Applied Biological Research 25(1): 127-131; (2023)
DOI: 10.5958/0974-4517.2023.00014.9

SHORT COMMUNICATION

ANALYSIS OF BIOACTIVE COMPOUNDS FROM *Sapindus marginatus* (Willd.) BY USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY TECHNIQUE

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(Received 29 October, 2022; accepted 11 January, 2023)

ABSTRACT

This experiment was performed to detect the beneficial compounds present in seed oil of *Sapindus marginatus*. The oil from the seeds of *S. marginatus* (Sapindaceae family) was extracted by the Soxhlet extraction technique using petroleum ether as solvent at 60-80^{0}C. 22.18% oil was recovered from seeds sample. The chemical compositions of seeds were examined by using GC-MS technique. The GC-MS analysis showed that oil of *S. marginatus* contained more than 40 compounds in total peaks area, out of which we discussed here mainly six monounsaturated compounds according their retention time (RT) and percentage of peak area, molecular formula and molecular weight. GC-MS study revealed the presence of cis-vaccenic acid ($C_{18:1}$), cis-13-octadecenoic acid ($C_{18:1}$) and trans-13-octadecenoic acid ($C_{18:1}$) at RT 22.18 and its peak area was 40.48% of the compounds present in high amounts; whereas cis-13-eicosenoic acid ($C_{20:1}$), cis-11-eicosenoic acid ($C_{20:1}$) and cis-10-nonadecenoic acid ($C_{19:1}$) at RT 23.56 and peak area 7.74 normally present in low amounts in seed oil, these are prominent components in the seed oil.

Keywords: Fatty acids, GC-MS technique, Sapindaceae, *Sapindus marginatus*, seed oil

INTRODUCTION

Sapindus marginatus is a small to medium sized tree that usually grows to 15 m belongs to the family Sapindaceae, native to warm temperature to tropical regions of the world and widely found in Bundelkhand region (India). Genus *Sapindus* includes both deciduous and evergreen species. Ritha (soap nuts) and soapberries are the popular names for members of this genus because soap is made from the pulp of fruit. The plant reportedly has high saponin content. The saponin moiety is characterised as the hederagenin group of glycosides. The soap nuts which contain saponins are commonly used as natural surfactants. The generic name *Sapindus* is derived from Latin word *saponis* that means "soap" and *indicus* means "India" (Quattrocchi, 2000)**.** In Asia and America, the native peoples have been using the raw materials of seeds of Sapindaceae family for thousands of years for the purpose of washing (Austin *et al*., 2004)**.**

Many species belonging to Sapindaceae family are commonly used commercially as food, soap production, traditional medicine, etc. Soap nuts are being studied and used for a variety of applications. A natural surfactant found in soap nuts have been utilised as detergent, shampoo cleansers and hair dressing. For instance, the various parts of *Sapindus trifoliatus* are known for their medicinal values (Arulmozhi *et al*., 2004; Arulmozhi *et al*., 2005; Peleigrini *et al*., 2008; Arulmozhi *et al*., 2009). Eicosanoic and 11-eicosenoic acids are common and highly occurring constituents of cyanolipids (Hopkins *et al*., 1967). In addition, other parts of seeds are eaten roasted. The arils of

Paullinia cupana contain caffeine and are used to prepare soft drinks and to relieve fatigue (Avato *et al*., 2003; Hamerski *et al*., 2013).

The chemical compositional study of sapindaceae family has been carried out by many researchers in India and abroad (Spitzer, 1996; Dinesh *et al*., 2011; Tava *et al*., 2014); Cis-13-eicosenoic (paullinic) acid is an omega-7 monounsaturated fatty acid find in variety of plant sources, including *P. cupana* from which it gets its name (Avato *et al.*, 2003)**.** It is one of a number of eicosenoic acids. The seeds of *P. elegans* are also a rich source of cis-13-eicosenoic acid (Spitzer *et al*., 1996**).** In present study we recovered the oil from *Sapindus marginatus* using hot Soxhlet extraction process and performed compositional studies by using GC-MS.

MATERIALS AND METHODS

Sample collection

Seeds of *Sapindus marginatus* (Fig. 1) were collected from a nursery at Handewadi, Pune (India). The seeds were washed with distilled water, air-dried for two weeks, and then crushed with the help of mortar grinder. The material was sieved to obtain particle size of 0.29 and 0.50 mm and collected in an airtight glass container for further study.

Extraction of oil

The seeds of *S. marginatus* were broken and kernels separated from shells in a glass container. Oil was extracted by crushing the seeds in a Soxhlet apparatus using petroleum ether. The powdered dirt-free soap nuts (185 g) and 600 mL petroleum ether was packed in a Soxhlet extractor, and heated at 60-80^{0}C in an electric heating mantle for 12 h. For condensation, separate water condenser was used to condense petroleum ether vapours and oil gathered in round bottom flask. The vapours of steamed oil layer on water top was separated with the help of analytical grade ether. The 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 (Rao *et al.*, 2005)**:**

Fig. 1: *Sapindus marginatus* seeds

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

Preparation of sample and GC-MS analysis

For GC-MS analysis, 1 mL extracted seed oil and 1 mL chloroform was taken in a glass vial and mixed well. The diluted sample was filtered through a 0.45 µm syringe filter. The filtered sample was used on a 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. The acquisition parameters conditions were as follow: the initial oven temperature 40^{0}C for 5 min, ramp 12 to 260°C min^{-1} with hold for 10 min, Injector-Bauto = 250°C. The sample injection volume utilized was 1 µL, split ratio = 50:1. Helium gas was used as carrier delay for 2 min. The MS transfer line was maintained at 180°C. The source temperature was maintained at 200^{o}C. The unknown component's spectrum was compared with the known component's spectrum stored in NIST library according their molecular weight, molecular formula and retention time.

Identification of compounds

The mass spectrum of GC-MS was interpreted using the National Institute of Standards and Technology (NIST) 2017 library data base (*https://pubchem.ncbi.nlm.nih.gov*) having 574,826 spectra

patterns (Fukuzawa *et al*., 2008). The spectrum of unknown components was compared to the spectrum of known components stored in NIST library according their molecular weight, molecular formula, retention time, % peak area and biological activities reported in different databases searched (NCBI, 2021a,b) with the help of PubChem, Drugbank, Chemical book, and Human metabolome databases. Different components spectra of NIST 2017 library (*https://pubchem.ncbi.nlm.nih.gov*).

RESULTS AND DISCUSSION

The GC-MS analysis of *Sapindus marginatus* seed extract in petroleum ether, revealed the presence of several chemical components which were identified on the basis of their retention time, peak area, molecular weight and molecular formula (Table 1). The separated compounds were matched with the mass spectrum database of NIST library 2017 using the retention time and molecular mass. The GC-MS spectrums of identified peaks of detected compounds are given in Fig. 2. The first separated retention time (RT) on 22.18 min covered 40.487% peak area and were identified as cis-vaccenic acid, cis-13-eicosenoic acid and trans-13-octadecenoic acid. These showed highest percentile area amongst all constituents. Second RT on 23.56 min covered only 7.74% peak area and contained the compounds

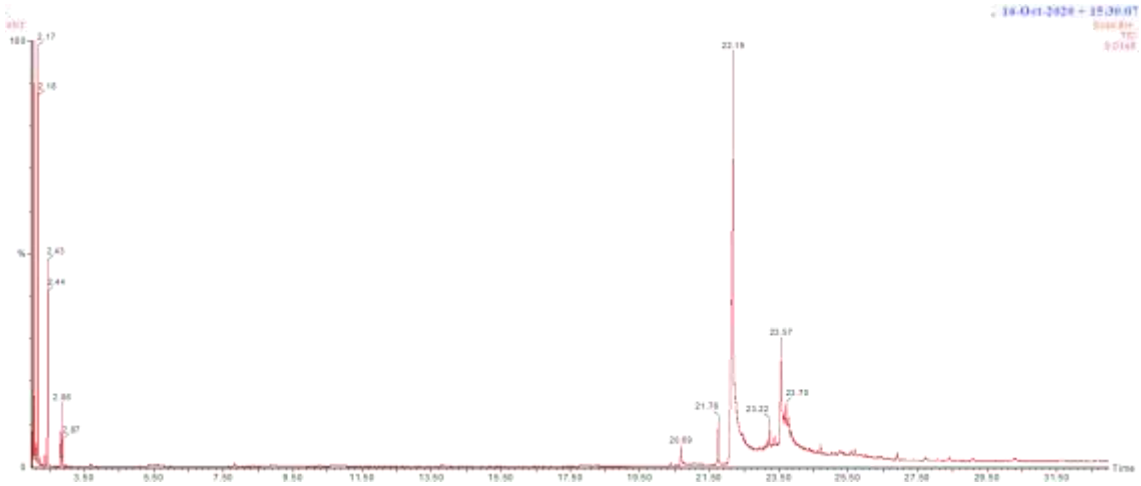
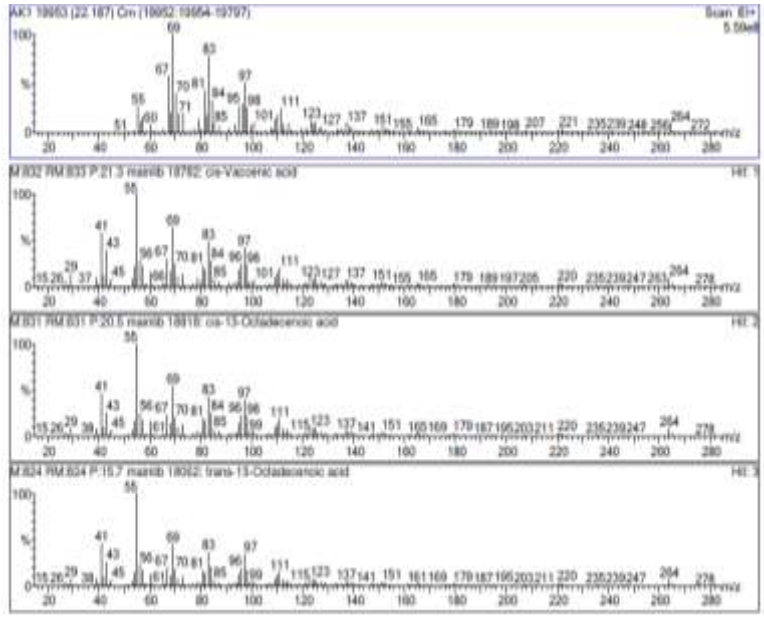


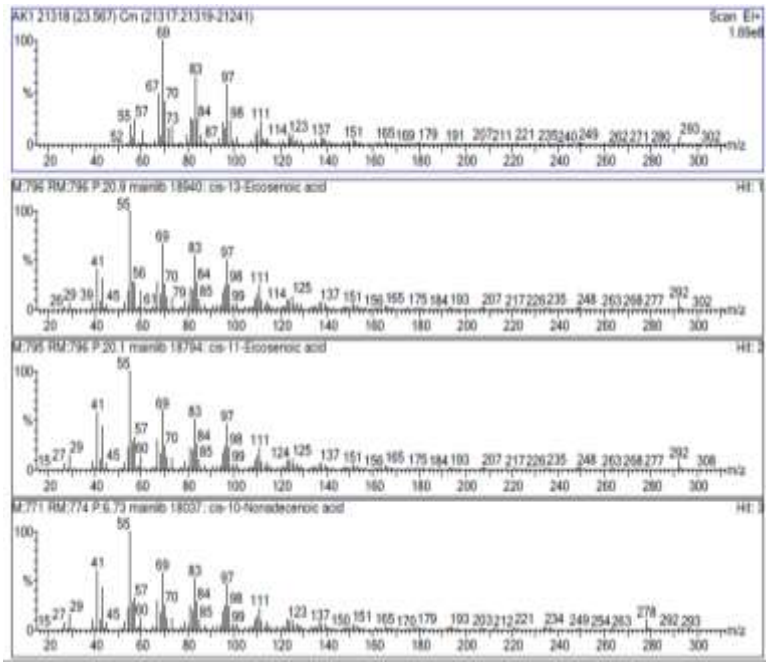
Fig. 2: GC-MS chromatogram of *Sapindus marginatus*

Table 1: Compounds identified by GC-MS analysis from *Sapindus marginatus* seed sample

RT (min)	Compounds name	Peak area %	Molecular formula	Molecular weight	Reported biological activities	References
22.18	Cis-vaccenic acid	40.487	$C_{18}H_{34}O_2$	282.5	Anti-hypercholesterolemic, anti-inflammatory	[Pintus *et al*., 2012]
22.18	Cis-13-octadecenoic acid	40.487	$C_{18}H_{34}O_2$	282.5	Therapeutic uses in medicine	[Sunita *et al.*, 2017]
22.18	Trans-13-octadecenoic acid	40.487	$C_{18}H_{34}O$	282.5	Role as a human Metabolites	[NCBI, 2021a]
23.56	Cis-13-eicosenoic acid	7.744	$C_{20}H_{38}O_2$	310.5	Role as a mouse and plant metabolites	[NCBI, 2021b]
23.56	Cis-11-eicosenoic acid	7.744	$C_{20}H_{38}O_2$	310.5	Role as a human and plant metabolite	[NCBI, 2021b]
23.56	Cis-10-nonadecenoic acid	7.744	$C_{19}H_{36}O_2$	296.5	Antitumor	[Fukuzawa *et al.*, 2008]



a) RT 22.187



a) RT 23.567

Fig. 3: Spectrum of possible hits of RT 22.87 and RT 23.567

as cis-13-eicosenoic acid, cis-11-eicosenoic acid and cis-10-nonadecenoic acid. GC-MS analysis showed three major compounds *viz*., cis-vaccenic acid, cis-13-octadecenoic acid and trans-13-octadecenoic acid in seed oil sample (Fig. 3a); whereas three compounds were in low amounts at RT 23.56 min; peak area 7.74%. Cis–vaccenic acid is an omega-7 fatty acid which shows anti-bacterial, antihypercholesterol-emic and anti-inflammatory activity. Cis-13-eicosenoic acid (also known as paullinic acid) has double bond at position 13 and has a role as a mouse and plant metabolite; whereas trans-13-octadecenoic acid has a role as a human metabolite. Cis-11-eicosenoic acid (also called gondoic acid) has a role as human and plant metabolite. Cis-10-non-adecenoic acid is a mono-unsaturated fatty acid ($C_{19:1}$) and has potential antitumor activity (Pintus *et al*., 2012). Cis-13-octadecenoic acid has therapeutic uses as well (Table 1). The spectrum of possible hits of RT 22.187 min and RT 23.567 min are shown in Fig. 3a,b).

Conclusion: This is the first detailed compositional study of MUFA (monounsaturated fatty acids) on the seed oil of *Sapindus marginatus*. Various biochemical compounds such as cis-vaccenic acid ($C_{18:1}$), cis-13-octadecenoic acid ($C_{18:1}$), trans-13-octadecenoic acid ($C_{18:1}$) cis-13-eicosenoic acid ($C_{20:1}$), cis-11-eicosenoic acid ($C_{20:1}$) and cis-10-nonadecenoic acid ($C_{19:1}$). The identified compounds have different biological activities.

Declaration of competing interest: The authors declare that there is no conflict and interest.

Acknowledgments: We are grateful to the Principal and Head, Department of Chemistry, Bipin Bihari College, Jhansi, Uttar Pradesh (India) for their kind assistance throughout the study.

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