ORIGINAL ARTICLE

Phytochemical analysis of nuts of *Semecarpus anacardium* using gas chromatography-mass spectrometry and high performance thin layer chromatography: Effect of Shodhana

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Abstract

Background: *Semecarpus anacardium* (SA) has been classified in Ayurveda as a toxic plant. Ayurvedic Pharmacopoeia has described the method of purification (known as Shodhana) to remove the toxic substances from this plant. **Objective:** SA nuts before and after Shodhana were treated with chromatographic methods to check the presence of phytoconstituents in it. **Materials and Methods:** Methanolic extracts of pre-shodhit methanolic (PSM) and shodhit methanolic (SM) SA nuts were studied for high performance thin layer chromatography (HPTLC) and gas chromatography-mass spectrometry (GC-MS). Nuts were measured for total phenolic and flavonoid content using gallic and quercetin as standard. **Results:** HPTLC studies have shown that PSM drug and SM drug R_f values were decreasing. GC-MS studies confirmed the presence of urushiol in PSM and anacardol in SM extracts. **Conclusion:** These research work states that Shodhana improves the yield, decreases the phenolic and flavonoid content and further converts toxic urushiol into nontoxic anacardol thereby reducing toxicity.

Key words: Anacardol, gas chromatography-mass spectrometry, high performance thin layer chromatography, *Semecarpus anacardium,* urushiol

INTRODUCTION

Semecarpus anacardium (SA) has been regarded as semi poisonous plant and found to be associated with contact dermatitis due to presence of a chemical called urushiol. Shodhana of fruits of this plant is carried out before systemic administration. Shodhana involves soaking of the nuts in cow's urine followed by cow milk and finally rubbing in brick powder as quoted in Ayurvedic Pharmacopoeia of India.[1,2]

In the current decades, phytochemistry has emerged up due to the progress of chromatographic techniques which involve separation, purification, and identification of enormous amounts of phytoconstituents present in the plant extracts. High performance thin layer chromatography (HPTLC) studies have been recognized as one of the unique techniques with respect to its application of identification and quantification of plant phytoconstituents. An important insight is its ability to distinguish components of the extract even in µg amount. Over the decades, this technique has emerged out to be one of the most powerful visualization techniques interpreted on the basis of its accuracy, preciseness, specificity, sensitivity, and reproducibility.[3] Gas liquid chromatography on coupling with mass spectrometry has emerged as an important technique which provides an insight to both qualitative and quantitative estimation of volatile components present in

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plant extracts based on the measurements interpreted by area under the peaks.[4]

SA nuts are reported to possess phenolic compounds, biflavonoids, etc.[5] Phenolic compounds, such as bhilavanol A (monoenepentadecyl catechol I) and bhilavanol B (dienepentadecyl catechol II), have been isolated from oil of SA.[6] Anacardoside (a glucoside) has been isolated from its seeds.[7] Some of the bioflavonoids isolated from this plant are biflavone (A1, A2, B and C), tetrahydromentoflavone, tetrahydrobustaflavone, semecarpuflavanone, jeediflavanone, galluflavanone, nallaflavanone, semecarpetin, and anacarduflavanone.[8,9] An anticancer catechol compound 3-(8' (Z), 11' (Z)-pentadecadienyl) catechol was isolated from the kernel of SA nut.[10] Its fruit contains bhilawanol which is a mixture of cis and trans isomers of ursuhenol. The black corrosive juice of the pericarp contains an oxyacid (anacardic acid) and a nonvolatile alcohol called cardol.[11]

There are limited studies on effect of Shodhana on phytoconstituents of SA. In this work, we identified and confirmed the presence of different constituents by gas chromatography-mass spectrometry (GC-MS) and HPTLC from shodhit methanolic (SM) and pre-SM (PSM) methanolic extracts of SA nuts to evaluate the effect of Shodhana on the phytoconstituents present.

MATERIALS AND METHODS

Collection, Shodhana, Maceration and Preliminary Phytochemical Screening

The dried fruits of SA were collected, authenticated, macerated as reported earlier[1] and subjected to preliminary phytochemical screening using standard methods.[12]

Thin Layer Chromatography (TLC)

Plate preparation

The plates were prepared by mixing adsorbent silica gel with a small amount of gypsum and water. This mixture was spread as thick slurry on an unreactive carrier sheet like thick aluminium foil. Then, the resultant plate was dried and activated by heating in an oven for 1 h at 100°C. The thickness of adsorbent layer was 0.1-0.25 mm for analytical purposes.

Spotting the plate

The thin end of the spotter was placed in the dilute solution of extracted drug. The solution was raised up in the capillary, and the starting line of the plate was touched briefly. The solvent was allowed to evaporate and at the same time; spot was taken on the plate in such a way that the spot should be far enough away from the edges and from each other as well. The small spot of solution containing the sample was applied to the plate about 1 cm from the base to avoid dissolution of the spot.

Preparation of solvent system

Chloroform and methanol was taken in ratio of 9.8:0.2 in a beaker, mixed properly and covered with aluminium foil to prevent evaporation of solvent.

Development of plate

The TLC plate was developed in a closed beaker or jar. Then, small amount of solvent system was placed in that beaker. The spotted plate was dipped in the solvent system. The solvent moved up the plate by capillary action and meets the sample mixture, which was carried up the plate by the solvent. Different compounds in the sample mixture travel at different rate due to the difference in their attraction to the stationary phase and because of differences in solubility in the solvent.

Visualization of spots

When the solvent front reached about 1 cm from the top of the plate (15-45 min), the plate was removed from the developing chamber. The position of the solvent front was marked, and the solvent was allowed to evaporate. Then, the plate was allowed to stand for a few minutes in a closed container in which the atmosphere was saturated with iodine vapor.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

HPTLC

The HPTLC was performed at the Anchrom Research Labs. Testing Pvt. Ltd., Mumbai, India. HPTLC plates silica gel 60 F254 (20 cm × 10 cm) was used. The samples were prepared by dissolving 50 mg each in 5 ml of methanol. Spots of SM and PSM methanolic extract both pre and post were applied on the plates with increasing concentrations of 0.5, 1 and 2 µg/l.

The instrument used in studies was CAMAG Automatic TLC sampler (Linomat 5) with sample solvent as methanol, dosage speed of 150 nl/s, 200 µl as pre-dosage volume and 25 µl as syringe size. There were a total of six tracks of SM and PSM plant extracts both pre and post with 0.5, 1 and 2 µg/L. Application position and band length were 8.00 mm and 15.00 mm, respectively. The calibration parameters consist of mode of single level, calibration curve with area and peak heights. The percentage was calculated as per the following formula:

$$\text{Percentage of constituent} = \frac{\text{Sample area} \times \text{Standard dilution} \times \text{Purity}}{\text{Standard area} \times \text{Sample dilution} \times 100} \times 100$$

GC-MS

The details of the instrument used in the studies are as follows:

- Instrument type: Clarus 500 Perkin

- Mass detector type: Turbo mass Gold-Perkin Elmer Turbomass 5.1 spectrometer
- Column type: Capillary column with Elite (100% dimethylpolysiloxane, 30 m × 0.25 mm ID × 0.25 µm)
- Injection temperature: 250°C
- Type of gas and flow rate: Helium and flow rate was 1.5 ml/min
- Ion source temperature: 230°C
- Injection mode with volume: Split less mode and the volume was 1 µl.

The initial temperature of the instrument was set at 70°C and maintained for 3 min. Then at a rate of 10°C/min, the temperature was increased to 300°C and maintained for 9 min. Electron ionization was used to obtain mass spectra of the compounds present in the extracts. The detector was operated in scan mode from 40 to 700 m/z. The MS start time, solvent cut time and end time were 3, 3 and 35 min, respectively. Identification was done by comparing with NIST libraries.

RESULTS

Maceration Yield and Preliminary Phytochemical Screening

The yield of PSM and SM drug was found to be 12.25% w/w and 15.41% w/w, respectively. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, phenolic compounds, steroids, and tannins.

TLC

TLC developed was visualized, and the spots were marked for both SM and PSM samples. R_f values calculated are depicted in Table 1.

HPTLC

The PSM (Tracks 1, 3 and 5) and SM (Tracks 2, 4 and 6) methanolic extracts showed well resolved spots on HPTLC plates [Table 2 and Figures 1, 2]. The maximum height and area were decreased in SM.

Flavonoid and Phenolic Content

Flavonoid and the phenolic content of PSM and SM are depicted in Table 3 as an equivalent of quercetin and gallic acid, respectively. Both flavonoid and phenolic content were decreased due to Shodhana.

GC-MS

The SM extract [Figure 3] were found to be elucidated for the presence of various components as identified by NIST libraries.

Table 1: R_f values of identified spots of PSM and SM extract of SA in TLC

Solvent system	Spots	R_f values	
		PSM	SM
Chloroform: Methanol (9.8:0.2)	1	0.307	0.352
	2	0.5	0.509
	3	0.557	0.549
	4	0.634	0.607
	5	0.692	0.686
	6	0.788	0.803

PSM: Pre-shodhit methanolic, SM: Shodhit methanolic extract, TLC: Thin layer chromatography, SA: *Semecarpus anacardium*

Table 2: HPTLC analysis of PSM and SM extract of SA

Track No	Details of standard and sample	R_f	Maximum height	Area
1	PSM extract 0.5 µg/L	0.37	133.3	3867.6
2	SM extract 0.5 µg/L	0.36	72.8	1899.9
3	PSM extract 1 µg/L	0.36	209.2	6522.0
4	SM extract 1 µg/L	0.35	122.2	3432.3
5	PSM extract 2 µg/L	0.36	308.2	10589.3
6	SM extract 2 µg/L	0.36	200	6147.3

PSM: Pre-shodhit methanolic, SM: Shodhit methanolic extract, SA: *Semecarpus anacardium*, HPTLC: High performance thin layer chromatography

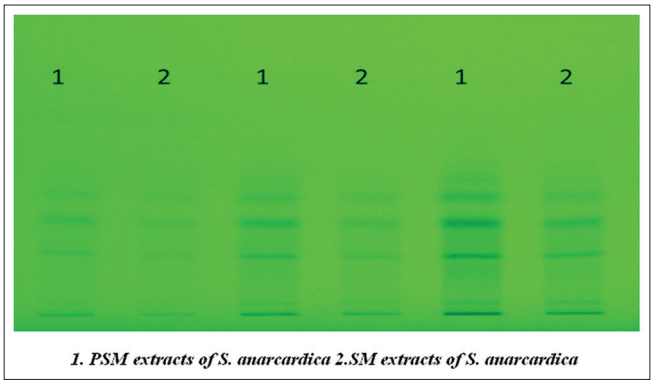

Figure 1: High performance thin layer chromatography of pre-shodhit methanolic (PSM) and shodhit methanolic extract (SM) of *Semecarpus anacardium* before derivatization in short Ultraviolet (254 nm). 1 – PSM extracts of SA, 2 – SM extracts of SA

n-hexadecanoic acid [Figure 4], (73.5%) with m/z 256 and fragment ions of 43, 57, 60, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 213, 227, 239 is noticed as main component. 8-octadecenoic acid, methyl ester [Figure 5] (9.42%) with m/z 296 and with fragment ions of 41, 55, 74, 83, 97, 110, 123, 137, 152, 166, 180, 194, 207, 222, 246, 264, 279 is observed. 9-octadecenoic acid, methyl ester [Figure 6] (20.1%) with m/z 282 and fragment ions of 41, 55, 69, 83, 97, 111, 125, 138, 151, 165, 180, 193, 207, 222, 235, 246, 264 are also seen. Propane

phosphonic acid, bis (trimethylsilyl) ester [Figure 7] (16.2%) with m/z 253 along with fragment ions of 27, 29, 33, 41, 43, 53, 55, 57, 69, 71, 77, 83, 85, 98, 111, 126, 140, 154, 169 and 45, 59, 73, 91, 147, 165, 181, 195, 211, 226 are seen 1As-(1a. alpha, 4b. beta, 8as)-4a, 8, 8-trimethyloctahydrocyclopropa (d) naphthalen-2(3H)-one [Figure 8] (13.7%) with m/z 123 and fragment ions of 41, 55, 69, 79, 93, 107,123, 135, 149, 163, 178, 191, 206 is also observed. All the components and their fragments with synonyms are depicted in Table 4.

Table 3: Flavonoid and phenolic content of PSM and SM extract of SA

Drug	Flavonoid content	Phenolic content
	µg of quercetin/mg of extract (w/w)	µg of gallic acid/mg of extract
PSM	5.882	574.166
SM	4.364	539.166

PSM: Pre-shodhit methanolic, SM: Shodhit methanolic extract, SA: *Semecarpus anacardium*

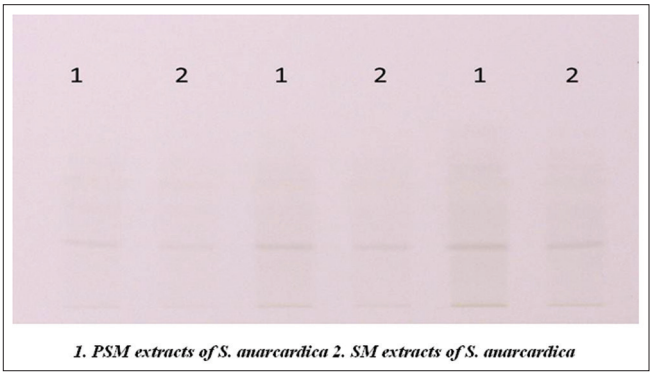

Figure 2: High performance thin layer chromatography of pre-shodhit methanolic (PSM) and shodhit methanolic extract (SM) of *Semecarpus anacardium* after derivatization in white light. 1 – PSM extracts of SA, 2 – SM extracts of SA

The PSM extracts were found to have the presence of following structures identified by NIST libraries [Figure 9]. Oleic acid [Figure 10] (26.6%) with m/z 282 and fragment ions of 41, 55, 69, 83, 97, 111, 123, 137, 151, 166, 179, 193, 208, 220, 235, 246, 264,282 are seen. In addition, 1As-(1aα, 4bβ. 8as)-4a, 8, 8-trimethyloctahydrocyclopropa (d) naphthalen-2(3H)-one [Figure 11] (11.2%), with m/z 206 with fragment ions of 41, 55, 69, 79, 93, 107, 123, 135, 149, 163, 178, 191, 206 is observed. Heptafluorobutyric acid, 4-methoxyphenylester [Figure 12], with m/z 320 and fragment ions of 41, 52, 69, 95, 107, 123, 150, 169, 245, 273, 289, 320 is also characterized prominently (77.9%) as the main component.

DISCUSSION

Phytochemical progress has been witnessed as one of the most rapid and accurate methods targeted for screening of the plants with the aid of chromatographic techniques. GC-MS has provided us with major breakthrough in phytochemical analysis. It has been put forth that none of the phytochemical

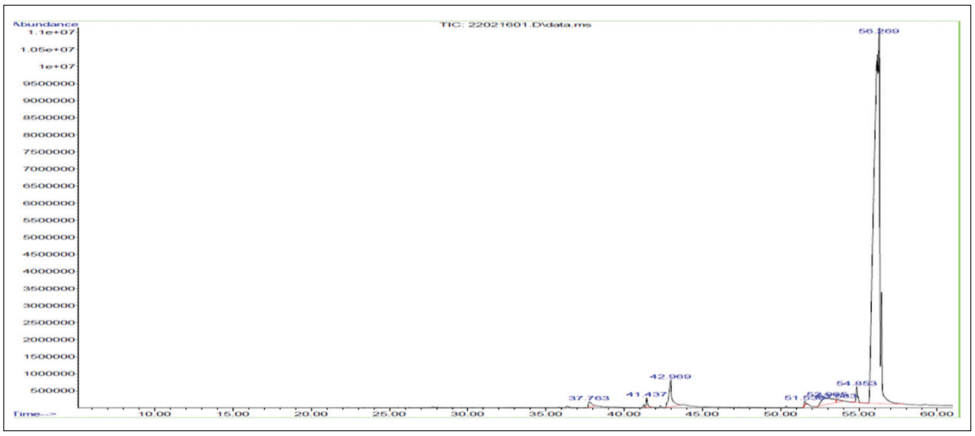

Figure 3: Mass spectrum of shodhit methanolic extract (SM) of *Semecarpus anacardium*

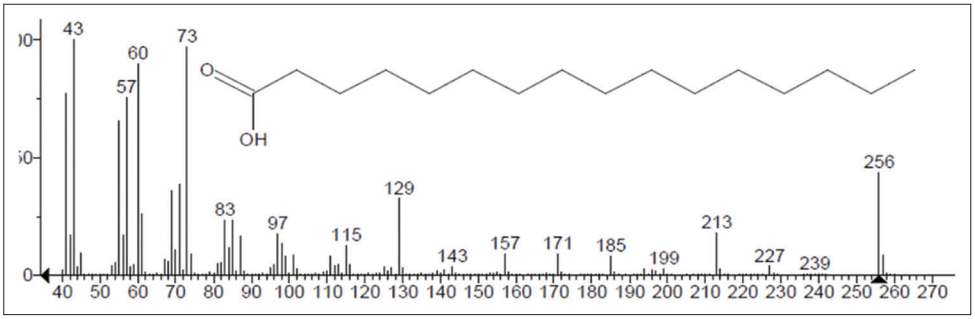

Figure 4: Mass spectrum showing presence of *n*-hexadecanoic acid in shodhit methanolic extract of *Semecarpus anacardium*

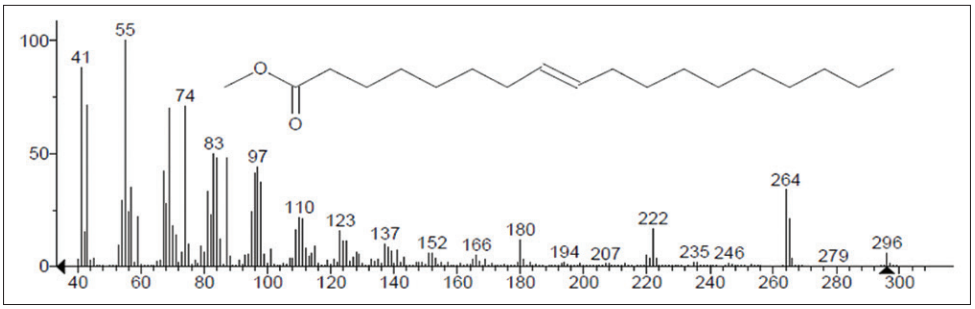

Figure 5: Mass spectrum showing presence of 9-octadecenoic acid, methyl ester in shodhit methanolic extract of *Semecarpus anacardium*

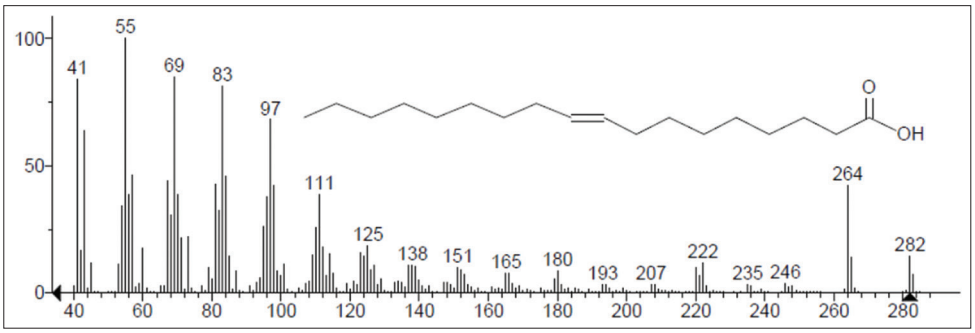

Figure 6: Mass spectrum showing presence of 8-octadecenoic acid, methyl ester in shodhit methanolic extract of *Semecarpus anacardium*

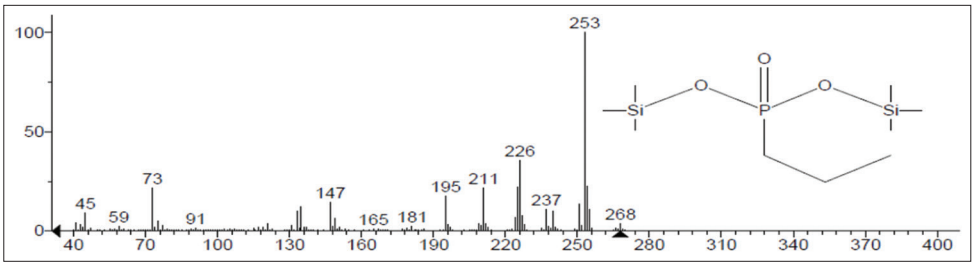

Figure 7: Mass spectrum showing presence of propanephosphonic acid, bis (trimethylsilyl) ester in shodhit methanolic extract of *Semecarpus anacardium*

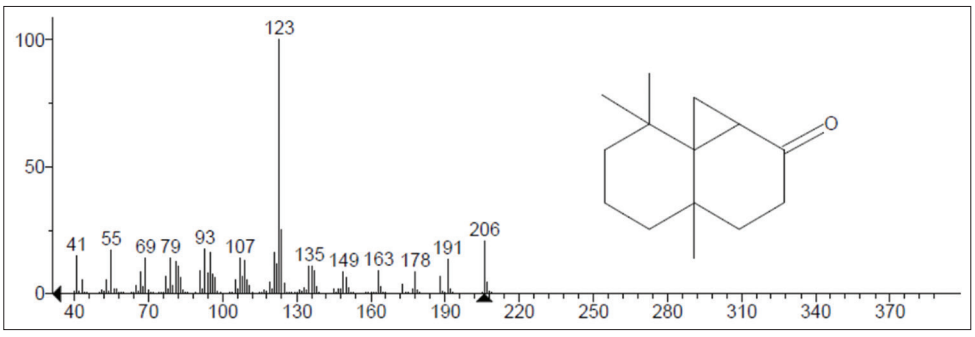

Figure 8: Mass spectrum showing presence of (1As-(1a.alpha. 4b.beta. 8as)-4a, 8, 8-trimethyloctahydrocyclopropa (d) naphthalen-2(3H)-one in shodhit methanolic extract of *Semecarpus anacardium*

analysis has been found to complete without the progress of HPTLC.[13,14] In this study, we have conducted GC-MS and HPTLC of both PSM and SM extract of SA to evaluate the effects of Shodhana on phytoconstituents present.

Shodhana of nuts improved the yield of methanolic extract of SA from 12% to 15% w/w. Again there is a decrease in flavonoid and phenolic content in SM extracts which are in agreement with earlier studies.[15] It was noticed that number of spots in TLC does not change. In addition, spot 1 R_f value matches with HPTLC studies, R_f values. However, maximum height and areas were found to be decreased in both PSM as well as SM which were noticed on observing the concentrations values. This interpretation puts forth

Table 4: Various components and their fragments in PSM and SM extract of SA

Retention time	Identity	Molecular formula	m/z	Synonyms	Figures
37.761	(1)	$C_{16}H_{32}O_2$	256	*n*-hexadecanoic acid	4
41.436	(2)	$C_{19}H_{32}O_2$	296	9-octadecenoic acid, 8-octadecenoic acid, methyl ester	5
42.967	(3)	$C_{18}H_{34}O_2$	282	Oleic acid, 9-octadecenoic acid	6
51.538	(4)	$C_9H_{25}O_3PSi_2$	253	Phenol, 3-pentadecyl- $$ phenol, *m*-pentadecyl- $$ *m*-pentadecylphenol $$ anacardol, tetrahydro- $$ cyclogallipharaol $$ hydrocardanol $$ hydroginkgol $$ tetrahydroanacardol $$ 3-*n*-pentadecylphenol $$ 3-pentadecylphenol, acetic acid, 4-methylphenyl ester (CAS) $$ narceol $$ *p*-tolylacetate $$ *p*-cresyl acetate $$ *p*-cresolacetate $$ 4-acetoxytoluene $$ *p*-acetoxytoluene $$ *p*-tolylethanoate $$ *p*-methylphenyl acetate $$ 4-methylphenyl acetate $$ acetic acid, *p*-tolyl ester $$ 2-pyridinamine, 5-methyl-(CAS) $$ 2-amino-5-methylpyridine $$ 2-amino-5-picoline $$ 3-picoline, 6-amino- $$ 5-methyl-2-pyridinamine $$ 5-methyl-2-aminopyridine $$ 5-methyl-2-pyridylamine dine $$ 5-methyl-2-pyridylamine $$ 6-amino-3-picoline	7
52.963	(5)	$C_{14}H_{22}O$	123	9,10-dihydroanthracene $$ anthracene, 9,10-dihydro- $$ anthracene, dihydro- $$ 9,10-dihydroanthracene[2,2'-binaphthalene]-5,5',8,8'-tetrone, 1,1'-dihydroxy-6,6'-dimethyl-(CAS) $$ 1,1'-dihydroxy-6,6'-dimethyl-2,2'-binaphthalene-5,5',8,8'-tetrone [1,2'-binaphthalene]-5,5',8,8'-tetrone, 1',4-dihydroxy-2,3'-dimethyl-, (-)-(CAS) $$ isodiospyrin $$ Isoldiospyrin	8
42.89	(6)	$C_{19}H_{30}O_3$	282	Adipic acid, di (*t*-butyldimethylsilyl) ester $$ hexanedioic acid, bis[(1,1-dimethylethyl) dimethylsilyl] ester, 4*H*-1-benzopyran-4-one, 5,6,7,8-tetrahydro-3-hydroxy-2-methyl- (CAS) $$ 5,6,7,8-tetrahydro-3-hydroxy-2-methylbenzopyrone, 9,10-dihydroanthracene $$ anthracene, 9,10-dihydro- $$ anthracene, dihydro- $$ 9,10-dihydroanthracene	10
54.854	(7)	$C_{16}H_{32}O_2$	206	10-oxo-5,5-dimethyl-5-sila-5H,10H, 11H-benzo[*e*]pyrido[3,4-*b*] azepine, 11-oxo-5,5-dimethyl-5-sila-5H,10H,11H-benzo[*b*] pyrido[4,3-*e*]azepine, 1H-Indene, 1,1'-(1,2-ethanediylidene) bis- (CAS)	11
56.270	(8)	$C_{25}H_{52}O_3S$	320	(*Z*,*Z*)-3-(Heptadeca-8',11'-dienyl)-benzene-1,2-diol $$ 1,3-benzenediol, 3-(8,11-heptadecadienyl)-, (*Z*,*Z*) (*Z*,*Z*)-4-(heptadeca-8',11'-dienyl)benzene-1,2-diol $$ 1,2-benzenediol, 4-(8,11-heptadecadienyl)-, (*Z*,*Z*) 3-(8'*Z*,11'*Z*)-pentadeca-8',11',14'-trienylbenzene-1,2-diol $$ 1,2-benzenediol, 3-(8,11,14-pentadecatrienyl)-, (*Z*,*Z*)-	12

PSM: Pre-shodhit methanolic, SM: Shodhit methanolic extract, SA: *Semecarpus anacardium*

that Shodhana does not have qualitative effect but has a quantitative effect on the phytoconstituents.

Our studies on GC-MS have elucidated with *n*-hexadecanoic acid, heptafluorobutyric acid, and 4-methoxyphenylester as major components in PSM and SM extract of SA nuts. There were three compounds present in PSM extract. One of them is urushiol like structure (1, 2-benzenediol, 3-(8, 11, 14-pentadecatrienyl)-, (Z, Z)-, retention time 56.270). Five compounds were found in SM extract. One of them is anacardol derivative (anacardol, tetrahydro-; retention time 51.538). Earlier reports have revealed that Shodhana helps in conversion of toxic urushiol into nontoxic anacardol.[2] Hence, the presence of anacardol derivative in SM extract and urushiol derivative in PSM extract further confirms that Shodhana helps in removal of toxic principle urushiol.

CONCLUSION

Thus, our studies shows that Shodhana of nuts of as per method investigated followed by Ayurvedic Pharmacopoeia of India improves the yield, decreases the phenolic and

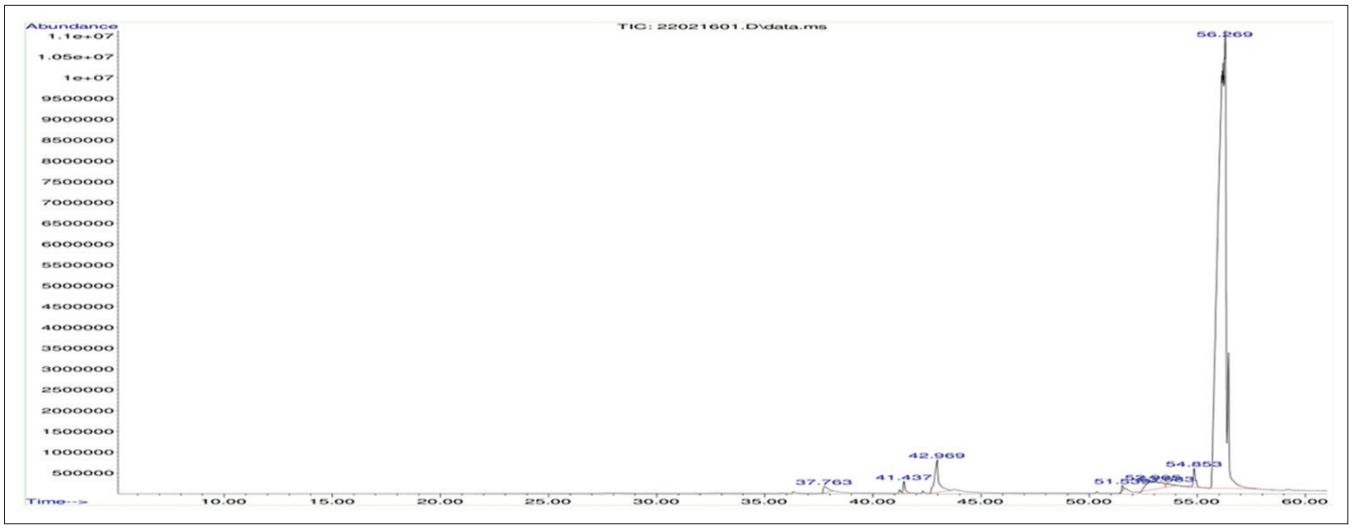

Figure 9: Mass spectrum of pre-shodhit methanolic extract of *Semecarpus anacardium*

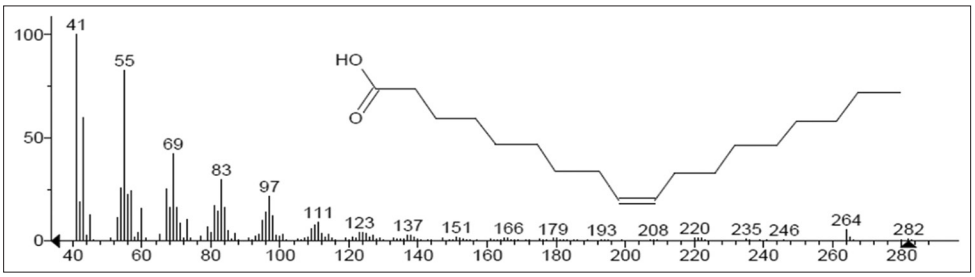

Figure 10: Mass spectrum showing presence of oleic acid in pre-shodhit methanolic extract of *Semecarpus anacardium*

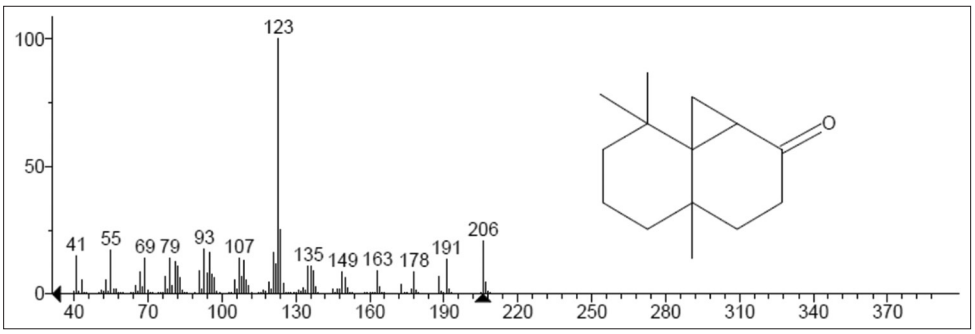

Figure 11: Mass spectrum showing presence of (1As-(1aα, 4bβ. 8as)-4a, 8, 8-trimethyloctahydrocyclopropa (d) naphthalen-2(3H)-one in pre-shodhit methanolic extract of *Semecarpus anacardium*

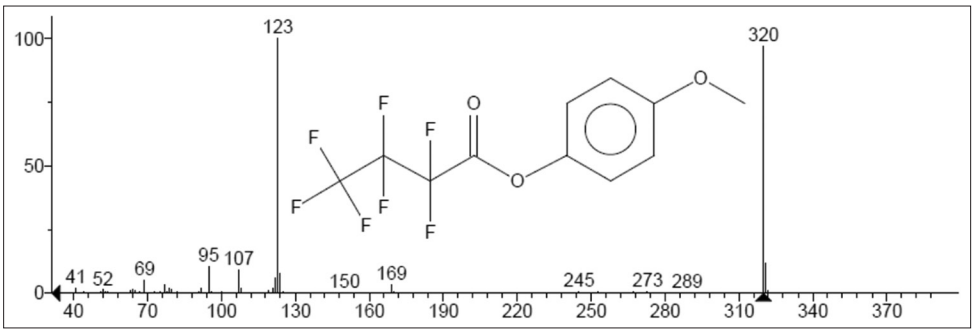

Figure 12: Mass spectrum showing presence of heptafluorobutyric acid, 4-methoxyphenyl ester in pre-shodhit methanolic extract of *Semecarpus anacardium*

flavonoid content; and increases the anacardol content thereby reducing toxicity.

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