Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

SHORT COMMUNICATION

Gas Chromatography-Mass Spectrometric Determination of Components of Leaves of *Aegle marmelos* and *Psidium guajava* and Seeds of *Nigella sativa* and Correlation with *In vitro* Antioxidant Activity

C. V. Jayachandran Nair, Sayeed Ahamad[1], Washim Khan[1], Varisha Anjum[1], Rajani Mathur

Department of Pharmacology, Delhi Institute of Pharmaceutical Science and Research, [1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Bioactive Natural Product Laboratory, Jamia Hamdard, New Delhi, India

ABSTRACT

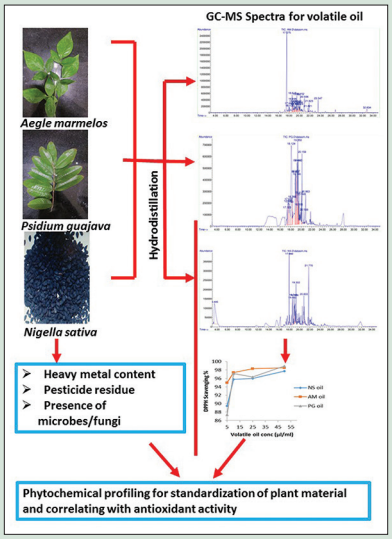
Objective: The aim of this study is to analyze the volatile components of the leaves of *Aegle marmelos, Psidium guajava*, and seeds of *Nigella sativa*, using gas chromatography-mass spectrometric (GC-MS) and correlate with their *in vitro* antioxidant activity. **Materials and Methods:** Plant material was analyzed for heavy metal content (HMC), pesticide residue (PR), and the presence of microbes/fungi. Following hydrodistillation, the volatile components (AM, PG, and NS) were subjected to GC-MS analysis and *in vitro* antioxidant assay (2,2-diphenyl-1-picrylhydrazyl). **Results:** The plant material passed the test for HMC, PR, microbial, and fungal contamination. Using GC-MS, the number of compounds identified in AM, PG, and NS were 62, 46, and 58, respectively. The antioxidant activity of AM, PG, and NS was 98.538%, 98.955%, and 97.755%, respectively. **Conclusion:** GC-MS-based methods can be successfully utilized for phytochemical profiling and standardization of plant material.

Key words: *Aegle marmelos*, antioxidant activity, gas chromatography-mass spectrometric, *Nigella sativa*, *Psidium guajava*, volatile compounds

SUMMARY

The leaves of *Aegle marmelos, Psidium guajava*, and seeds of *Nigella sativa*, were analyzed for heavy metal content, pesticide residue, and the presence of microbes/fungi and their volatile oil content was profiled by gas chromatography-mass spectrometric and correlated with *in vitro* antioxidant activity.

Abbreviations Used: AM: Volatile oil of *Aegle marmelos* Correa.; GC-MS: Gas chromatography-mass spectrometry; HMC: Heavy metal content; NS: Volatile oil of *Nigella sativa* Linn.; PG: Volatile oil of *Psidium guajava* Linn.; PR: Pesticide residue.



Correspondence:
Dr. Rajani Mathur,
Delhi Institute of Pharmaceutical Sciences and Research, Pushp Vihar, Sec III, MB Road, New Delhi - 110 017, India.
E-mail: mathurajani@gmail.com
DOI: 10.4103/pr.pr_93_17

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Since time immemorial, plants have been an exemplary source of drugs. The traditional system of medicine like Ayurveda contains extensive mention of formulations that contain *Nigella sativa* Linn. (Ranunculaceae), *Aegle marmelos* (L.). Correa (Rutaceae), and *Psidium guajava* Linn. (Myrtaceae).[1-3] Recent pharmacological studies have also established the medicinal properties of these plants,[4,5] that can be mainly attributed, to the presence of wide array of chemical moieties such as tannins, alkaloids, essential oil, quinones, sterols, triterpenoids, and flavonoids.

Thorough validation and standardization of the medicinal plant-derived drugs are essential but cumbersome and technically challenging. Thus, there is an urgent need to set-up simple methods that can be utilized for quality analysis of medicinal plant-based products. Here, we report a simple gas chromatography-mass spectroscopy-based method that was developed in accordance with ICH guidelines[6] to address this lacuna. In addition, the phytochemical constituents were pharmacodynamically assessed for *in vitro* antioxidant activity.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh leaves of *Aegle marmelos* (L.) Correa. and *Psidium guajava* Linn. were locally collected (Herbal garden, DIPSAR) and the seeds of *Nigella*

Cite this article as: Jayachandran Nair CV, Ahamad S, Khan W, Anjum V, Mathur R. Gas chromatography-mass spectrometric determination of components of leaves of *Aegle marmelos* and *Psidium guajava* and seeds of *Nigella sativa* and correlation with *In vitro* antioxidant activity. Phcog Res 2018;10:230-5.

Table 1: Qualitative determination of the plant leaves of *Psidium guajava* Linn., *Aegle marmelos* (L) Correa and seeds of *Nigella sativa* Linn

Description	P. *guajava* leaf	A. *marmelos* leaf	N. *sativa* seed	LOQ	Method
Pesticide residues					
DDT (all isomers-sum of p,p'-DDT,p,p'-DDE,p,p'-TDE(DDD)) exp as DDT	ND	ND	ND	0.005 ppb	GC-MS/MS
Hexachorocyclohexane (alpha, beta, Gamma-isomers) (mg/kg)	ND	ND	ND	0.005 ppb	GC-MS/MS
Endosulfan (sum of isomers)	ND	ND	ND	0.005 ppb	GC-MS/MS
Malathion	ND	ND	ND	5.0 ppb	GC-MS/MS
Parathion	ND	ND	ND	0.005 ppb	GC-MS/MS
Total viable aerobic count (cfu/g)	<10	255	125	-	BP
Total enterobacteriaceae count (cfu/g)	<10	<10	<10		BP
Total fungal count	230	Less than 10	ND		BP
Total aflatoxin	ND	ND	ND		
Aflatoxin B1	ND	ND	ND	1.0 ppb	LC-MS/MS
Aflatoxin B2	ND	ND	ND	1.0 ppb	LC-MS/MS
Aflatoxin G1	ND	ND	ND	1.0 ppb	LC-MS/MS
Aflatoxin G2	ND	ND	ND	1.0 ppb	LC-MS/MS
Metallic elements					
Arsenic	0.176 mg/kg	1.282 mg/kg	ND		LC-MS/MS
Lead	0.558 mg/kg	1.047 mg/kg	ND		LC-MS/MS
Mercury	ND	2.302 mg/kg	ND		LC-MS/MS
Cadmium	ND	ND	ND		LC-MS/MS
Iron	171.949 mg/kg	322.191 mg/kg	9.163 mg/kg		LC-MS/MS
Zinc	8.394 mg/kg	4.512 mg/kg	3.47 mg/kg		LC-MS/MS
Potassium	1.72 mg/kg	1.37 mg/kg	164.5 mg/kg		LC-MS/MS
Calcium	904.318 mg/kg	3.40 mg/kg	155.7 mg/kg		LC-MS/MS

ND: Not detected; BP-British pharmacopeia; LC: Liquid chromatography; MS: Mass spectrometry; GC: Gas chromatography; LOQ: Limit of quantitation; cfu: Colony forming unit; ppb: Parts per billion; DDT: Dichloro diphenyl trichloro ethane; p,p'-DDT: Analogue of Clofenotane; p,p'-DDE:Dichloro diphenyl dichloroethene; p,p'-TDE: Tetra chloro diphenyl ethane or DDD (Dichlorodiphenyl dichloro ethane)

Table 2: Composition of essential oil from leaves of *Aegle marmelos* (L.) Correa (AM)

Retention (min)	Area (%)[a]	Compound name	CAS number
4.918	0.04	Naphthalene	91-20-3
5.31	0.22	2,6-Dimethyl-3,5,7-octatriene-2-ol	999071-68
5.79	0.23	Trans-Carveol	1197-07-5
6.535	0.33	2-Cyclohexen-1-one	99-49-0
8.883	0.05	Limonene epoxide	4680-24-4
10.575	0.05	p-Vinylguaiacol	7786-61
11.624	0.09	Alpha-A-Cubebene	17699-14-8
13.505	2.36	Copaene	3856-25-5
15.159	8.73	Beta elemene	515-13-9
16.245	7.67	Caryophyllene	87-44-5
16.675	2.02	Bicyclo(3.1.1)hept 2 ene	17699-05-7
16.826	2.5	beta-sesquiphellandrene	20307-83-9
16.978	1.88	Alpha-Humulene	6753-98-6
17.142	4.25	Beta Farnesene	502-60-3
17.672	1.56	Alpha-Cedrene	469-61-4
17.874	2.67	2-Epi-Alpha-Cedrene	35944-22
17.88	1.49	Delta-Cadinene	483-76-1
18.102	0.88	2,6-bis (1,1-dimethylethyl)-4-methyl	128-37
18.24	4.53	Beta Bisabolene	495-61-4
18.442	1.72	Nerolidol	7212-44-4
18.809	1.82	Cis-Z-alpha-Bisabolene epoxide	999235-76-2
18.682	1.23	3-Bromohomoadamantane	14504-84-8
18.809	1.82	3,5 Cyclooctadien-1-one	96743-76-9
19.011	2.57	Alpha-Chamigrene	19912-83-5
19.15	2.25	Beta-Maaliene	489-29-2
19.238	1.98	Bergamotene	55123-21-2
19.364	1.99	Alpha.H-cis-Eudesma-6-ene-12-al	999235-93-6
19.554	4.01	Geranylgeraniol	24034-73-9
19.743	1.31	(S)-(+)-Xanthorrhizol	999229-60-2
19.844	2.22	Myristinic acid	544-63-8
19.933	3.11	Fumaric acid	999688-01-6
20.147	2.1	Hexahydrofarnesyl acetone	502-69-2
20.362	0.8	4,7 Dimethylpentacyclododecane-4,7-diol	999234-93-8
20.551	0.74	Palmitic acid	112-39

Contd...

Table 2: Contd...

Retention (min)	Area (%)[a]	Compound name	CAS number
20.614	0.3	7,9-di-tert-butyl-1-oxaspirodeca-6,9-diene-2,8-dione	82304-66-3
20.678	0.34	trans-Z-alpha-Bisabolene epoxide	999235-76-1
20.917	4.87	Hexadecanoic acid	57-10-3
21.22	0.228	(E, E)-Farnesol	106-28-5
21.296	0.31	Corymbolone	97094-19-4
21.435	0.11	2 Methyl-Z, Z-3,13-Octadecadienol	999404-90-2
21.524	0.29	5,9-Diamino-2,4-dimethyl-7H-pyrazolo naphthyridine	999256-99-1
21.625	0.26	n-Heneicosane	629-94-7
21.688	0.2	(+)-trans alpha-himachalene	999192-35-7
21.814	1.89	2-Hexadecen-1-ol	150-86-7
22.067	2.13	Linoleic acid	60-33-3
22.231	0.55	Stearic acid	57-11-4
22.445	0.33	n-docosane	629-97
22.597	0.18	Cis-12-Octadecadienoic acid	60-33-3
22.698	0.25	11,13-Dimethyl-12-tetradecen-1-ol acetate	999410-16-2
22.976	0.59	Alpha-Terthienyl	1081-34-1
23.228	0.23	Majorenolide	999404-17-6
23.544	0.61	Phytan	638-36-8
24.011	0.33	Linoleic acid	60-33-3
24.34	0.47	Thianthrene	92-85-3
24.933	0.21	n-Tetracosane	646-31-1
26.373	0.03	13-Tetradece-11-yn-1-ol	999203-20-2
26.461	0.04	6-Methylindole	03420-2-8
26.827	0.22	n Pentacosane	629-99-2
28.14	0.03	1,2-Benzenedicarboxylic acid	4376-20-9
29.327	0.04	n-Eicosane	112-95-8
31.121	0.04	3-Methyl-1,3 thiazole-2-thione	999218-30-2
32.825	0.45	Heptacosane	593-49-7

[a]Percentage are the means of three runs and were obtained from electronic integration measurements

Table 3: Composition of essential oil from leaves of *Psidium guava* Linn (PG)

Retention (min)	Area (%)[a]	Compound name	CAS number
13.227	0.54	Alpha-Copaene	3856-25-5
16.852	23.84	Beta-Caryophyllene	87-44-5
17.256	4.71	Alpha-Humlene	6753-98-6
17.319	1	(-) Alloaromadendrene	25246-27-9
17.533	3.84	Alpha-Amorphene	23515-88-0
17.887	4.07	Cis-alpha-Bisabolene	17627-44-0
18.19	9.03	Delta-Cadinene	483-76-1
18.683	10.34	Nerolidol	7212-44-4
18.834	3.47	(-)-Caryophyllene oxide	01139-30-6
18.986	3.4	1,4 Methano-1H Indene	087064-18-4
19.124	3.39	Naphthalene	87064-18-4
19.225	7.76	Torreyol	19435-97-3
19.566	0.64	2,6,10-Dodecatrien-1-ol	04602-84-0
19.693	0.17	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo (2,4) octane	999229-88-3
19.832	0.52	3-phenyl-1,3 Dihydro 2-Benzofuran 1 ol	999213-90-1
20.046	0.23	1-Naphthalenol	055012-72-1
20.16	0.56	Hexahydrofarnesyl acetone	0502-69-2
20.324	0.12	1,2-Benzenedicaroxylic acid	84-69-5
20.387	0.17	n-Nonadecane	629-92-5
20.564	0.55	Palmatic acid	112-39-0
20.69	0.09	1-Hexadecen-3-ol	505-32-8
20.854	1.13	Hexadecanoic acid	57-10-3
21.221	0.05	gamma-cis-sesquicyclogeraniol	108287-11-2
21.309	0.06	(-) Pinane-3-carboxylic acid	64284-84-0
21.486	0.07	n-Nonadecane	629-92-5
21.65	0.72	9,12 Octadecadienoic acid (z, z)-methyl ester	112-63-0
21.789	0.35	Phytol	150-86-7
22.029	0.55	Linoleic acid	60-33-3
22.231	0.2	Ethyl linolenate	1191-41-9
22.395	0.07	Linoleic acid	60-33-3

Contd...

Table 3: Contd...

Retention (min)	Area (%)[a]	Compound name	CAS number
22.509	0.07	Farnesol	999281-50-3
22.685	0.05	Neophadiene	504-96-1
22.887	0.02	Geranyl methyl ether	2565-82-4
22.976	0.03	Geranyl linalool isomer	999241-79-2
23.052	0.04	Farnesol isomer a	288246-53-7
23.216	0.02	cis-11,14,17 Eicosatrienoic acid	999509-74-3
23.569	0.06	14-methyl-8-hexadecyn-1-ol	64566-18-3
23.973	0.01	2-Propenoic acid	5466-77-3
24.138	0.01	Campherenone	18530-02-4
24.264	0.03	Diethyl 3 Cyclopentene-1,2-dicarboxylate	999212-25-9
24.845	0.01	Heptacosyl acetate	999704-75-8
26.461	0.01	14B-Pregnane	999427-18
26.815	0.03	n-pentacosane	629-99-2
28.014	0.37	n-noncosane	630-03-5
32.737	0.02	Eicosane	112-95-8
33.116	0.06	L ycopersen	502-62-5

[a]Percentage are the means of three runs and were obtained from electronic integration measurements

Table 4: Composition of essential oil from seed of *Nigella sativa* Linn (NS)

Retention (min)	Area (%)[a]	Compound name	CAS number
3.492	0.65	gamma-Terpinene	000099-85-4
4.628	0.64	3- Cyclohexen-1-ol	000562-74-3
8.202	0.08	Bornyl acetate	76-49-3
8.543	0.49	Thymol	89-83-8
9.06	4.52	Carvacrol	499-75-2
11.573	1.92	Alpha-Longipinene	1493692
13.429	5.06	Alpha-Copaene	3856-25-5
15.551	9.51	D Longifolene	475-20-7
16.283	17.57	Caryophyllene	87-44-5
16.422	0.1	Beta-Gurjunene	30021-74-0
16.612	0.08	Alpha Farnesene	026560-14-5
16.94	3.27	1,4,7,-Cycloundecatriene	999192-21-1
17.054	1.07	(-)-Alloaromadendrene	025246-27-9
17.344	1.21	Alpha-Amorphene	023515-88-0
17.458	0.67	Alpha-Selinene	473-13-2
17.71	1.78	Trans-Alpha Bisabolene	017627-44-0
17.786	1.39	Beta-Bisabolene	495-61-4
17.975	4.69	Delta-Cadinene	483-76-1
18.064	1.65	L Naphthalene	16728-99-7
18.165	0.51	Calacorene	999114-01-2
18.253	0.61	7-Dodecatriene	999095-39-9
18.417	7.26	Nerolidol	999242-28-8
18.594	3.1	Caryophylene oxide	1139-30-6
18.859	0.61	Azobenzene	103-33-3
19.036	5.48	Alpha-Cubebene	17699-14-8
19.225	1.2	Caryophyllenol-II	32214-89-4
19.301	1.71	(+)-Alpha-Bisabolol	23178-88-3
19.516	0.3	trans-Caryophyllene	87-44-5
19.604	0.34	12-Nor-Vittatalactone	999292-92-1
19.731	0.31	Cyclododecene	1501-82-2
19.832	0.94	Pentafluoropropionic acid	4-4-6222
20.021	0.2	1,4-trans-6-hydroxyisocalamenene	999230-04-0
20.122	0.41	Hexahydrofarnesyl acetone	502-69-2
20.299	0.46	1-(1-Cyanocyclohexyl) piperidine	3867-15-0
20.539	0.46	hexadecanoic acid	112-39-0
20.602	0.2	7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	82304-66-3
20.905	2.51	(2s, 3s/R)-2-Methyl-3 vinyl-3,6-dihydro-2H-pyran-3-ol	0000-00-0
21.006	0.22	1R-Alpha-Pinene	7785-70-8
21.094	0.43	Farnesol (E)	999281-50-3
21.284	0.29	3-methyl cyclohexanol	591-23-1
21.637	0.54	Linoleic acid	112-63-0
21.764	4.25	Cyclohexene, 3methyl-6-(1-methylethyl)-trans	1124-26-1
22.016	1.92	9,12-Octadecadienoic acid (z, z)	60-33-3

Contd...

Table 4: Contd...

Retention (min)	Area (%)[a]	Compound name	CAS number
22.206	0.25	z, z-8,10-Hexadecadien-1-ol	999287-67-1
22.281	0.38	Eicosane	112-95-8
22.4333	0.24	Pentadecane	1921-70-6
22.685	0.16	Farnesol Isomer A	999241-79-2
22.913	0.15	2-Dodecen-1-yl(-) succinic anhydride	19780-11-1
22.988	0.15	2,6,10,14,18,22, Tetracosahexaene	111-02-4
23.38	0.12	Alpha-selinene	473-13-2
23.582	0.12	9,12-Octadecadienoic acid (z, z)	60-33-3
24.213	0.02	n-Docosane	629-97-0
24.693	0.02	14-Beta-H-Pregna	999427-17-9
24.845	0.07	7-beta-hydroxydihydrodrimenin Eicosane	065882-99-7
24.933	0.06	n-Heneicosane	629-94-7
26.461	0.05	Beta Methyl indole	83-34-1
26.827	0.05	Quinoline	17299-24-0
31.777	0.58	Heneicosane	629-94-7

[a]Percentage are the means of three runs and were obtained from electronic integration measurements

sativa Linn. were procured from the local market. Identification and authentication of the plant material were carried out by Principle Scientist, Resources (Indian Council of Agricultural Research), New Delhi, India, and vouchers were provided (NHCP/NBPGR/2014-5-7).

Extraction of plant material

The leaves of *Aegle marmelos* (L.) Correa. and *Psidium guajava* Linn. and seed of Nigella sativa Linn. were weighed (100 g), grounded (28 mesh size), and micronized separately. In Clevenger apparatus, each plant material was separately soaked in distilled water (1 L) at 90°C for 6 h. Using hydrodistillation, the volatile components were collected by the addition of equal volumes of n-hexane, dried over anhydrous sodium sulfate, stored in the amber colored bottle (−20°C) and clearly labeled as AM, PG, and NS, respectively. The extract yield was calculated as 0.50%, 0.39%, and 0.41% (w/v) for AM, PG, and NS, respectively.

Analysis for pesticide residue, heavy metal, and microbial content

The dried leaves and seeds were tested for residues of pesticide [2,2-bis (p-chlorophenyl)-1,1,1trichloroethane (DDT); 1,2,3,4,5,6-hexachloro cyclohexane(HCH), a mixture of isomers; Endosulfan (α, β and sulphate isomers); Malathion and Parathion], aflatoxin (B1, B2, G1, G2), metallic elements (arsenic, lead, mercury, cadmium, iron, zinc, potassium, calcium) using liquid chromatography-mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS). The plant material was also analyzed for the total aerobic microbial count, enterobacteria, fungal count, in accordance with British Pharmacopeia.[7]

Gas chromatography-mass spectrometric analysis and identification of volatile constituents

Gas chromatography-mass spectrometric analysis was carried out after extraction using hexane and filtered through 0.25 μM PTFE filter. A volume of 2 μL was injected at a split ratio of 1:5 into Agilent 7890 GC System, USA equipped with a CT-PAL autosampler attached to silica column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with an electron ion spray mass detector (m/z 50–700 at 0.5 s/scan). The interface was set to 285°C and ion source was adjusted to 200°C with carrier gas (Helium @ 2 mL/min). After a 3 min solvent delay time at 70°C, the oven temperature was raised to 5°C/min–310°C. The volatile constituents were identified by matching the retention index and fragmentation pattern data with those of the standards using WILEY and NIST.[8]

In vitro antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl Assay

The antioxidant activity of volatile constituents was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[9] In brief, to different concentrations of the sample (1–50 μl/ml), DPPH· solution (2 ml, 0.1 mM) and Trolox (20–1000 μM) were added. The mixture was vortexed and kept undisturbed at room temperature for 30 min. The absorbance of the mixture was read at 517 nm against the blank using UV-Vis spectrophotometer (UV-1700 double beam, Shimadzu). The activity was calculated using the equation-DPPH scavenging effect (%) = [(A0–A1)/A0 × 100] where, A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of the sample or standard. The extract concentration versus DPPH scavenging effect (%) was calculated from the graph. The decrease in the absorbance indicates an increase in DPPH radical scavenging activity.

RESULTS AND DISCUSSION

The analysis of PG, AM, and NS did not detect presence of pesticide residues, microbial, and fungal contamination. The samples of AM, PG but not NS, tested positive for the presence of arsenic, lead, iron, zinc, potassium, and calcium. Cadmium was not detected in any of the samples [Table 1].

The chemical composition of the hydrodistilled volatile components of AM as analyzed using GC-MS showed the presence of 62 compounds [Figure 1a]. The major constituent was β-elemene (8.73%) [Table 2]. The other constituents were β-Farnesene (4.25%), β-caryophyllene (7.67%), myristinic acid, fumaric acid and hexadecanoic acid.

Forty-six compounds were identified in PG by GC-MS analysis [Figure 1b] of which the major were β caryophyllene (23.4%), δ-cadinene, nerolidol (10.34%), torreyol (7.71%), followed by α-copaene, α-humlene, α-amorphene, cis-α-bisabolene [Table 3].

The chromatogram of 58 compounds was obtained by GC-MS analysis of NS [Figure 1c]. The major compounds that could be identified were D-longifolene and β caryophyllene, followed by carvacrol (4.52%), α-cubebene (5.48%), n-heneicosane, farnesol isomer A, penta fluroproponicacid, and nor-vittalalactone [Table 4].

Interestingly, the three samples (AM, PG, and NS) also detected positive for L naphthalene.

The antioxidant activity of AM, PG, and NS was calculated to be 98.538, 98.955 and 97.755%, respectively [Figure 1d].

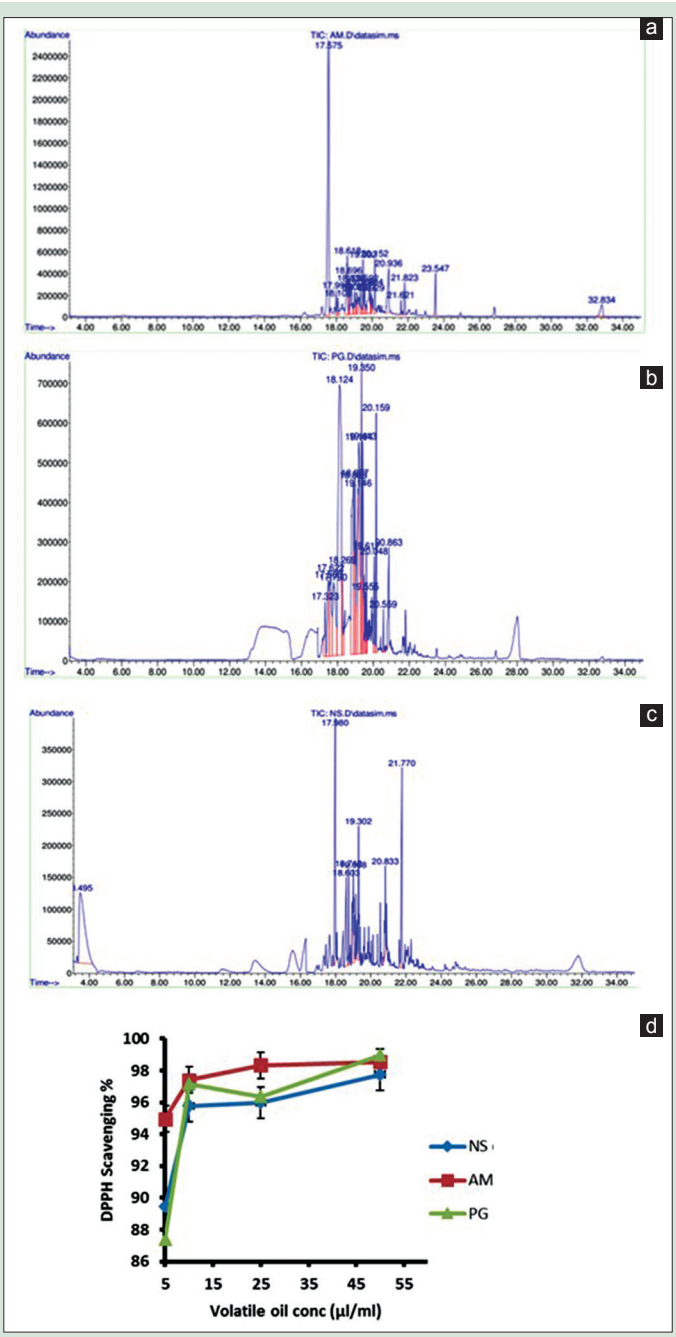


Figure 1: (a) Gas chromatography-mass spectrometric Spectra of volatile oil *Aegle marmelos* (L.) Correa. (b) Gas chromatography-mass spectrometric Spectra of volatile oil *Psidium guava* Linn. (c) Gas chromatography-mass spectrometric Spectra of volatile oil *Nigella sativa* Linn. (d) 2,2-diphenyl-1-picrylhydrazyl scavenging activity on volatile oils

There is a deep-seated faith of all humans worldwide, in plant-derived medicines as generally safe, effective, and cheap means of healthcare. Thus, quality control and standardization of herbal medicines are of utmost importance and modern-day analytical techniques such as GC-MS help to achieve the same. The leaves of *Aegle marmelos* and *Psidium guajava* and seeds of *Nigella sativa* enjoy well-established pharmacological activity that mainly stems from scavenging property.[10-12] In conclusion, the volatile oil components of *A. marmelos*, *P. guajava* and *N. sativa* were analysed by GC-MS, and correlated with their antioxidant activity. Of the three, AM demonstrated the highest antioxidant activity. The GC-MS profile of volatile oils can be used for herbal standardization of the three plants.

Financial support and sponsorship

The authors would express their appreciation to department of biotechnology (DBT No. BT/PR9068/MED/97/139/2013) and All India Council of Technical Education for financial support.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. The controller of publications, Ministry of AYUSH, Government of India. The ayurvedic pharmacopoeia of India. 2001;I (Ist ed):27-119.
2. Maity P, Hansda D, Bandyopadhyay U, Mishra DK. Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos* (L.) corr. Indian J Exp Biol 2009;47:849-61.
3. Mahaseth RK, Kumar S, Dutta S, Sehgal R, Rajora P, Mathur R, *et al.* Pharmacodynamic study of interaction of aqueous leaf extract of *Psidium guajava* linn. (Myrtaceae) with receptor systems using isolated tissue preparations. Indian J Pharm Sci 2015;77:493-9.
4. Burkil HM. The Useful Plants of West Tropical Africa.London,UK: Royal Gardens Kew; 1994. p. 21-150.
5. Gutiérrez RM, Mitchell S, Solis RV. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2008;117:1-27.
6. www.ich.org.Current Step 4version Dated 4 June, 2008. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q10/Step4/Q10_Guideline.pdf. [Last accessed on 2018 Jan 25].
7. The Stationery Office. British Pharmacopoeia. 2013;6:A475-5.
8. Khan W, Chester K, Anjum V, Ahmad W, Ahmad S, Narwaria A, *et al.* Chromatographic profiling of pancharishta at different stages of its development using HPTLC, HPLC, GC-MS and UPLC-MS. Phytochem Lett 2017;20:391-400.
9. Okawa M, Kinjo J, Nohara T, Ono M. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. Biol Pharm Bull 2001;24:1202-5.
10. Wang L, Wu Y, Huang T, Shi K, Wu Z. Chemical compositions, antioxidant and antimicrobial activities of essential oils of *Psidium guajava* L. Leaves from different geographic regions in China. Chem Biodivers 2017;14:e1700114.
11. Ramadan MF, Kroh LW, Mörsel JT. Radical scavenging activity of Black cumin (*Nigella sativa* L.), Coriander (*Coriandrum sativum* L.), and Niger (*Guizotia abyssinica* Cass.) Crude Seed Oils and Oil Fractions. J Agric Food Chem 2003;51:6961-9.
12. Baliga MS, Bhat HP, Joseph N, Farhan F. Phytochemistry and medicinal uses of the bael fruit (*Aegle marmelos* Correa): A concise review. Food Res Int 2011;44:1768-75.