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Analysis of bioactive compounds from *Sapindus emarginatus* in petroleum ether solvent using gas chromatography-mass spectrometry technique

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Abstract: The seeds of the *Sapindus emarginatus* (Sapindaceae family) were extracted through the Soxhlet extraction technique using petroleum ether as extraction solvent. The kernels were separated from the seed shells. 5.31% oil was recovered from *Sapindus emarginatus* seeds. GC-MS technique was used to evaluate the chemical composition of seed oil. According to the GC-MS study, *Sapindus emarginatus* seed oil contain more than 30 compounds in total peaks area out of which six monounsaturated compounds were found, based on their retention time (RT), percentage of peak area, molecular formula and molecular weight. GC-MS study revealed that the presence of cis-Vaccenic acid (C18:1), cis-13-Octadecenoic acid (C18:1) and trans-13-Octadecenoic acid (C18:1) at RT 22.174 and its peak area 29.121% of compounds are present in rich amount, whereas cis-13-Eicosenoic acid (C20:1),cis-11-Eicosenoic acid (C20:1) and cis-10-Heptadecenoic acid (C17:1) at RT23.568 and peak area 4.711, normally present in relatively small amounts in seed oil, these are prominent components present in the seed oil of *Sapindus emarginatus.*

Keywords: *Sapindus emarginatus* , Sapindaceae , GC-MS technique , oil extraction, , Biochemical analysis, Fatty acids.

1. Introduction:

The medicinal plants always play an important role for the better development of human health. According to the world health organization (WHO) medicinal plants would be the best source to obtain a large variety of medicinal compounds and drug molecules About 80% of population in developed countries used traditional medicine. The medicinal plants are reliable sources for the treatment of many health problems. People have dependent on herbs in the past and even in the present time. Several studies have reported that elemental contents in plant extracts, which are consumed by human beings, either as an herbal heath drink or medicine [1].Herbal medicine based on their traditional uses in the form of powder, liquids or mixtures has been reported for the treatment of various ailments in India since ancient time [2].

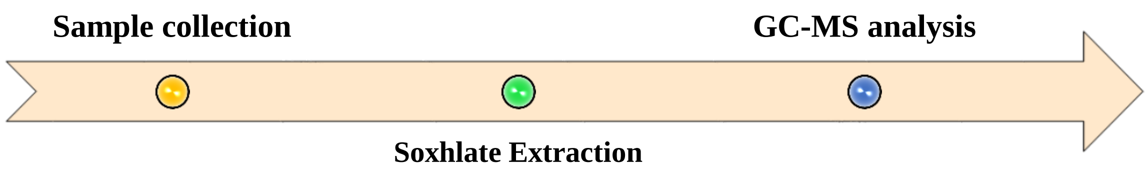
In recent years, Gas Chromatography-Mass Spectrometry studies have been increasingly applied for the analysis of medicinal compounds present in medicinal plants, with this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids [3]and alkaloids [4].

Sapindus emarginatus is also a medicinal plantand small to medium-sized tree that grows to 30 feet tall and belongs to the Sapindaceae family. It is native to tropical climates in India. The Sapindus genus includes both deciduous and evergreen species. Sapindaceae family is also known for its traditional medicinal uses as stimulate, diuretic, natural surfactant and dermatitis in the world. The chemical investigations of the sapindaceae family have led to the isolation of saponins, diterpenes and flavonoids among other secondary metabolites. Several saponins, fatty acids and acyclic sesquiterpene and diterpene oligo glycosides have been isolated as main secondary metabolites of several Sapindaceae species used in traditional oriental medicine [5]. Plant of *Sapindus emerginatus* has been reported for its high content of saponins. The saponin moiety is characterised as the hederagenin group of glycosides. Sapindaceae families which contain saponins are commonly used as natural surfactants.

In Asia and America, the native peoples have been used raw materials of seeds of Sapindaceae family for thousands of years for the purpose of washing [6].Many species belonging to Sapindaceae family, are commonly used commercially as food, soap production, besides, are used in traditional medicine. For instance, the various parts of *Sapindus trifoliatus* are well known for their medicinal values [7-11].The arils of *Nephelium lappaceum* (Sapindaceae) which are a part of the seeds, are well known and eaten fresh or cooked [12]. In addition, other parts of the seeds are also eaten roasted. The arils of *Paullinia cupana* (Sapindaceae) contain caffeine and are used to prepare soft drinks and to relieve fatigue [13-14].The chemical compositional investigation of sapindaceae family was carried out by the many researchers in India and abroad [15-19]. Cis-13-Eicosenoic (Paullinic) acid is anomega-7 monounsaturated fatty acid find in variety of plant sources, including *Paullinia cupana* (Sapindaceae) from which it gets its name [13]. It is one of a number of eicosenoic acids. The seed of *Paullinia elegans* (Sapindaceae) is also consista rich source of cis-13-Eicosenoic acid [16].

In the present study we recovered oil from the seeds of *Sapindus emarginatus* using soxhlet extraction technique and the qualitative analysis carried out by using GC-MS technique. After this study revealed that the monounsaturated fatty acids (MUFA) isolated from the oil of *S. emarginatus* consist of high content cis-Vaccenic acid (C18:1), cis-13-Octadecenoic acid (C18:1) and trans-13-Octadecenoic acid (C18:1) whereascis-13-Eicosenoic acid (C20:1) cis-11-Eicosenoic acid (C20:1) and cis-10-Heptadecenoic acid (C17:1) normally present in relatively small amounts in the seed oil.

2. Materials and Method:



2.1 Sample Collection:

Seeds of *Sapindus emarginatus* were purchased from www.seedseller.in.For the removal of dust particles from seed sand were washed with distilled water then followed by air dry for two weeks under sunlight shade, and then dried seeds were crushed into fine small pieces with the help of mortar grinder. The crushed seeds of *Sapindus emarginatus* was sieved and collected in an airtight glass container for further processing.

Fig.1.Seeds of *Sapindus emarginatus*

2.2 Extraction of oil from Seeds:

The seeds of *Sapindus emarginatus* were crushed and kernels were separated from shells into a glass container. Oil was extracted from crushed seeds material by Soxhlet apparatus using petroleum ether as solvent. The dirt-free seed powder 73gm.into 600 mL Petroleum ether packed into soxhlet extractor, and heated at 60^0C-80^0C with an electric heating mantle for 12 hours for condensation use separate water condenser to condense Petroleum ether vapors' and Oil gathered in a round bottom flask. The vapors of steamed oil layer on top of the water, which was separated with the help of analytical grade ether. Now, oil layer was cooled and concentrate through anhydrous Na_2SO_4(sodium sulphate). The concentrated oil was preserved in a dry glass vial and put into desiccator to absorb remained moisture.

The % yield of oil was calculated by using the following formula [20].

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

Fig.2. Extraction oil sample for GC-MS analysis

2.3 Sample preparation and GC-MS parameters for analysis:

For GC-MS analysis, we took 1ml. seed oil and 1 ml. Chloroform in ratio of (1:1) into 5ml. glass test tube and then mix well. Now diluted sample filtered by using 0.45 µm syringe filter and transfer into 2 ml. glass vial. Sample ready for the analysis, analysis was carried out on sophisticated instrument brand-Perkin Elmer (GC-Clarus 680) and (MS-SQ 8C) for chromatographic analysis with mass spectrometer using electron capture detector (ECD) and flame ionization detector(FID). 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 40^{0}C for 5 minutes, ramp 12 ^{0}C/min to 260 ^{0}C, hold for 10 minutes, Injector-Bauto=250^{0}C. The sample injection volume utilized was 1 µL, split ratio = 50:1, the helium gas was used as carrier delay for 2 minutes.

The MS transfer line was maintained at 180^{0}C temperature. The source temperature was maintained at 200^{0}C. The unknown component's spectrum was compared to the known component's spectrum stored in NIST library according their molecular weight, molecular formula, and retention time.

2.4 Identification of the compounds

The mass spectrum of *Sapindus emarginatus* was interpreted using the National Institute of Standards and Technology (NIST) 2017 library, database having 574,826spectra patterns [21]. 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.

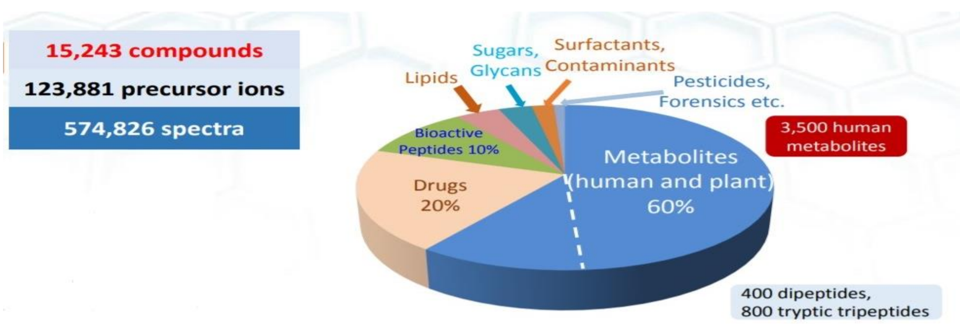


Fig.3.Updated Spectra of NIST 2017 library

3. Results and Discussion:

After, the successful study by Gas chromatography and mass spectroscopy analysis of *Sapindus emarginatus* seed soil extract in petroleum ether. GC-MS studies were used to separate and identify the volatile components. The compounds were identified according their retention time, peaks area; molecular weight and molecular formula are demonstrated (Table 1). The identified compounds were matched with the National Institute Standard Technology (NIST) Mass Spectrum database using the retention time and molecular mass. The GC-MS Spectrums of the identified peaks of detected compounds are shown in Fig. 4.

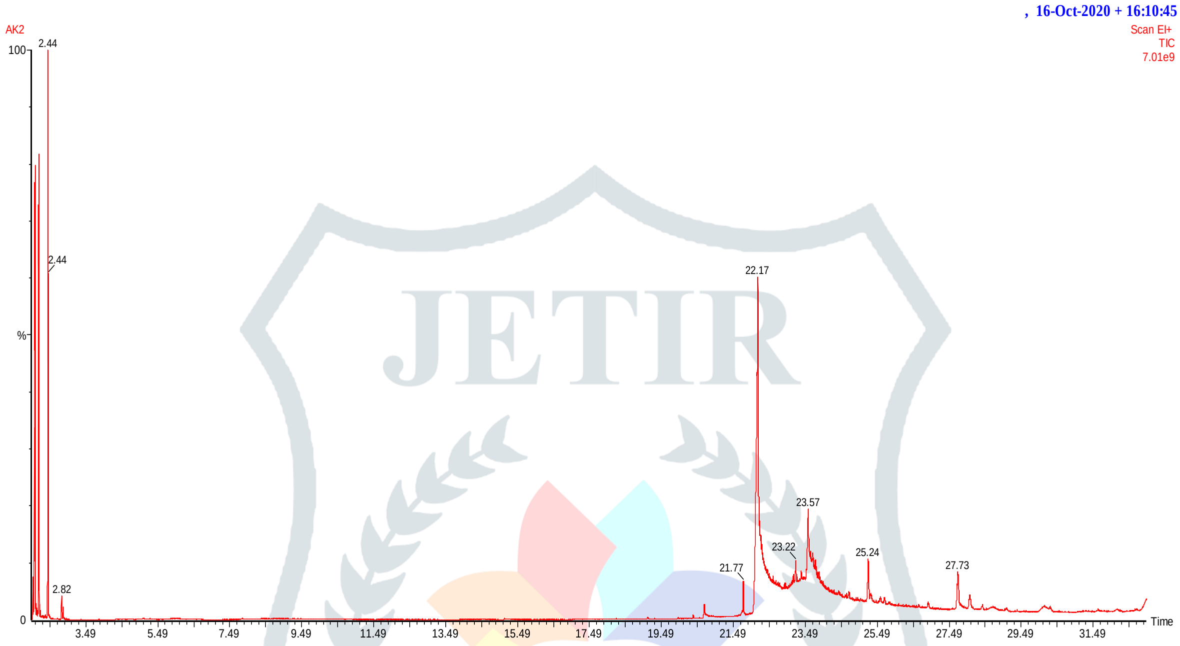


Fig.4.GC-MS chromatogram of *Sapindus emarginatus*

Most separated compounds hit retention time on 22.174that covered29.121% peak area identified as cis–Vaccenic acid, cis-13-Eicosenoic acid and trans-13-Octadecenoic acid. The highest percentile area of amongst all constituents. Second RT 23.568 covered 4.711%peak area contained compounds as cis-13-Eicosenoic acid, cis-11-Eicosenoic acid and cis-10- Heptadecenoic acid comparatively present in small amount.

The GC-MS analysis showed three major compounds found in the seed oil sample. For instance, cis-Vaccenic acid (RT 22.174; peak area 29.121%), cis-13 –Octadecenoic acid (RT 22.174; peak area 29.121) and trans-13-Octadecenoic acid (RT 22.174; peak area 29.121%) whereas three compounds were found in low amounts e.g., cis-13-Eisosenoic acid (RT 23.568; peak area 4.711%), cis-11-Eisosenoic acid (RT 23.568; peak area 4.711%) and cis-10-Heptadecenoic acid (RT 23.568; peak are 4.711). cis–Vaccenic acid is an omega -7 fatty acid which shows antibacterial, anti-hypercholesterolemic and anti-inflammatory activity.Cis-13-Eicosenoic acid is also known as Paullinic acid which has double bond at position 13 and has a role as a mouse and plant metabolite whereas trans-13- Octa decenoic 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 C17:1 monounsaturated fatty acid and it has anti-tumor activity. Cis-13-Octadecenoic acid has therapeutic uses in medicine.

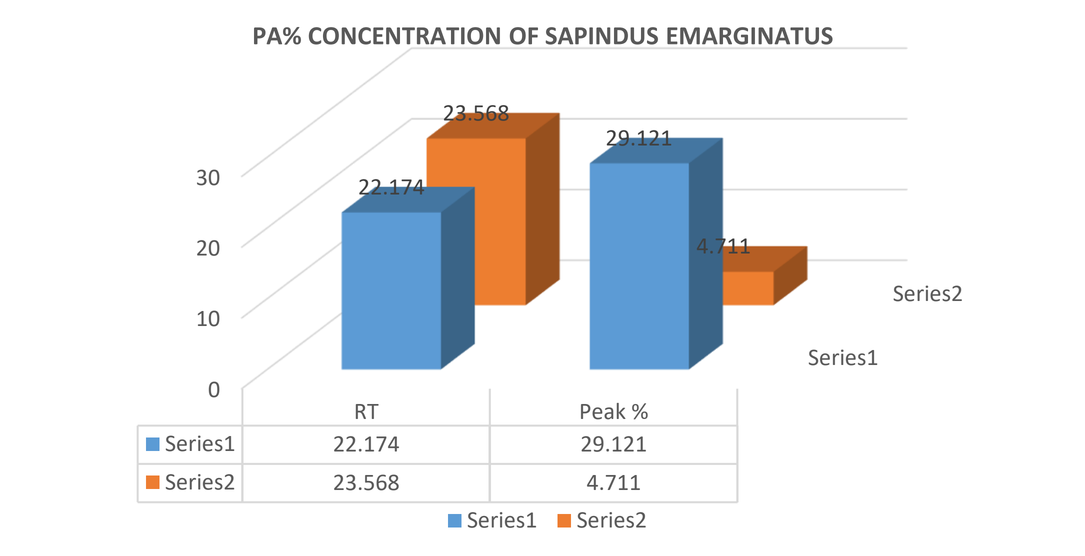


Fig. 5.RT wise Peak Area % Concentration of *Sapindus emarginatus*

Table1:Detailed of compounds identified by GC-MS analysis from sample.

Sr. No	RT	Compounds Name	Peak Area %	Mole. Formula	M. Weight	Reported Biological Activities	Ref.
1.	22.174	Cis-Vaccenic acid	29.121	$C_{18}H_{34}O_2$	282.5	Anti-hypercholesterolemic, anti-inflammatory	[23]
2.	22.174	Cis-13-Octadecenoic acid	29.121	$C_{18}H_{34}O_2$	282.5	Therapeutic uses in medicine.	[24]
3.	22.174	Trans-13-Octadecenoic acid	29.121	$C_{18}H_{34}O$	282.5	Role as a human metabolites	[25]
4.	23.568	Cis-13-Eicosenoic acid	4.711	$C_{20}H_{38}O_2$	310.5	Role as a mouse and plant metabolites	[26]
5.	23.568	Cis-11-Eicosenoic acid	4.711	$C_{20}H_{38}O_2$	310.5	Role as a human and plant metabolite	[27]
6.	23.568	Cis-10-Heptadecenoic acid	4.711	$C_{17}H_{32}O_2$	268.4	Anti-tumor	[22]

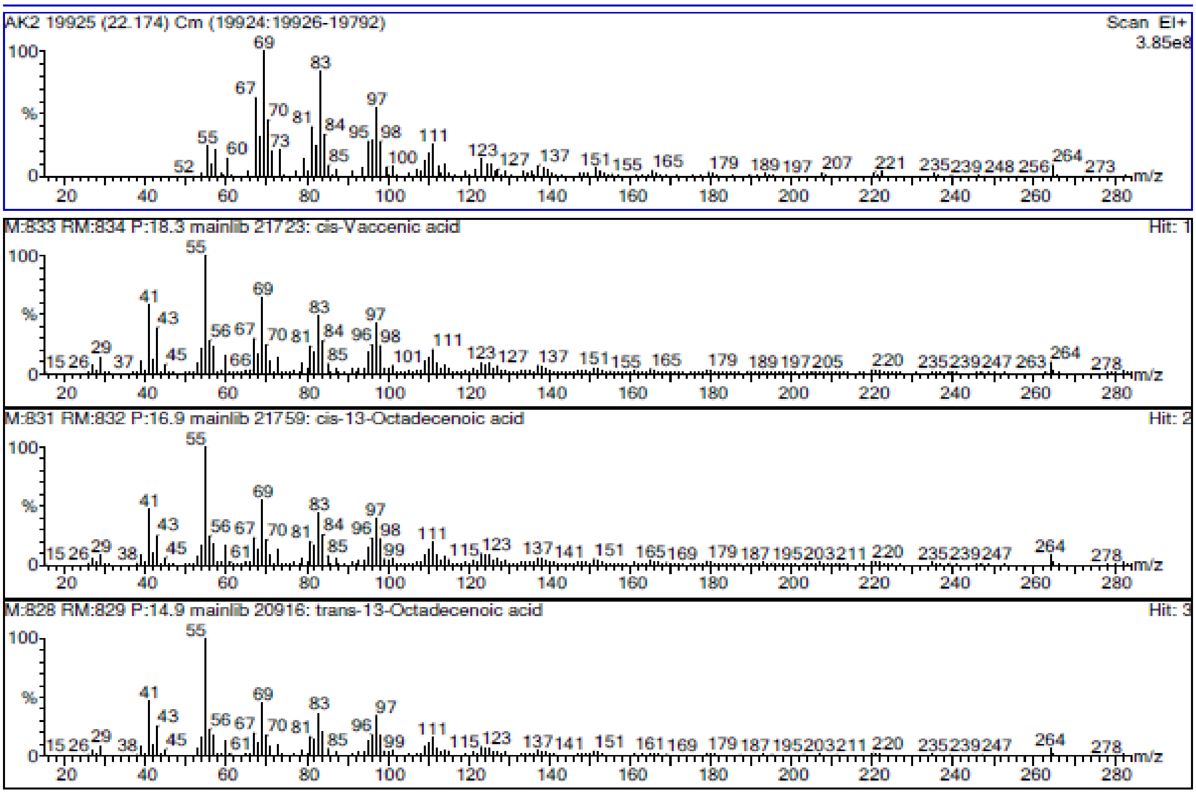


Fig.6. Spectrum of possible Hits onRT 22.174

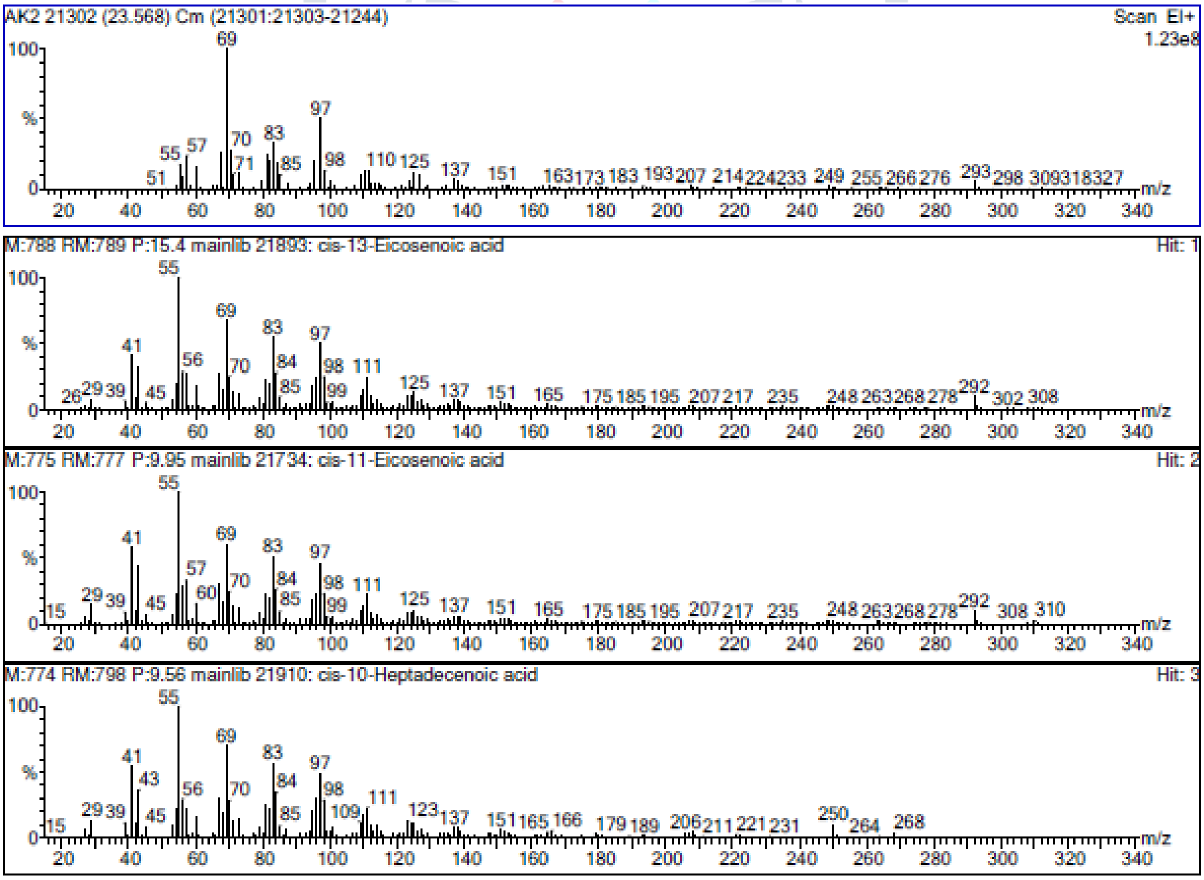


Fig.7. Spectrum ofpossible hits on RT 23.568

4. Conclusion:

On the basis of GC-MS study of seed oil extract of *sapindus emarginatus* in petroleum ether, conclude that MUFA (monounsaturated fatty acids) were found from seed oil that responsible for the treatment of Anti-hypercholesterolemic, Anti-inflammatory, Therapeutic uses in medicine , Anti-tumor and Role as a human and plant metabolite.

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Reference:

1. Kumar, A., Nair, A.G.C., Reddy, A.V.R., Garg, A.N. (2009). Analysis of essential elements in Pragya-peya- a herbal drink and its constituents by neutron activation. J. Food Properties. 12: 286-294(https://doi.org/10.1016/j.jpba.2004.11.051).
2. Arora, D.S., Kaur, G.J., Kaur, H. (2009). Antibacterial activity of tea and coffee, their extracts and preparation. Int. J Food Properties. 12: 286 -294 (https://doi.org/10.1080/10942910701675928).
3. Jie, M.S.F., Choi, C.Y.C. (1991). Characterization of picolinyl and methyl ester derivatives of isomeric thiafattyacids. J. Int. Fed. Clin. Chem. 3: 122 (https://doi.org/10.1002/bms.1200201007).
4. 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 MáHuáng. J AOAC Int. 80(2): 303-315 (https://doi.org/10.1093/jaoac/80.2.303).
5. Perry, L.M. (1980). Medicinal plants of East and Southeast Asia: attributed properties and uses. The MIT Press Cambridge Massachusetts and London, 376 (https://doi.org/10.1007/bf02858311).
6. Austin, D.F., Honychurch, P.N. (2004). Florida Ethnobotany, CRC Press, London. 601-603 (https://www.routledge.com/Florida-Ethnobotany/Austin/p/book/9780849323324#).
7. Ojha, P., Maikhuri. J.P., Gupta, G. (2003). Effect of spermicides on Lactobacillus acidophilusin vitro - nonoxynol-9 vs. Sapindussaponins, Contraception.68(2): 135–138 (https://doi.org/10.1016/s0010-7824(03)00138-0).
8. Arulmozhi, D.K., Veeranjaneyulu, A., Bodhankar, S.L., Arora, S.K. (2004). Pharmacological studies of the aqueous extract of Sapindustrifoliatus on central nervous system: possible antimigraine mechanisms. Journal of Ethnopharmacology. 97(3): 491–496 (https://doi.org/10.1016/j.jep.2004.12.012).
9. Arulmozhi, D.K., Veeranjaneyulu, A., Bodhankar, S.L., Arora, S.K. (2005). Pharmacological investigations of Sapindustrifoliatus in various in vitro and in vivo models of inflammation, Indian J Pharmacol.37(2): 96-102(https://doi.org/10.4103/0253-7613.15109).
10. Denise, D., PELEGRINI, Joyce K., TSUZUKI, Ciomar, A.B., AMADO, Diógenes, A.G., CORTEZ &Izabel, C.P., FERREIRA. (2008). Biological activity and isolated compounds in S. saponaria L. and other plants in genus Sapindus. Lat. Am. J. Pharm.27: 922-932 (http://www.latamjpharm.org/previous_issue.php?vol=27&num=6).

11. Arulmozhi, D.K., Veeranjaneyulu, A., Bodhankar, S.L., Arora, S.K. (2009). Effect ofSapindustrifoliatus on hyperalgesic in vivo migraine models. Brazilian Journal ofMedical and Biological Research. 38 (3): 469–475 (https://doi.org/10.1590/s0100-879x2005000300019).
12. Hopkins, C.Y.,Swingle, R. (1967).Eicosenoic acid and other fatty acids ofSapindaceae seed oils. Lipids 2: 258-260 (https://doi.org/10.1007/bf02532565).
13. Avato,P., Pesante, M.A., Fanizzi,F.P., Aimbiréde MoraesSantos, C. (2003). Seed oil Composission of Pauniacupana var. sarbilis (Mart) Ducke. Lipids. 38: 773-780 (https://doi.org/10.1007/s11745-003-1126-5).
14. Hamerski, L., Somner, G.V., Tamaio, N. (2013).PaulliniacupanaKunth (Sapindaceae): a review of its ethnopharmacology, phytochemistry and pharmacology. J Med Plants7:2221-2229 (https://doi.org/10.5897/jmpr2013.5067).
15. Ucciani, E., Mallet, F., Zahra, J.P. (1994).Cyanolipids and fatty acids of Sapindustrifoliatus (Sapindaceae) seed oil. FettWissTechnol.96: 69-71 (https://doi.org/10.1002/lipi.19940960210).
16. Spitzer, V. (1995). GLC-MS Analysis of the fatty acids of the seed oil, triglycerdes and cyanolipid of Paulliniaelegans (Sapindaceae) a rich source of cis-13-Eicosenoic acid (paullinic acid). J High Resolute Chromatogram. 18: 413-416 (https://doi.org/10.1002/jhrc.1240180704).
17. Spitzer, V. (1996). Fatty acid composition of some seed oils of the Sapindaceae. Phytochemistry. 42: 1357-1360 (https://doi.org/10.1016/0031-9422(96)00140-9).
18. Kumar, D., Hasan, S.Q.(2011).Cyanolipids in Sapindaceous seed oils. Asian Journal of Chemistry23(6): 2589-2591(https://asianjournalofchemistry.co.in/User/ViewFreeArticle.aspx?ArticleID=23_6_49).
19. Tava, A., Avato, P. (2014). Analysis of cyanolipids from Sapindaceae seed oils by gas chromatography–EI-mass spectrometry. Lipids.49: 335-345 (https://doi.org/10.1007/s11745-014-3885-8).
20. Ra, B.R.R., Kaul, P.N., Syamasundar, K.V., Ramesh, S. (2005). Chemical profiles of primary and secondary essential oils of palmarosa (Cymbopogonmartinii (Roxb.). Wats var. motia Burk.). Industrial Crops and Products.21: 121-127 (https://doi.org/10.1016/j.indcrop.2004.02.002).
21. Yang, X., Neta, P. et al. (2017). Extending a tandem mass spectral library to include MS^2 spectra of fragment ions produced in-source and MS^n spectra. J. Am. Soc. MassSpectrom. 28(11): 2280–2287 (https://doi.org/10.1007/s13361-017-1748-2).
22. Fukuzawa, M., Yamaguchi, R., Hide, L. et al. (2008). Possible involvement of long chain fatty acids in the sporce of Ganodermalucidum (ReishiHoushi) to its anti-tumor activity. Biological and Pharmaceutical Bullentin 31(10): 1933-1937 (https://doi.org/10.1248/bpb.31.1933).
23. Pintus, S., Murru, E., Carta, G., Cordeddu, B., Batteta, B.et. al. (2012). Sheep cheese naturally enriched in α-linolenic, conjugated linoleic and vaccenic acids improves the lipid profile and reduces anandamide in the plasma of hypercholesterolaemic subjects. Br. J. Nutr.24: 1-10(https://doi.org/10.1017/s0007114512003224).

24. Arora, S., Kumar, G.,Meena, S. (2017). Screening and Evaluation of Bioactive Components of CenchrusCiliaris L. By Gc-Ms Analysis. Int. Res. J. Pharm. 8: 69-76(http://dx.doi.org/10.7897/2230-8407.08699).
25. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 6161490, trans-13-Octadecenoic acid. Retrieved March 3, 2022 from *https://pubchem.ncbi.nlm.nih.gov/compound/trans-13-Octadecenoic-acid*.
26. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 5312518, Paullinic acid. Retrieved March 7, 2022 from *https://pubchem.ncbi.nlm.nih.gov/compound/Paullinic-acid.*
27. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 5282768, cis-11-Eicosenoic acid. Retrieved March 3, 2022 from *https://pubchem.ncbi.nlm.nih.gov/compound/cis-11-Eicosenoic-acid.*