

Phytochemical screening of oil extracted from two categories of fruit of *Semecarpus anacardium* Linn. by using traditional Indian oil extraction method

Ritesh Watekar¹, Dhirajsingh Rajput^{2*}, Bharat Rathi³, Anita Wanjari⁴

¹ MD Scholar, Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved college Hospital and Research Centre, Datta Medhe Institute of Medical Sciences (Deemed to be University), Wardha, Maharashtra, India

² Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved college Hospital and Research Centre, Datta Medhe Institute of Medical Sciences (Deemed to be University), Wardha, Maharashtra, India

^{3,4} Professor, Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved college Hospital and Research Centre, Datta Medhe Institute of Medical Sciences (Deemed to be University), Wardha, Maharashtra, India

Abstract

Oil extracted from fruits of *Semecarpus anacardium* Linn. is widely known for its therapeutic utility especially in traditional Indian System of Medicine (ISM). Analytical studies on *S. anacardium* have established its anti-infective, anti-fungal, anti-bacterial properties. However, in ISM, a test based on sinking and floating of fruits of *S. anacardium* has been mentioned and it has been claimed that only sunk fruits yield better therapeutic potential. Based on this concept, present work was planned. In this work, fruits of *S. anacardium* were divided into two categories by separating procured fruits and subjecting them for sinking test in water. The sunken fruits are considered as acceptable and the floating fruits are considered as unacceptable. The oil extraction was done as per ISM method by using earthen pot and invert distillation. The obtained oil from acceptable fruits (SAO-1) and unacceptable fruits (SAO-2) was analyzed by Gas chromatography and mass spectroscopy (GC-MS). Total 44 and 32 phytoconstituents are detected in SAO-1 and SAO-2 respectively. The numbers of toxic constituents in SAO-1 is detected less compared to SAO-2. Therapeutic potentials such as anti-microbial, anti-bacterial, anti-fungal, HIV type 1 type entry inhibitor, fungicidal, hemolytic, hyperconjugation and homohyperconjugation are found based on the chemical composition of the compounds detected in GC-MS. The findings represent need of more exploratory researches on oil of *S. anacardium* extracted based on concept of ISM.

Keywords: *S. anacardium*, Extracted oil, Indian system of medicine, Gas chromatography and mass spectroscopy

Introduction

In Ayurveda, a Indian System Medicine (ISM) i.e. Ayurveda, *Semecarpus anacardium* Linn. is one of the well-known medicinal herb frequently utilized as rejuvenator, anti-pyretic, anti-fungal, aphrodisiac and as anti-diabetic. Seed and oil extracted from fruit of *S. anacardium* are the commonly parts of this plant. The seed is reported to have anti-inflammatory ^[1], antiulcer ^[2], spermatogenic ^[3] and anti-oxidant ^[4] activity. Similarly, oil extracted from *S. anacardium* fruit has been also studied for its various therapeutic potentials. However, in ISM, a specific test is mentioned to select therapeutically utilizable fruit of *S. anacardium*. According to this test, well ripe fruits of *S. anacardium* are to be taken after removing their receptacles. Handfuls of these fruits are to be placed in water filled big vessel. Fruits which sink and settle at the bottom are considered as good quality acceptable fruits (AF) and fruits floating on water surface are considered as inferior quality and unacceptable (UF). Later the selected fruits are subjected for traditional procedure known as "Purification (*Shadhana* in Sanskrit)". As in ISM metals-minerals and poisonous herbs are subjected for different procedures of purification to remove or minimize their toxicity and to make them suitable for pharmaceutical processing.^[5] As this testing method as well as traditional purification is followed in ISM hence like concepts of ISM, this test may have

specific scientific rationality which can affect quality and thereby therapeutic utility of extracted oil.

Quality of a medicine can be well judged based on its chemical constituents. Mass spectroscopy is one of the technologies which provides information of almost all chemical constituents present in a given sample ^[6]. Phytochemical screening by using Gas chromatography and mass spectroscopy (GC-MS) is mostly utilized method in analytical chemistry. Therefore, in present work an attempt has been made to extract oil from AF and UF fruits of *S. anacardium* by traditional oil extraction method and testing the chemical constituents of both samples of oils by using GC-MS.

Material and Method

Well ripe fruits of *S. anacardium* (10 kg) were procured from authentic Ayurveda raw drug supplier. Dried receptacles from these fruits were removed and the obtained quantity (9,727 gm) was taken for separation of AF and UF through sinking test in water. 10 litre's water was taken in big stainless-steel vessel. Handful of receptacles removed *S. anacardium* fruits were dropped in water. This was done for three times. All floating *S. anacardium* fruits were separated. Similarly, the *S. anacardium* fruits which sank at the bottom were also collected separately. Obtained quantity of AF and UF was 5866 gm (60.30%) and 3861 gm (39.70 %) respectively.

As per the concept of ISM, fruits of *S. anacardium* are to be subjected for traditional purification procedure before extraction of oil. In the purification method, separately all fruits from both samples was 10 times punctured by a needle and kept in brick powder for 7 days. Later all fruits were removed and cleaned by cotton cloth. Such purified AF and UF fruits were kept separately in big earthen pot. The mouth of the pot was covered by a stainless-steel mesh. Two pits were prepared on earth surface by using cement bricks. The size of each pit was 2 feet in diameter and 2 feet in height. Stainless steel vessel of size which properly fits the mesh covered mouth of earthen pot, was kept at the centre of bottom of the each pit. The earthen post were carefully placed (mouth on stainless steel vessel) on the steel vessel. Pieces of cement bricks were placed nearby steel vessel and up to the covering of mesh. Then cow dung cakes were systematically placed around the pot and over each pot. These cow dung cakes were ignited by using some kerosene. On next day, after complete cooling, earthen pots and vessels were removed carefully and the oil obtained in the steel vessel was collected in two separate glass bottle. In such way *S. anacardium* oil from acceptable fruits (SAO-1) and unacceptable fruits (SAO-2) of was extracted. SAO-1 and SAO-2 were analyzed by GC-MS to identify the chemical constituents present in them as well as to know the difference in chemical composition of these two categories of fruits of *S. anacardium*.

Instruments and Chromatographic conditions for GC – MS analysis

Turbo-Mass Gold-Perkin-Elmer was used as mass-detector and Turbo-Mass ver-5.2 software was used to handle mass spectra. In GC-MS analysis, ionization energy of 70 eV was applied. Helium gas (99.999%) was used as a carrier gas (flow rate of 1 ml/min) and injection (1 µl) of methanolic and chloroform extracts were employed separately. As per

standard procedure the injector temperature (260°C), ion source temperature (200°C), oven temperature (80°C) with sequential increase of 10°C/min to 280 °C was maintained during the procedure. Mass spectra were obtained at 70eV; at scan interval of 0.5 sec. and fragments from 45 to 450 Da. The solvent delay was 0 to 1.5 min, and the total GC/MS running time was 42 min.

Identification of components

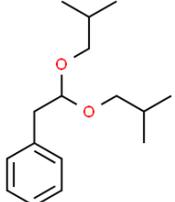
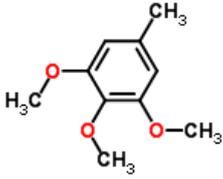
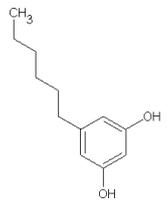
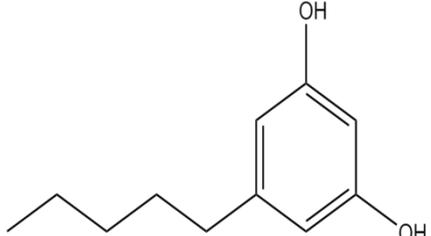
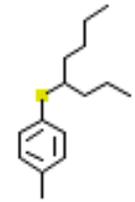
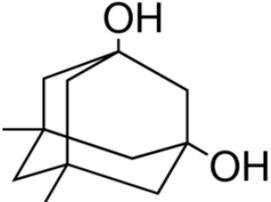
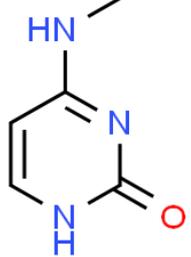
The interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST).

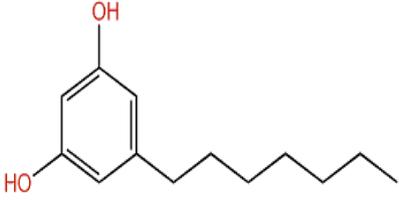
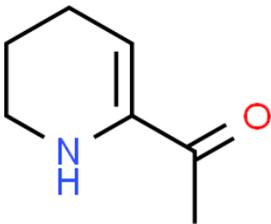
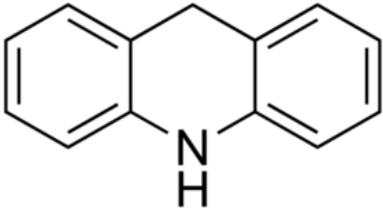
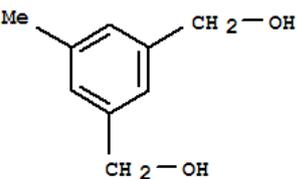
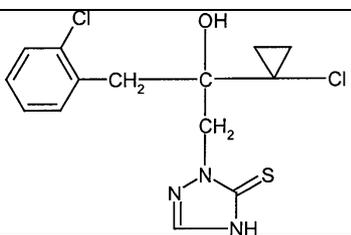
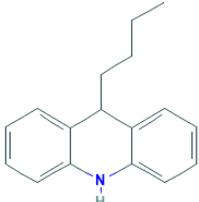
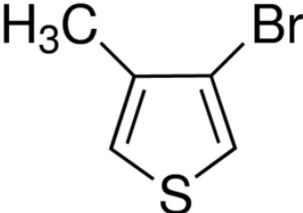
Observation and Results

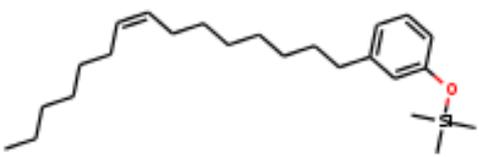
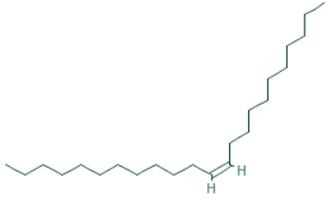
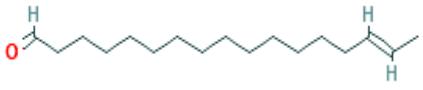
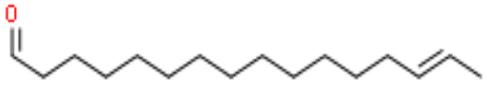
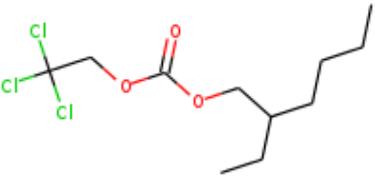
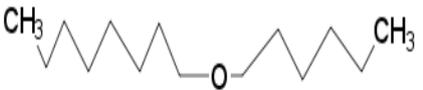
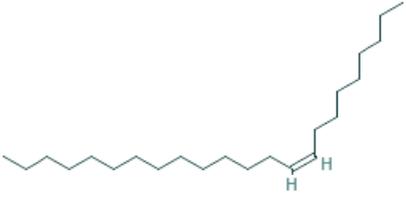
44 and 32 phytoconstituents are detected in GC-MS of SAO-1 and SAO-2 respectively. (Table 1 and 2) Structure and utility of 6 and 8 compounds from SAO-1 and SAO-2 respectively are not found known. (Table 3) In GC-MS analysis both samples showed constituents having antimicrobial, anti-fungal, anti-bacterial activities along with cytotoxic, peripheral blood mononuclear cells poisonous effect and skin irritant actions. However, compare to the toxic effects, therapeutic potentials are found much more in SAO-1 than SAO-2. Few constituents in SAO-1 has reported to have actions such as HIV type 1 type entry inhibitor, utility in human pancreatic cancer, anti-cancer, Anesthetic & Antipruritic activity. In SAO-2 presence of Carbazole indicated Anti-diabetic activity. However, all these activities need to be studied by extracting therapeutically active principles and filtering the unwanted toxic constituents. In such way *S. anacardium* Taila may prove as source of a novel therapeutic agent and may bring new ways for treating severe chronic illness. Further researches on pharmacokinetic, pharmacodynamic and experimental grounds are required to elaborate findings of present study. (Table 4)

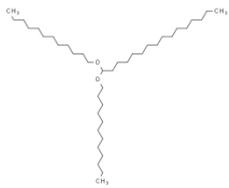
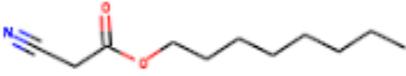
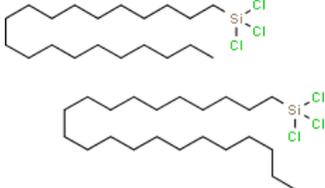
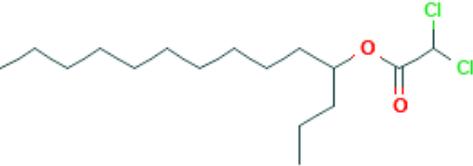
Table 1: GC-MS of oil extracted from acceptable fruits of *S. anacardium* (sample SAO-1)

Sr.	Compound Name	Formula	Structure
1.	3H,8H-DIPYRROLO[1,2-A:2,1-B] IMIDAZOLE-3,8-DIONE, HEXAHYDRO-	C ₉ H ₁₂ O ₂ N ₂	
2.	1,3-BENZENEDIOL, 5-PENTADECYL-	C ₂₁ H ₃₆ O ₂	
3.	(Z)-5-(PENTADEC-8-EN-1YL) BENZENE-1,3-DIOL	C ₂₁ H ₃₄ O ₂	

4.	SCLAREOLIDE	C ₁₆ H ₂₆ O ₂	
5.	CIS-2,5-DIALLYLOXY-2,5-DIHYDROFURAN	C ₁₀ H ₁₄ O ₃	
6.	5-TRIDECYLBENZENE-1,3-DIOL	C ₁₉ H ₃₂ O ₂	
7.	1,3-BENZENEDIOL, 5-HEXYL-	C ₁₂ H ₁₈ O ₂	
8.	1,3-BENZENEDIOL, 5-PENTYL-	C ₁₁ H ₁₆ O ₂	
9.	BENZENE, 1-METHYL-4-[(1-PROPHYL)PENTYL] THIO]	C ₁₅ H ₂₄ S	
10.	5,7-DIMETHYL-1,3-ADAMANTANEDIOL	C ₁₂ H ₂₀ O ₂	
11.	4N-METHYLCYTOSINE	C ₅ H ₇ O _N ₃	

12.	5-HEPTYLRESORCINOL	C ₁₃ H ₂₀ O ₂	
13.	2-ACETYL-1,4,5,6-TETRAHYDROPYRIDINE	C ₇ H ₁₁ O _N	
14.	ACRIDINE, 9,10-DIHYDRO-	C ₁₃ H ₁₁ N	
15.	1,3-BENZENEDIMETHANOL, 5-(DIMETHYLAMINO)	C ₁₀ H ₁₅ O ₂ N	
16.	[1,2,4] TRIAZOLE-3-THIONE, 4-ETHYL-5-(THIOPHEN-2-YL)-2-(9H-XANTHEN-9-YL)-2,4-	C ₂₁ H ₁₇ O _N ₃ S ₂	
17.	ACRIDINE, 9-BUTYL-9,10-DIHYDRO-	C ₁₇ H ₁₉ N	
18.	HYDROGINKGOL (TMS)	C ₂₄ H ₄₄ O _{Si}	
19.	THIOPHENE, 3-BROMO-4-(1,1-DIMETHYLETHOXY)-	C ₈ H ₁₁ OBrS	

20.	GINKGOL (TMS)	C ₂₄ H ₄₂ O _{Si}	
21.	11-TRICOSENE	C ₂₃ H ₄₆	
22.	E-15-HEPTADECENAL	C ₁₇ H ₃₂ O	
23.	E-14-HEXADECENAL	C ₁₆ H ₃₀ O	
24.	CARBONIC ACID, HEXADECYL 2,2,2-TRICHLOROETHYL ESTER	C ₁₉ H ₃₅ O ₃ Cl ₃	
25.	HEXADECYL OCTYL ETHER	C ₂₄ H ₅₀ O	
26.	TRIFLUOROACETIC ACID, PENTADECYL ESTER	C ₁₇ H ₃₁ O ₂ F ₃	
27.	9-TRICOSENE	C ₂₃ H ₄₆	

28.	HEXADECANE, 1,1-BIS(DODECYLOXY)-	C ₄₀ H ₈₂ O ₂	
29.	PENTAFLUOROPROPIONIC ACID, PENTADECYL ESTER	C ₁₈ H ₃₁ O ₂ F ₅	
30.	CYANOACETIC ACID, HEPTADECYL ESTER	C ₂₀ H ₃₇ O ₂ N	
31.	SILANE, TRICHLORODOCOSYL-	C ₂₂ H ₄₅ Cl ₃ Si	
32.	DICHLOROACETIC ACID, TETRADECYL ESTER	C ₁₆ H ₃₀ O ₂ Cl ₂	
33.	DODECYL OCTYL ETHER	C ₂₀ H ₄₂ O	
34.	3-EICOSENE	C ₂₀ H ₄₀	
35.	DICHLOROACETIC ACID, TRIDECYL ESTER	C ₁₅ H ₂₈ O ₂ Cl ₂	

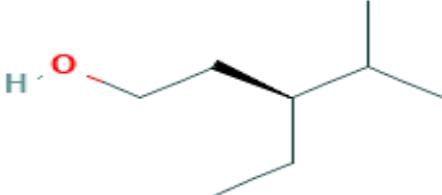
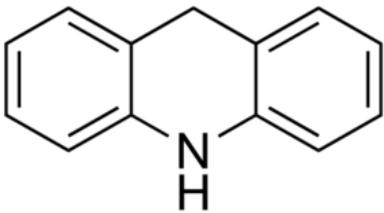
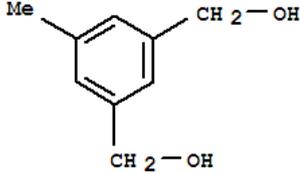
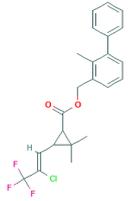
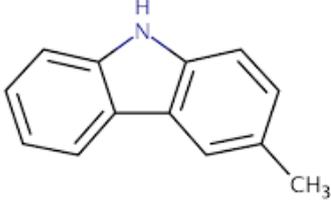
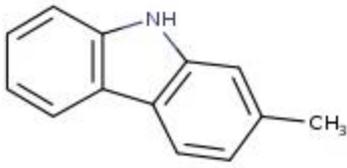
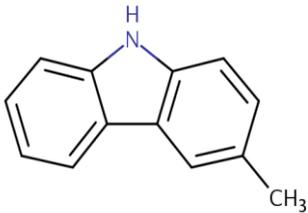
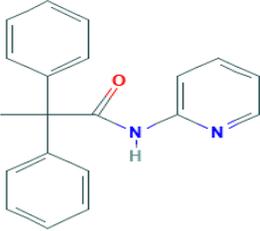
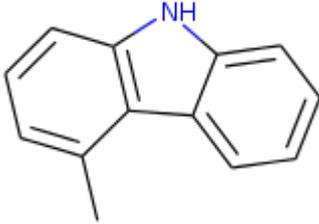
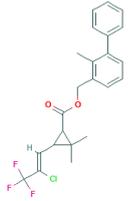
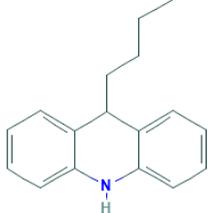
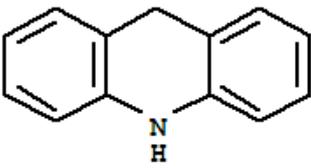
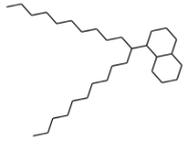
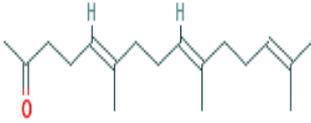
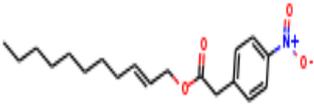
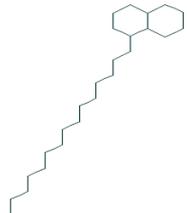
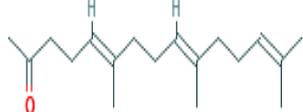
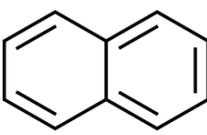
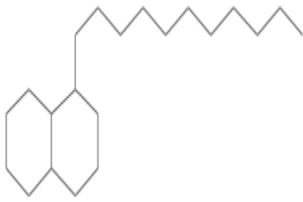
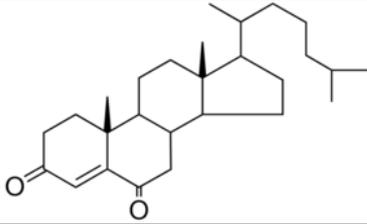
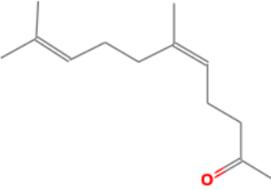
36.	TETRADECYL TRIFLUOROACETATE	C ₁₆ H ₂₉ O ₂ F ₃	
37.	1-DECANOL, 5,9-DIMETHYL-	C ₁₂ H ₂₆ O	
38.	(S)-3-ETHYL-4-METHYLPENTANOL	C ₈ H ₁₈ O	

Table 2: GC-MS of oil extracted from unacceptable fruits of *S. anacardium* (sample SAO-2)

Sr.	Compound Name	Formula	Structure
1.	ACRIDINE, 9,10-DIHYDRO	C ₁₃ H ₁₁ N	
2.	1,3-BENZENEDIMETHANOL, 5-(DIMETHYLAMINO)	C ₁₀ H ₁₅ O ₂ N	
3.	BIFENTHRIN	C ₂₃ H ₂₂ O ₂ ClF ₃	
4.	3-METHYLCARBAZOLE	C ₁₃ H ₁₁ N	
5.	9H-CARBAZOLE, 2-METHYL-	C ₁₃ H ₁₁ N	

6.	3-METHYLCARBAZOLE	C ₁₃ H ₁₁ N	
7.	PROPIONAMIDE, 2,2-DIPHENYL-N-(2-PYRIDINYL)	C ₂₀ H ₁₈ O ₂ N	
8.	4-METHYLCARBAZOLE	C ₁₃ H ₁₁ N	
9.	BIFENTHRIN	C ₂₃ H ₂₂ O ₂ ClF ₃	
10.	ACRIDINE, 9-BUTYL-9,10-DIHYDRO-	C ₁₇ H ₁₉ N	
11.	10-ACETYL-9,10-DIHYDROACRIDINE	C ₁₅ H ₁₃ O ₂ N	
12.	NAPHTHALENE, 1-(1-DECYLUNDECYL) DECAHYDRO-	C ₃₁ H ₆₀	
13.	5,9,13-PENTADECATRIEN-2-ONE, 6,10,14-TRIMETHYL-	C ₁₈ H ₃₀ O	

14.	BENZENEACETIC ACID, 4-NITRO-, UNDEC-2-EN-1-YL ESTER	C ₁₉ H ₂₇ O ₄ N	
15.	NAPHTHALENE, DECAHYDRO-1-PENTADECYL	C ₂₅ H ₄₈	
16.	NAPHTHALENE, 2-DECYLDECAHYDRO-	C ₂₀ H ₃₈	
17.	5,9,13-PENTADECATRIEN-2-ONE, 6,10,14-TRIMETHYL-, (E,E)-	C ₁₈ H ₃₀ O	
18.	NAPHTHALENE		
19.	NAPHTHALENE, DECAHYDRO-1-UNDECYL-	C ₂₁ H ₄₀	
20.	CHOLEST-4-ENE-3,6-DIONE	C ₂₇ H ₄₂ O ₂	
21.	5,9-UNDECADIEN-2-ONE, 6,10-DIMETHYL-, (Z)-	C ₁₃ H ₂₂ O	
22.	5,9-UNDECADIEN-2-ONE, 6,10-DIMETHYL-, E-	C ₁₃ H ₂₂ O	Same as above
23.	5,9-UNDECADIEN-2-ONE, 6,10-DIMETHYL-	C ₁₃ H ₂₂ O	Same as above

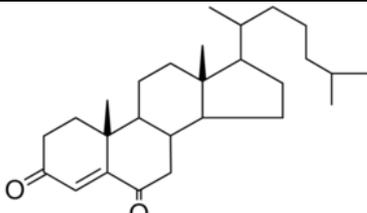
24.	CHOLEST-4-ENE-3,6-DIONE	C27H42O2	
-----	-------------------------	----------	---

Table 3: Compounds in SAO-1 and SAO-2 whose structures is not identified

Sr.	Sample	Compound name	Chemical formula
1.	SAO-1	2-METHYL-N-(1-METHYLETHYL METHYLIDENE)-4-[(1E)-1-PENTADECENYL]-1,3,2-DIOXABORI	C22H42O2NB
2.	SAO-1	BORINIC ACID, DIETHYL-, ANHYDRIDE	C8H20O2
3.	SAO-1	2-PROPANOL, 1,3-DIBROMO-	C3H6ONr2
4.	SAO-1	10-ACETYL-9,10-DIHYDROACRIDINE	C15H13ON
5.	SAO-1	N-PENTANE, 2-CYCLOHEXYL-5-[1-CYCLOAZAPROPYL]-	C13H25N
6.	SAO-1	5,9,13-TRIMETHYLTETRADECANOIC ACID2,2,2-TRIFLUOROETHYL ESTER	C19H35O2F3
7.	SAO-2	4AH-BENZENEDIMETHANOL, 5-(DIMETHYLAMINO)	C13H11N
8.	SAO-2	9-ANTHRACENEPROPANOIC ACID, 1,2,3,4-TETRAHYDRO-, METHYL ESTER	C18H20O2
9.	SAO-2	1,2,3,4-TETRAHYDRO-4-PHENANTHRENACETIC ACID, 2,2,3,3,4,4,4-HEPTAFLUOROB	C20H17O2F7
10.	SAO-2	THIOPHENE, 3-BROMO-4-(1,1-DIMETHYLETHOXY)	C8H11OBrS
11.	SAO-2	1H-INDENE, 1-(1,5-DIMETHYL-2-HEXENYL) OCTAHYDRO-7A-METHYL-, [1R-[1. ALPHA.	C18H32
12.	SAO-2	1-OXA-3-AZASPIRO [4.5] DECANE-3-ACETAMIDE, N-(4-FLUOROPHENYL)-4-HYDROXY	C17H21O4N2F
13.	SAO-2	2,2-DIMETHYL-1-(3-OXO-BUT-1-ENYL)-CYCLOPENTANECARBOXALDEHYDE	C12H18O2
14.	SAO-2	ZINC, BIS[2-(1,1DIMETHYL-2-PROPENYL)-3,3-DIMETHYLCYCLOPROPYL]-, [1. ALPHA	C20H34Zn

Table 4: Actions of individual compounds among phyto-constitutes detected in SAO-1 and SAO-2

Sr.	Name of compound	Sample	Action	Reference
1.	Pentadecenyl	SAO-1 & SAO-2	Cytotoxicity and moderate genotoxicity, increases apoptosis rate, in gastric cancer cells.	Alam <i>et al.</i> [7]
2.	Imidazolo	SAO-1	Anti-bacterial, Anti-fungal	Maurice <i>et al.</i> [8]
3.	Pyrrole	SAO-1	HIV type 1 type entry inhibitor	Jiang <i>et al.</i> [9]
4.	Dioxabori and borinic acid	SAO-1 & SAO-2	Anti-microbial	Borokhov <i>et al.</i> [10]
5.	Sclareolide	SAO-1	Enhances gemcitabine-induced cell death through mediating the NICD and Gli1 pathways in gemcitabine-resistant human pancreatic cancer	Chen <i>et al.</i> [11]
6.	Dihydrofuran	SAO-1	Anti-tumor effect	Zhang <i>et al.</i> [12]
7.	Benzenediol	SAO-1 & SAO-2	Peripheral blood mononuclear cells poisonous effect	Bukowska <i>et al.</i> [13]
8.	Adamantanediol	SAO-1	Hyperconjugation and homohyperconjugation	Sunko <i>et al.</i> [14]
9.	4N- Methylcytosine	SAO-1	DNA-methyletion, gene regulation and cancer.	Riggs <i>et al.</i> [15]
10.	Resorcinol	SAO-1	Disrupt thyroid hormone synthesis and can produce goitrogenic effects	Lynch <i>et al.</i> [16]
11.	Tetrahydropyridine	SAO-1	Can produces unalloyed parkinsonism	Langston <i>et al.</i> [17]
12.	Acridine	SAO-1 & SAO-2	Cytotoxic and useful in human bladder cancer	Lin <i>et al.</i> [18]
13.	Triazole	SAO-1	Fungicidal	Ly <i>et al.</i> [19]
14.	Thiophen	SAO-1 & SAO-2	Irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath, Phototoxic, Anti-microbial	Towers <i>et al.</i> [20]
15.	Hydroginkgol	SAO-1	Skin-lightening agent. It bleaches the skin, which can be helpful when treating different forms of hyperpigmentation, Causes subcutaneous dark collections of pigment, nephrotoxicity and carcinogenic	Kooyers <i>et al.</i> [21]
16.	Heptadecenal	SAO-1	Antibacterial compound that can inhibit the growth of K. pneumoniae ATCC 13883 strain	Supardy <i>et al.</i> [22]
17.	Carbonic acid	SAO-1	Makes the blood acidic, increases oxidizing power of the liver cells cytoplasm	Mel'nychuk <i>et al.</i> [23]
18.	Trichloroethyl ester		Relatively short-term exposure resulted in harmful effects on the nervous system, liver, respiratory system, kidneys, blood, immune system, heart, and body weight. Exposure to trichloroethylene in the workplace may cause scleroderma in some people	
19.	Tricosene	SAO-1	Major component of the sex pheromone, being responsible for inducing the courtship ritual and the males' striking activity	Feyereisen <i>et al.</i> [24]
20.	Dodecyloxy	SAO-1	Anesthetic & Antipruritic	Gosselin <i>et al.</i> [25]
21.	Cyanoacetic acid	SAO-1	Skin and eye corrosivity and respiratory irritation for cyanoacetic acid, at high doses effects on hematology, liver, adrenals and sperms after repeated dosing, embryotoxic or fetotoxic effects	SIAM report [26]
22.	Eicosene	SAO-1	Antioxidant activity	Vijayamuthuramalingam <i>et al.</i> [27]

23.	Benzenedimethanol	SAO-2	Irritant, Skin sensitivity	Material Safety Data Sheet ^[28]
24.	Bifenthrin	SAO-2	Pyrethroid poisoning, caused anaemia, elevated white blood cell count (WBC), elevated alanine transaminase (ALT), superoxide dismutase (SOD), and decreased glutathione peroxidase (GPx) activity	Nieradko <i>et al.</i> ^[29]
25.	Carbazole	SAO-2	Anti-diabetic	Eseyin <i>et al.</i> ^[30]
26.	Anthracenepropanoic acid	SAO-2	Severe irritation, cause damage to the skin	Tarafdar <i>et al.</i> ^[31]
27.	Naphthalene	SAO-2	Increases susceptibility of the red blood cell to hemolysis	Sudakin <i>et al.</i> ^[32]

Discussion

Herbal medicines have been used since the beginning of civilization to sustain health and treat disease^[33]. Evidences of traditional medicines in various countries clearly represent rich heritage of ancient knowledge. In ISM, not only herbs but also metals-minerals and poisonous herbs are utilized for therapeutic purpose. *S. anacardium* is one of such herb classified as semi-poisonous herb based on toxic effect of oil extracted from its fruits. Regarding sinking test in water, *S. anacardium* fruits are rich source of oil which also contain some volatile constituents. As an organic plant material, it should float on water. Moreover, oil has tendency to float over water due to its less specific gravity but it has more molecular weight and density. However, if a substance with more molecular weight and density is packed in container and then placed on water surface then even having low specific gravity than water, the substance will settle at the bottom. In clear words, proportion of molecular weight, density and the occupied space are the variables which needed to be considered for reasoning of floating and sinking mechanism. In case of acceptable *S. anacardium* (AF) it is evident that the oil content is packed within walls of outer of surface of the fruits and there are no or very less empty cells lacking oil. This results in increasing weight of *S. anacardium* fruits even if it is looking of same size to that of unacceptable *S. anacardium* (UF). On the other hand, UF may have less significant number of partial empty cell, which may occur due environmental factors such as heat, less nutritional supply while maturing and the duration collection and storage. These empty spaces can force *S. anacardium* fruits to float over water.

GC-MS of SAO-1 and SAO-2 showed highly complex nature and richness of phyto-constituents in both samples. Total 44 and 32 chemical compounds are detected in SAO-1 and SAO-2 respectively. Structural information of most compounds is available however few compounds the structure of remaining compounds was not clearly known. These compounds may be the result of chemical differences in soil, nourishment of *S. anacardium* fruits, the adopted purification procedure or the effect of heat applied during extraction of oil.

Due to highly complex nature of all constituents, it is difficult to interpret the probable role of each constituent as researches on exact similar compounds are not available. However, each compound is formed from combination of two or more individual compounds and interpretations can be made based on these individual compounds. The resultant compound may or may not have similar action. Therefore, to avoid complication in information as well as any misleading information; only few individual compounds are interpreted in table 4.

GC-MS of SAO-1 and SAO-2 revealed significant information by detecting large number of pharmacological compounds, most of which are yet to be studied. Compounds such as Pentadecenyl, Benzenediol and

Acridine which are detected in higher proportion in SAO-1 and SAO-2 possess toxic potentials which are narrated for the toxic effect of *S. anacardium*. Dioxaborin, Thiophen and borinic acid are the common compounds in both samples having Anti-microbial activity. Few compounds found in SAO-1 such as Imidazole (Anti-bacterial, Anti-fungal), Pyrrole (HIV type 1 type entry inhibitor), Adamantanediol (Hyperconjugation and homohyperconjugation) and Triazole (Fungicidal); need more attention for their separation and detail pharmacological evaluation. In SAO-2, Benzenedimethanol (Irritant, Skin sensitivity) and Naphthalene (Increases susceptibility of the red blood cell to hemolysis) are found in more proportion indicating more toxic nature of SAO-2 compared to SAO-1.

The analytical findings are supportive of traditional criteria of selection of good quality fruits of *S. anacardium* by performing a simple test in water. Therefore, it can be claimed that the water sinking test narrated in ISM have scientific basis as the oil extracted from acceptable fruits have more phytoconstituents compared to unacceptable (floating) fruits. Presence of less number of toxic and corrosive constituents in acceptable fruits also supports the rationality of ISM. Separation of each constituent and studying its pharmacokinetic and pharmacodynamic actions is needed to establish preclinical evidences for therapeutic utility of oil of *S. anacardium*.

Conclusion

Oil extracted from acceptable fruits and unacceptable fruits of *S. anacardium* contain combination of therapeutically active as well as toxic compounds, however more proportion of toxic compounds in oil extracted from unacceptable fruits and more proportion of therapeutic compounds in oil extracted from acceptable fruits supports the rightness of ISM claim of accepting only water sinkable fruits of *S. anacardium* and rejecting Unacceptable i.e. floating fruits. Thus this simple test can be further used for extracting quality control of oil of *S. anacardium*. GC-MS has identified several compounds of which the chemical information is completely unavailable which represent great scope in drug discovery and development. Based on the major indications of the compounds found in oil extracted from acceptable fruits in severe systemic chronic diseases such as cancer, HIV and gene regulation, it is vivid that *S. anacardium* oil need much more exploratory studies to establish its therapeutic potential.

References

1. Selvam C, Jachak SM. A cyclooxygenase (COX) inhibitory biflavonoid from the seeds of *Semecarpus anacardium*. *Journal of ethnopharmacology*. 2004; 95(2-3):209-12.
2. Manoj K, Meenu J, Sunil S. Evaluation of antiulcer activity of various extracts of *Semecarpus anacardium* seeds. *Pharmacognosy Journal*. 2011; 3(20):39.

3. Jain P, Sharma HP. A potential ethnomedicinal plant *Semecarpus anacardium* Linn. A review. *Int J Res Pharm Chem*. 2013; 3(3):564-72.
4. Ali MA, Wahed MI, Khatune NA, Rahman BM, Barman RK, Islam MR, *et al*. Antidiabetic and antioxidant activities of ethanolic extract of *Semecarpus anacardium* (Linn.) bark. *BMC Complementary and Alternative Medicine*. 2015; 15(1):138.
5. Rajput DS, Patgiri B, Shukla VJ. Standardization of Shodhita Naga with special reference to thermogravimetry and infra-red spectroscopy. *Ayu*. 2014; 35(3):316.
6. Doshi GM, Nalawade VV, Mukadam AS, Chaskar PK, Zine SP, Somani RR, *et al*. Structural elucidation of chemical constituents from *Benincasa hispida* seeds and *Carissa congesta* roots by gas chromatography: Mass spectroscopy. *Pharmacognosy research*. 2015;7(3):282
7. Alam-Escamilla D, Estrada-Muniz E, Solís-Villegas E, Elizondo G, Vega L. Genotoxic and cytostatic effects of 6-pentadecyl salicylic anacardic acid in transformed cell lines and peripheral blood mononuclear cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 2015; 777:43-53.
8. Maurice M, Pichard L, Daujat M, Fabre I, Joyeux H, Domergue J, *et al*. Effects of imidazole derivatives on cytochromes P450 from human hepatocytes in primary culture. *The FASEB Journal*. 1992; 6(2):752-8.
9. Jiang S, Lu H, Liu S, Zhao Q, He Y, Debnath AK, *et al*. N-substituted pyrrole derivatives as novel human immunodeficiency virus type 1 entry inhibitors that interfere with the gp41 six-helix bundle formation and block virus fusion. *Antimicrobial agents and chemotherapy*. 2004; 48(11):4349-59.
10. Borokhov O, Schubert D. Antimicrobial properties of boron derivatives. *New Biocides Development*, 2007; 20:412-35.
11. Chen S, Wang Y, Zhang WL, Dong MS, Zhang JH. Sclareolide enhances gemcitabine-induced cell death through mediating the NICD and Gli1 pathways in gemcitabine-resistant human pancreatic cancer. *Molecular medicine reports*. 2017; 15(4):1461-70.
12. Zhang Y, Zhong H, Wang T, Geng D, Zhang M, Li K, *et al*. Synthesis of novel 2, 5-dihydrofuran derivatives and evaluation of their anticancer activity. *European journal of medicinal chemistry*, 2012; 48:69-80.
13. Bukowska B, Michałowicz J, Marczak A. The effect of catechol on human peripheral blood mononuclear cells (in vitro study). *Environmental toxicology and pharmacology*. 2015; 39(1):187-93.
14. Sunko DE, Hirs-Starcevic S, Pollack SK, Hehre WJ. Hyperconjugation and homohyperconjugation in the 1-adamantyl cation. Qualitative models for gamma-deuterium isotope effects. *Journal of the American Chemical Society*. 1979; 101(21):6163-70.
15. Riggs Arthur D, Peter A Jones. "5-methylcytosine, gene regulation, and cancer." *Advances in cancer research*, 1983; 40:1-30
16. Lynch BS, Delzell ES, Bechtel DH. Toxicology review and risk assessment of resorcinol: thyroid effects. *Regulatory toxicology and pharmacology*. 2002; 36(2):198-210.
17. Langston JW. I. MPTP neurotoxicity: an overview and characterization of phases of toxicity. *Life sciences*. 1985; 36(3):201-6.
18. Lin YC, Lin JF, Tsai TF, Chen HE, Chou KY, Yang SC, *et al*. Acridine orange exhibits photodamage in human bladder cancer cells under blue light exposure. *Scientific reports*. 2017; 7(1):1-1.
19. Lv X, Pan L, Wang J, Lu L, Yan W, Zhu Y, *et al*. Effects of triazole fungicides on androgenic disruption and CYP3A4 enzyme activity. *Environmental pollution*, 2017; 222:504-12.
20. Towers GN, Arnason T, Wat CK, Graham EA, Lam J, Mitchell JC, *et al*. Phototoxic polyacetylenes and their thiophene derivatives [effects on human skin]. *Contact Dermatitis*. 1979; 5(3):140-4.
21. Kooyers TJ, Westerhof W. Toxicological aspects and health risks associated with hydroquinone in skin bleaching formula. *Nederlands tijdschrift voor geneeskunde*. 2004; 148(16):768-71.
22. Supardy NA, Ibrahim D, Sulaiman SF, Zakaria NA. Inhibition of *Klebsiella pneumoniae* ATCC 13883 cells by hexane extract of *Halimeda discoidea* (Decaisne) and the identification of its potential bioactive compounds. *Journal of microbiology and biotechnology*. 2012; 22(6):872-81.
23. Mel'nychuk DO, Skoryk LV, Scholz C, Mitsyk EV, Guly MF. Effect of carbonic acid concentration in blood on content of keto-acids and redox state of nicotinamide coenzymes in rabbit tissues. *Ukrains'kyi biokhimichnyi zhurnal*. 1977; 49(5):86-93.
24. Iga M, Kataoka H. Recent studies on insect hormone metabolic pathways mediated by cytochrome P450 enzymes. *Biological and Pharmaceutical Bulletin*. 2012; 35(6):838-43.
25. Gosselin RE, Hodge HC, Smith RP, Gleason MN. *Clinical Toxicology of Commercial Products*. 4th ed. Baltimore: Williams and Wilkins, 1976, p. 180
26. SIAM 23, report dated 17-20 October 2006, available at <https://hpvchemicals.oecd.org/ui/handler.axd?id=D8489E4D-D25A-4BED-B770-99ADA9011581>, last accessed on 04-05-2020 at 9.45 PM
27. Vijayamuthuramalingam UD, Rajaram R, Kuppusamy KM, Jonnalagadda B, Arokiasamy S. Anti-hyperglycemic and antioxidant potential of *Croton bonplandianus*. *Bail fractions in correlation with polyphenol content*. *Iranian Journal of Basic Medical Sciences*. 2017; 20(12):1390.
28. Material Safety Data Sheet, available at <http://datasheets.scbt.com/sc-222909.pdf>, last accessed on 04-05-2020 at 9.50 PM
29. Nieradko-Iwanicka B, Borzecki A, Jodłowska-Jedrych B. Effect of subacute poisoning with bifenthrin on locomotor activity, memory retention, haematological, biochemical and histopathological parameters in mice. *J Physiol Pharmacol*. 2015; 66(1):129-37.
30. Eseyin OA, Edem E, Johnson E, Ahmad A, Afzal S. Synthesis and in vitro antidiabetic activity of some alkyl carbazole compounds. *Tropical Journal of Pharmaceutical Research*. 2018; 17(3):537-41.
31. Tarafdard A, Sinha A, Mastro RE. Biodegradation of anthracene by a newly isolated bacterial strain, *Bacillus thuringiensis* AT. ISM. 1, isolated from a fly ash deposition site. *Letters in applied microbiology*. 2017; 65(4):327-34.
32. Sudakin DL, Stone DL, Power L. Naphthalene mothballs: emerging and recurring issues and their

- relevance to environmental health. *Current topics in toxicology*, 2011; 7:13
33. Khan MB, Rathi BJ, Rajput D, Wanjari A. A review on classical Vajikarana formulations of Shweta Musali. *Journal of Indian System of Medicine*. 2019; 7(4):205.