chemicals elute in proportion to their retention times. The peak with the greatest height represents the relative concentration of chemicals contained in the plant. The mass spectrometer analyses the molecules eluted at different periods in order to determine their type and structure. The transformation of larger compound fragments into smaller fragments results in the development of peaks with varying m/z ratios. These mass spectra are the compound's fingerprint, which can be identified using the data repository. Generally, the effectiveness of a medicinal plant is determined by comparing its phytochemical constituents with its biological activity [22]. The GC-MS analysis of the seed petroleum ether extract of *S. emarginatus* revealed the presence of thirteen major components in the present investigation. In medicine, these substances have anti-hypercholesterolemic, antifungal, antibacterial, anti-inflammatory, anti-tumor, anti-cancer, anti-proliferative and therapeutic applications. Therefore, the seed extract of *S. emarginatus* included a variety of biologically active chemicals with therapeutic applications.

Table-1: Detail of compounds identified by GC-MS Analysis of seed extract of *S. emarginatus*

SL N0.	Compound	Retention time	Peak area %	Mol formula	Mol weight	Known Biological activity
1	Cis- Vaccenic acid	22.174	29.121	$C_{18}H_{34}O_2$	282.5	Anti-hypercholesterolemic anti-inflammatory [23]
2	Cis-13-Octadecanoic acid	22.174	29.121	$C_{18}H_{34}O_2$	282.5	Therapeutic uses in medicines [24]
3	Trans-13-Octasecanoic acid	22.174	29.121	$C_{18}H_{34}O_2$	282.5	Role as human metabolites [25]
4	Oleic acid	22.174	29.121	$C_{18}H_{34}O_2$	282.5	Anti-fungal, anti-inflammatory, antibacterial [26]
5	Cis-13-Eicosenoic acid	23.568	4.711	$C_{20}H_{38}O_2$	310.5	Role as a mouse and plant metabolites [27]
6	Cis-11-Eicosenoic acid	23.568	4.711	$C_{20}H_{38}O_2$	310.5	Role as a human and plant metabolites [28]
7	Cis-10-Heptadecenoic acid	23.568	4.711	$C_{17}H_{32}O_2$	268.4	Anti-tumor [21]
8	n-hexadecanoic	20.684	0.686	$C_{16}H_{32}O_2$	256.4	Anti-inflammatory [29]
9	Pentadecanoic acid	20.684	0.686	$C_{15}H_{30}O_2$	242.4	Anti-Cancer [30]
10	Tridecanoic acid	20.684	0.686	$C_{13}H_{26}O_2$	214.3	Antimicrobial [31]
11	Tetradecanoic acid	20.684	0.686	$C_{14}H_{28}O_2$	228	Anti-cancer, Antioxidant Hypocholesterolemic [23]
12	Sigmasterol	30.143	0.778	$C_{29}H_{48}O$	412.7	Anticancer activity [32]
13	. 7, 22-Ergostadienol	30.143	0.778	$C_{28}H_{46}O$	398.7	Anti-proliferative activity [33]

CONCLUSTION

In the present study, the seeds of *Sapindus emarginatus,* were analysed for petroleum ether soluble chemical constituents using gas chromatography-mass spectrometry (GC-MS) technique. The seed extract showed various biochemical compounds such as cis-Vaccenic acid ($C_{18:1}$), cis-13-Octadecenoic acid ($C_{18:1}$), trans-13-Octadecenoic acid ($C_{18:1}$), Oleic acid ($C_{18:1}$), cis-13-Eicosenoic acid ($C_{20:1}$), cis-11-Eicosenoic acid ($C_{20:1}$), Cis-10-Heptadecenoic acid ($C_{17:1}$), n-hexadecanoic acid (C16:0), Pentadecanoic acid (C15:0), Tridecanoic acid (C13:0), Tetradecanoic acid, Sigmasterol, 7, 22-Ergostadienol. The study revealed that, all the compounds found in the seed oil of *S. emarginatus* have different biological importance which shows

therapeutic potency. Therefore, the seeds of *S. emarginatus* may be used for medicinal purposes. Thus, it can be concluded that the plant *S. emarginatus* need more focus and further study in order to get more medicinal value in near future. This plant can be used to find out others bioactive natural compounds that may serve as leads for the development of new pharmaceuticals to unmet remedial needs.

ACKNOWLEDGEMENT

All analyses were carried out at the Research Laboratory Department Chemistry, Bipin Bihari College, Jhansi and Innovation Centre, Bundelkhand University, Jhansi Uttar Pradesh. We are indeed grateful to the Principal and Head department of chemistry, Bipin Bihari College, Jhansi for their kind assistance throughout the study process.

REFERENCES

1. Rafiqul, I., Rahman, M. S., Rahman, S. M. (2015). GC-MS analysis and antibacterial activity of *Cuscuta reflexa* against bacterial pathogens. Asian Pacific Journal of Tropical Disease, v. 5 (5), 399-403.
2. Paul, J. P., Venkastesan, M., Yesu J. R. (2012). The antibacterial activity and phytochemicals of the Leaves of Stylosanthes Fruticosa Linn. International Journal of phytopharmacy, v. 2 (4), 98-106.
3. Moronkola, D. O., Faruq, U. Z., Adeseun, O. A., Ajiboye, C. O. (2017). Essentiol oil composition of leaf, stem-bark, stem, root, flower and fruit with seed of *Blighia unijugata* Baker (Sapidaceae). African Journal of Pharmacy and Pharmacology, v. 11(7), 108-119.
4. Awonyemi, I. O., Abegunde M. S., Olabiran, T. E. (2020). Analysis of bioactive compounds from *Raphia taedigera* using gas chromatography-mass spectrometry. Eurasian chemical communication, v. 2, 938-944.
5. Kushwaha, P., Dwivedi, L., Yadav, S. S. (2019). GC-MS analysis of bioactive compounds in methanolic extract of *Ziziphus mauritiana* fruit. International Journal of Pharmaceutical Sciences and Research, v. 10(6), 2911-2916.
6. Kumar, A., Nair A. G. C., Reddy, A.V. R., Garg, A. N. (2004). Analysis of essential elements in Pragya-peya- a herbal drink and its constituents by neutron activation. Journal of Pharma Biomed Analysis, v. 37 (4), 631-638.
7. Andrew, M. (2007) Role of advances in chromatographic technique in phytochemistry, Phytochemistry, v. 68, 2785-2797.
8. Jie, M. S. F., Christie, W. W., Brechany, E. Y., Bakare, O. (1991). Mass Spectral Characterisation of picolinyl and methyl ester derivatives of isomeric thia fatty acids. Biological Mass Spectrometry, v. 20, 629-635.
9. Betz, J. M., Gay, M. L., Mossoba M. M., Adams, S., Portz, B. S. (1997). Chiral Gas Chromatographic Determination of Ephedrine-Type Alkaloids in dietary Supplements Containing Ma Huang. Journal of AOAC International, v. 80(2), 303-315
10. Abegunde, S. M. (2018). Asian Journal of chemical sciences, v. 5, 1-8
11. Oboh, G., Ogunsuyi, O. B., Awonyemi, O. I., Atoki, V. A. (20180). Effect of alkaloids extract from African Jointfir (*Gnetum africanum*) leaves on manganese –induced toxicity in *Drosophila melanogaster*. Journal of Oxidative Medicine and Cellular Longevity, 1-10
12. Mahesh, B., Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agriculture Sciences, v. 4S, 839-843.
13. Archana, B., Kotesh, P., Sree, R., Nikhitha, P., Kumar, K. S., Afroj, S., Jiyarul, S. k. (2022). Antidiabetic activity of ethanolic extract of seed kernel of *Sapindus emarginatus* in rats. Journal of drug delivery and therapeutics, v. 12(3-S), 15-25.
14. Perry, L. M. (1980). Medicinal plants of East and Southeast Asia: attributed properties and uses. The MIT Press Cambridge Massachusetts and London, 376.
15. Saleem, A., Nasir, S., Rassol, N., Bokhari, T.H., Rizwan, K., Shahid, M., Abbas, M., Zubair, M. (2015). In vitro antimicrobial and haemolytic studies of *Kalanchoe Pinnata* and *Callistemon viminalis*. International journal of Chemical and Biological Sciences, v. 7, 29-34.
16. Arora, B., Bhadauriya, P., Tripathi, D., Sharma, A. (2012). Sapindus emarginatus: Phytochemistry & Various Biological activites. Indo Global Journal of Pharmaceutical Sciences, 2(3), 250-257.
17. Jasper, P. M., Sumithra, S. (2020). Adsorption kinetics and isotherm study on phosphate removal from aqueous solution by *Sapindus emarginatus*. EM Inernational, v. 39(2), 278-282.
18. Kumar, S. V., Kavimani, V. (2021). Sapindus emarginatus extract embedded with gold nanoparticles: An antiproliferative agent against MCF7 breast cancer cell line, v. 4(2), 1-7.
19. Rao, B. R. R., Kaul, P. N., Syamasundar, K. V., Ramesh, S. (2005). Chemical profiles of primary and secondary essential oils of palmarosa (*Cybopogon martinii* (Roxb.) Wats Var. m otia Burk.) Industrial Crops and Products, v. 21(1), 121-127.
20. Yang, X., Neta, P., Stephen, E., Stein (2017). Extending a tandem mass spectral library to include MS^2 spectra of fragment ions produced in-source and MS^n spectra. Journal of the American Society for Mass Spectrometry, v. 28, 2280-2287.
21. Fukuzawa, M., Yamaguchi, R., Hide, I., Chen, Z., Hirai, Y., Sugimoto, A.,Yasuhara, T., Nakata, Y. (2008). Possible involvement of long chain fatty acids in the spores of Ganoderma lucidum (Reishi Houshi) to its anti-tumor activity. Biological and Pharmaceutical Bulletin, v. 31(10), 1933-1937.
22. Perry, L. M. (1980). Medicinal plants of East and Southeast Asia: attributed properties and uses. The MIT Press Cambridge Massachusetts and London, 622.

23. Pintus, S., Murru, E., Carta, G., Cordeddu, L., Batteta, B., Accossu, S., Pistis, D., Uda, S., Ghiani, M. E., Mele, M., Secchiari, P., Almerighi, G., Pinuts, P., Banni, S. (2012). Sheep cheese naturally enriched in alpha linolenic, conjugated linoleic and vaccenic acids improves the lipid profile and reduces anandamide in the plasma of hypercholesterolaemic subjects. British Journal of nutrition, v. 109, 1453-1462.
24. Arora, S., Kumar, G., Meena, S. (2017). Screening and evaluation of bioactive Components of *cenchrus Ciliaris* by GC-MS analysis. International Research Journal of Pharmacy, v. 8(6), 69-76.
25. National Center for Boitechnology Information (2021). PubChem. reports for the https://pubchem. ncbi.nlm.nih.gov/compound/trans-13-Octadecenoic acid. Compound Summary for CID 6161490, trans-13-Octadecenoic acid. Accessed 11 December, 2021.
26. Sales-Campos, H., Souza, P. R., Peghini, B. C., Salva Da, J. S., Cardosa, C. R. (2013). An overview of the modulatory effects of oleic acid in health and disease. Medicinal Chemistry, v. 13(2), 201-210.
27. National Center for Biotechnology Information (2021). PubChem reports for the https://pubchem.ncbi. nlm.nih.gov/ compound/Paullinic-acid. Compound Summary for CID 5312518, Paullinic acid. Accessed 7 December, 2021.
28. National Center for Biotechnology Information (2021). PubChem reports for the https://pubchem.ncbi.nlm.nih.gov/compound/cis-11-Eicosenoic-acid. Compound Summary for CID 5282768, cis-11-Eicosenoic acid. Accessed 7 December, 2021.
29. Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., haridas, M. (2012) Anti- Inflammatory property of n-hexadecanoic acid: Structural Evidence and Kinetic Assessment. Chemical Biology and Drug design, v. 80: 434-439.
30. To, N. B., Nguyen, Y.T-K., Moon, J. Y., Ediriweera, M. K., Cho, S. K. (2020). Pentadecanoic acid, an odd-Chain Fatty Acid, Suppresses the Stemness of MCF-7/SC Human Breast Cancer Stem-Like Cells through JAK2/STAT3 Signaling. Nutrients, v. 12(6), 1663-1682.
31. Chowdhury, S. K., Dutta, T., Chattopadhyay, A. P., Ghosh, N. N., Chowdhury, S., Mandal, V. (2021). Isolation of antimicrobial tridecanoic acid from Bacillus sp. LBF-01 and its potentialization through silver nanoparticles synthesis: A combined experimental and theoretical studies. Journal of Nanostrutcture in Chemistry, v. 11(4), 573-587.
32. Ashraf, R., Bhatti, H. N. (2021). A Centum of valuable Plant Bioactives. Academic press chapter 10 –Stigmasterol, 213-232.
33. Oyugi, D. A., Ayorinde, F. O., Gugssa, A., Allen, A., Izevbigie, E. B., Eribo, B., Anderson, W. A. (2011). Biological Activity and Mass Spectrometric Analysis of *Vernonia amygdalina* Fractions. Journal of Bioscience and technology, v. 2(3), 287-304.

CITATION OF THIS ARTICLE

D Kumar, A Khan. GC-MS Analysis of *Sapindus emarginatus* (Sapindaceae) Seed Extract.Bull. Env.Pharmacol. Life Sci., Vol 12 [1] December 2022: 66-71