HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY AND GC-MS PROFILE OF *Aegle marmelos* (L.) Correa AND *Tamarindus indica* (L.)

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1. Abstract:
Aegle marmelos L., belongs to Rutaceae family, is a sacred plant described in Hindu scriptures. It is both therapeutic and cultural significance. *Tamarindus indica* belongs to family Leguminosae The pulp is also used in traditional medicine, seeds are also reported to have many therapeutic value. Chromatographic fingerprinting of herbal drugs is a complete qualitative approach for species identification and its authentication, qualitative evaluation and assuring the stability of herbal drugs and their related products. In the present research work HPTLC finger printing and GC-MS profile of *Aegle marmelos* and *Tamarindus indica* were carried out. Butanol: Acetic acid: Water (2:1:7) was used as mobile phase for *Aegle marmelos* and Chloroform: Glacial acetic acid: Methanol: Water (6.4:3.4:1.2:0.8) was used for *Tamarindus indica*. The developed TLC plates shown many peaks at 254 nm, 366 nm and after derivatization, indicates the presence of variety of chemical compounds in the extract. GC-MS profile for *Aegle marmelos* leaf extract reported around 42 different chemical constituents, whereas *Tamarindus indica* seed extract showed around 55 chemical constituents.

Keywords: HPTLC, GC-MS, *Aegle marmelos* and *Tamarindus indica*.

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2. INTRODUCTION

Aegle marmelos Family-Rutaceae commonly called as 'Bael' in Hindi and "bilva"in Kannada is indigenous to India. Leaves, fruits, stem and roots of *Aegle marmelos* have been used in ethnomedicine for several medicinal properties antidiabetic, antimicrobial, anti-inflammatory, antipyretic, analgesic, cardio protective, aphrodisiac, anticancer, radio protective, antioxidant, antidiarrheal, antidysenteric, haemostatic and as an antidote to snake venom. Phytochemical screening of *Aegle marmelos* (AM) leaf extracts revealed the presence of alkaloids, saponins, flavonoids, phenolic compounds, phytosterols, skimmianine (tannin; trace amount), aeglin, rutin, Y-sitosterol, β-sitosterol, flavone, lupeol, cineol, citral, glycoside, O-isopentenyl, halfordiol, marmeline, mitronellal, cuminaldehyde phenylethyl cinnamamides, eugenol, marmesinin [1, 2]. AM crude leaf extract has been reported to regenerate damaged pancreatic beta cells in diabetic rats and increased the activities of peroxidase in the liver tissues of isoproterenol treated rats. Leaf extract of AM was found to be a potential antioxidant drug, which reduces the blood sugar level. It was found to be as effective as insulin in the restoration of normal blood glucose level in diabetic rats [3-6]. It has been reported that both aqueous and alcohol extract of Aegle marmelos leaves have got antihyperglycemic and hypolipidemic effect [7-9].

Tamarindus indica (family-Leguminosae) commonly called 'Imli'in Hindi and "Hunase Hannu/Huliis" in Kannada is found almost all over India. The tree carries brittle, ligneous pods about the size of a human digit, containing up to 10 shiny seeds surrounded by a sticky, sour pulp that is used in food and drinks. It is rich in phytochemicals, and hence the plant is reported to possess antidiabetic, antimicrobial, antivenomic, antioxidant, antimalarial, hepatoprotective, antiasthmatic, laxative, and hypolipidemic activity [10,11]. Phytochemical analysis of methanolic extract of *Tamarindus indica* seed extract revealed high concentrations of rutin, quercetin, Gallic acid, epicatechin, taxifolin, apigenin, luteolin flavonoid, tannins, carbohydrate, phenols, termendiol and steroids [12]. The aqueous extract of *Tamarindus indica* seeds has shown antihyperglycemic and hypolipidemic effect in streptozotocin induced diabetic rats [13,14].

Scientific classification of *Aegle marmelos* and *Tamarindus indica* was given in Table 1.

***Aegle marmelos* L.**	***Tamarindus indica L.***
Kingdome – Plantae	Kingdom: Plantae
Order – Spindale	Order: Fabales
Family – Rutaceae	Family: Leguminosae
Subfamily: Aurantioideae	Subfamily: Caesalpiniaceae
Genus – Aegle L.	Genus: Tamarindus L
Species: *Aegle marmelos*	Species; *Tamarindus indica*
Botanical Name – *Aegle marmelos L*	Botanical Name: *Tamarindus indica L.*

Standardization of herbal drugs using high- performance thin- layer chromatography (HPTLC) is one of the best analytical methods to standardize most of the herbal drugs, which gives an information about the active constituent in that drug. [15,16]

3. MATERIAL AND METHODS

3.1. Authentication and Herbarium

Mature leaves of *Aegle marmelos* (L.) and seeds of *Tamarindus indica* were collected from Udupi district of Karnataka. The identification of *Aegle marmelos* (L.) (family-Rutaceae) and *Tamarindus indica* (family Caesalpiniacae) was authenticated by Dr. K Gopalkrishnan Bhat professor of Botany (Rtd.). Duly authenticated herbarium was kept in the Department of Pharmacognosy at Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education. PP622A Specimen number is given for *Aegle marmelos* and PP617A is given for *Tamarindus indica* respectively.

3.2. Extraction Process

Aegle marmelos leaves: The leaves were cleaned with water and shade dried and made into powder. 100 g powdered extract of *Aegle marmelos* leaves was taken and extracted with 50% ethanol & 50 % distilled water by maceration technique. Coarse powder of *Aegle marmelos* leaves (100 g) was subjected to maceration for 7 days at room temperature using 500 ml ethanol and 500 ml of distilled water. The extracts obtained were filtered separately using Whatman No.1 and the solvent was subjected to rotary vacuum evaporator under reduced pressure and temperature and further dried using water bath paste residue of *Aegle marmelos* leaves were obtained. The hydro extract was then stored in a desiccator until further use. *Tamarindus indica*: Seeds were cleaned with water and shade dried and made into powder. 100 g powdered extract of *Tamarindus indica* seeds was taken and extracted

3.5. Pre-wash and activation of pre- coated

with 50% ethanol & 50 % distilled water by maceration technique. Coarse powder of *Tamarindus indica* seeds (100 g) was subjected to maceration for 7 days at room temperature using 500 ml ethanol and 500 ml distilled water. The extracts obtained were filtered separately using Whatman No.1 and the solvent was subjected to rotary vacuum evaporator under reduced pressure and temperature and further dried using water bath powder residue of *Tamarindus indica* seeds were obtained. The hydro alcoholic extract was then stored in a desiccator until further use.

3.3. HPTLC finger printing analysis

Fingerprint analysis approach using High-Performance Thin-Layer Chromatography (HPTLC) has become the most important and potent tool for testing the quality of herbal medicines, because of its simplicity and reliability. It can serve as a tool for identification, authentication, and quality control of herbal drugs. HPTLC give information about the existence of chemical constituents in the extract. Each separated band on the chromatographic plate is the indication of individual chemical constituents present in the extract and has specific Rf value for the selected mobile phase. Even it is possible to quantify the most important chemical constituents present with the help of pure isolated marker compounds using HPTLC densitometric scanner and confirm the presence by Rf value.

The HPTLC works on the principle of separation is adsorption. The mobile phase or solvent flows through the capillary action. The analytes move according to their affinities towards the stationary phase (adsorbent). The higher affinity component travels slower towards the stationary phase. A low-affinity component travels rapidly toward the stationary phase. On a chromatographic plate, then, the components are separated. HPTLC is the commonly used fingerprint method for herbal extract/fraction with the following steps;

3.4. Pre-wash and activation of pre- coated plates

Percolated plates with sorbent thickness of 100-250 µm were pre-washed by dipping method using solvent of Butanol: Acetic acid: Water (2:1:7) for *Aegle marmelos* (AM) and for *Tamarindus indicus* Chloroform: Glacial acetic acid: Methanol: Water (6.4:3.4:1.2:0.8). To activate, plates were placed in an oven at 110 ° C - 120 ° C for 30 minutes before the sample application.

Activation at higher temperature for a longer period was avoided as it may lead to very active layers and risk of the samples being decomposed.

Figure 6. Shows HPTLC chromatogram of methanol extract of *Tamarindus indica*. Figure 7.

plates

Percolated plates with sorbent thickness of 100-250 µm were pre-washed by dipping method using solvent of Butanol: Acetic acid: Water (2:1:7) for *Aegle marmelos* (AM) and for *Tamarindus indicus* Chloroform: Glacial acetic acid: Methanol: Water (6.4:3.4:1.2:0.8). To activate, plates were placed in an oven at 110 ° C - 120 ° C for 30 minutes before the sample application. Activation at higher temperature for a longer period was avoided as it may lead to very active layers and risk of the samples being decomposed.

3.6. Sample Preparation and its application

Aegle marmelos (AM) and *Tamarindus indicus* (TI) sample were prepared at a concentration of 5 mg/mL concentration in methanol. HPTLC Silica gel 60 F254 plate with a dimension of 10 x 10 cm served as the stationary phase. Mobile phase for *Aegle marmelos* was prepared by saturating the 10 x 10 Twin trough chamber with 10 mL of Butanol: Acetic acid: Water (2:1:7) for twenty minutes. While. mobile phase for *Tamarindus indicus* was prepared by saturating the 10 x 10 Twin trough chamber with 10 mL of Chloroform: Glacial acetic acid: Methanol: Water (6.4:3.4:1.2:0.8) for twenty minutes. Sample solutions were loaded onto the sample applicator with a 100 µL HPTLC syringe. After application, the plate was air dried and

3.7. Spotting, Development and photo documentation

Plates were spotted using the Camag Linomat 5 sample applicator, 2 µL, 4 µL, 6 µL and 8 µL per sample was sprayed in the form of bands (band length of 6 mm) onto the HPTLC plate. Plates were air - dried and placed in the developing chambers, which contained smaller volumes (10 -15 ml) of the mobile phase. After the development, the plates were removed from the chamber and air -dried.

For visualization of spot, the air- dried plates were sprayed with various reagents. Using Camag TLC Scanner 3, the HPTLC plate was scanned densitometrically under a scanning speed of 20 mm/sec at 254, 366 nm and at 540 nm after spraying with Anisaldehyde (ANS) reagent.

4. RESULTS AND DISCUSSION

4.1. HPTLC finger print of *Aegle marmelos*

There were 8 peaks observed at 254 nm, 9 peaks observed at 366 nm and 7 spots observed when plate is sprayed with Anisaldehyde - sulfuric acid. Figure 1. shows the HPTLC chromatogram of methanolic extract of *Aegle marmelos*. Whereas Figure 2. shows 3D overlay of chromatogram at 254 nm, 366 nm and white light after derivatization. Figure 3, 4 and 5 shows the densitometric scan of *Aegle marmelos* at 254, 366 and at white light respectively.

4.3. GC-MS analysis of hydroalcoholic extracts

Shows 3D overlay chromatogram of *Tamarindus indica* at 254 nm, 366 nm and white light after derivatization. Figure 8, 9 and 10 the densitometric scan of *Tamarindus indica* at 254, 366 and at white light respectively.

4.2. HPTLC finger print of *Tamarindus indica*

There were 9 peaks observed at 254 nm, 7 peaks observed at 366 nm and 10 spots observed when plate is sprayed with Anisaldehyde - sulfuric acid.

of *Aegle marmelos* leaves:

Gas chromatography-mass spectroscopy (GC-MS) analysis of the extract. GCMS was carried out using Shimadzu GCMS-QP2010S instrument. The study was carried out at Analytical Research & Metallurgical Laboratories Pvt. Ltd. (ARML), Bengaluru, India by following process:

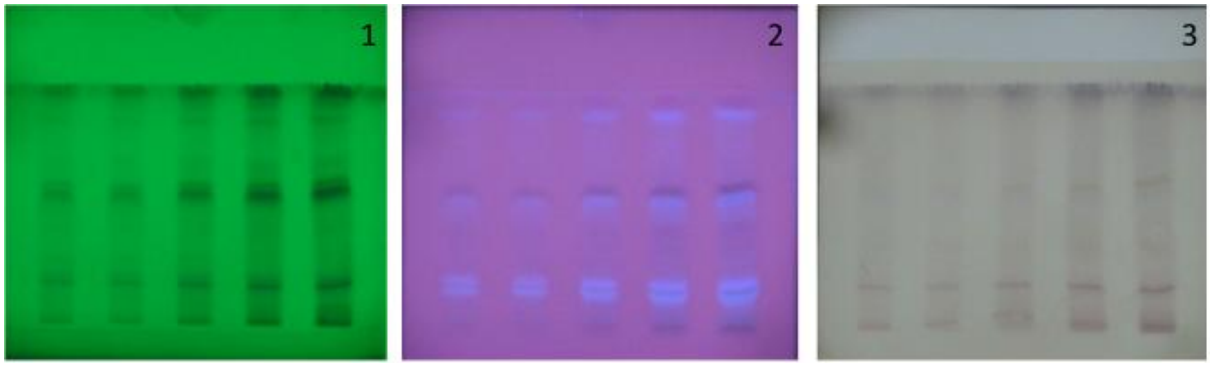

Figure 1: HPTLC chromatogram of *Aegle marmelos* methanol extract at 254 nm (1), 366 nm (2) and white light after derivatization (3)

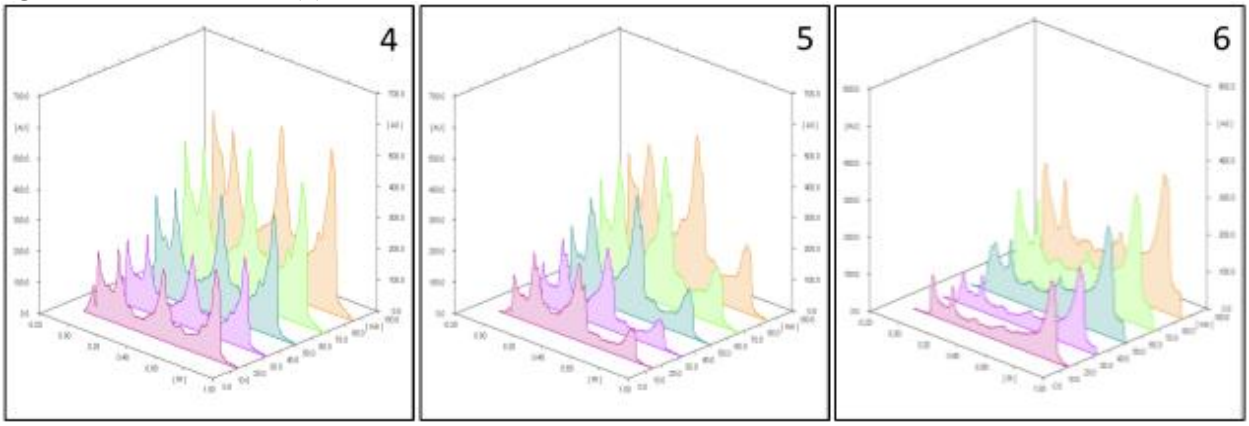

Figure 2: 3D overlay of chromatogram of *Aegle marmelos* at 254 nm (4), 366 nm (5) and white light after derivatization (6)

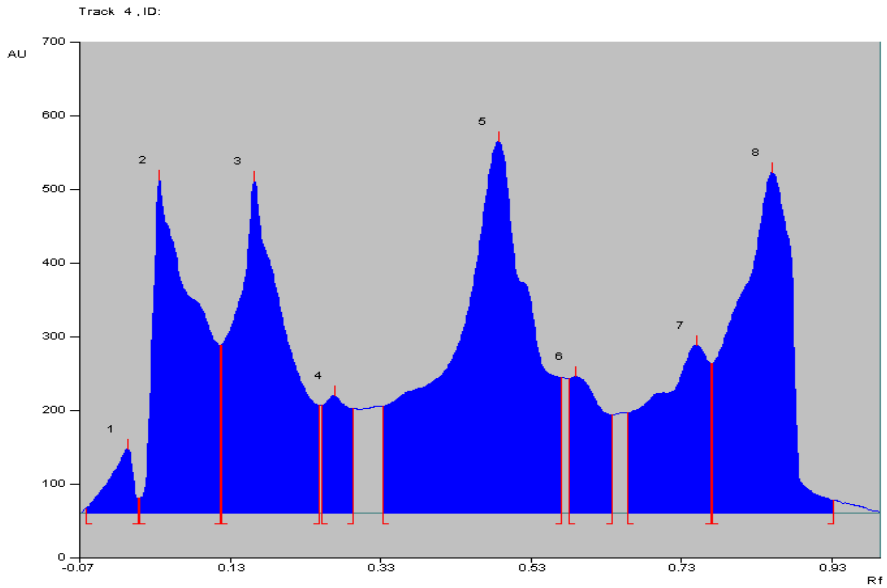

Figure 3: Densitometric scan of *Aegle marmelos* [track 4 (6 µL)] at 254 nm

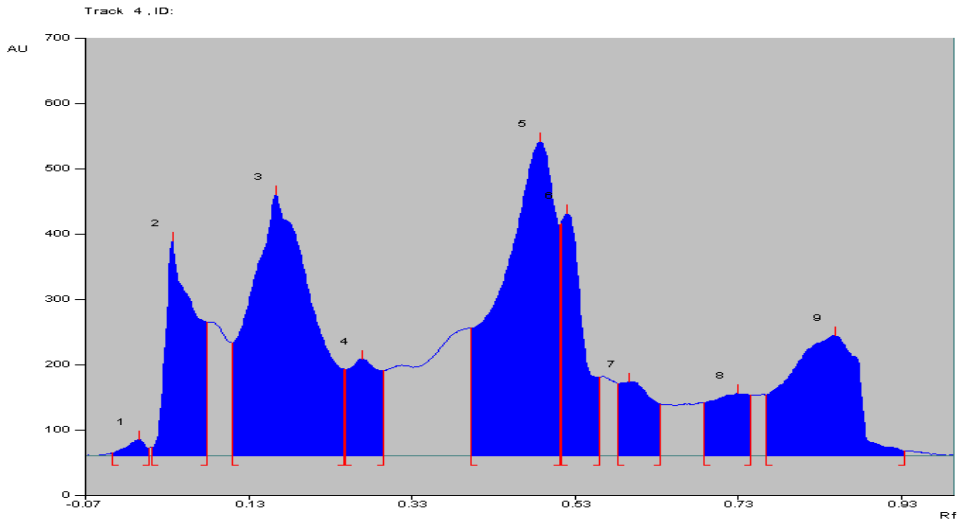

Figure 4: Densitometric scan of *Aegle marmelos* [track 4 (6 µL)] at 366 nm

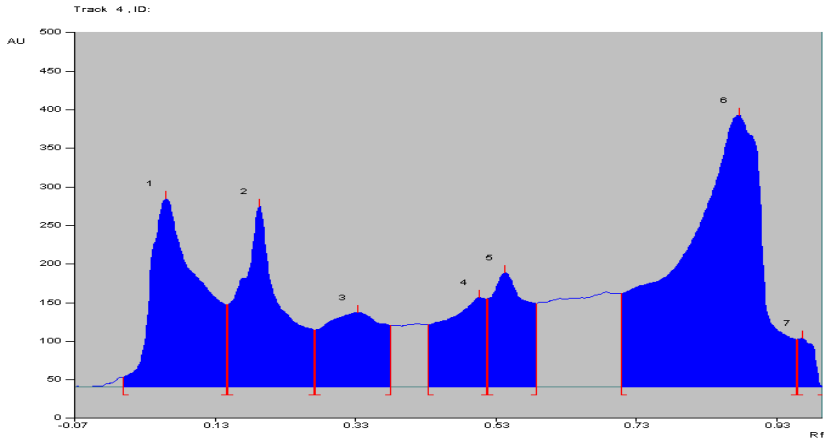

Figure 5: Densitometric scan of *Aegle marmelos* [track 4 (6 µL)] in white light

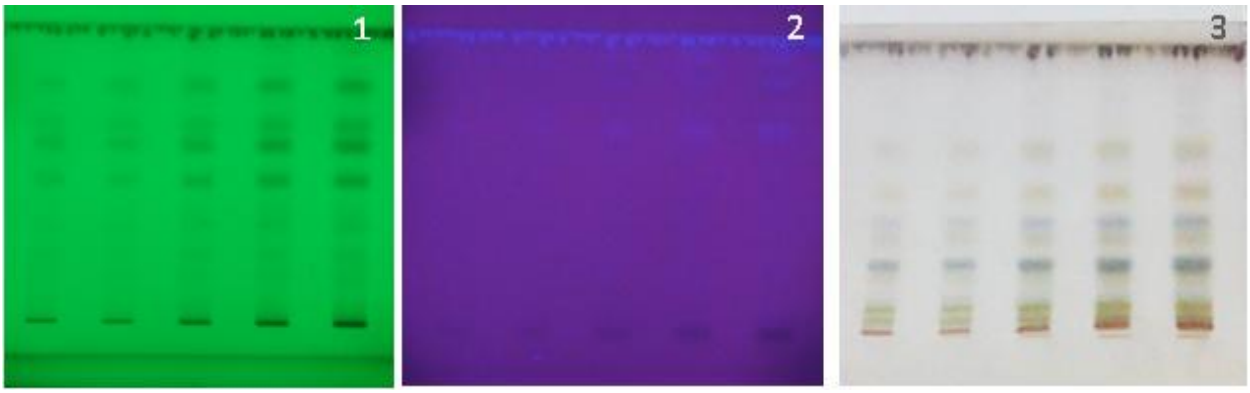

Figure 6: HPTLC Chromatogram of *Tamarindus indica* methanol extract at 254 nm (1), 366 nm (2) and white light after derivatization (3)

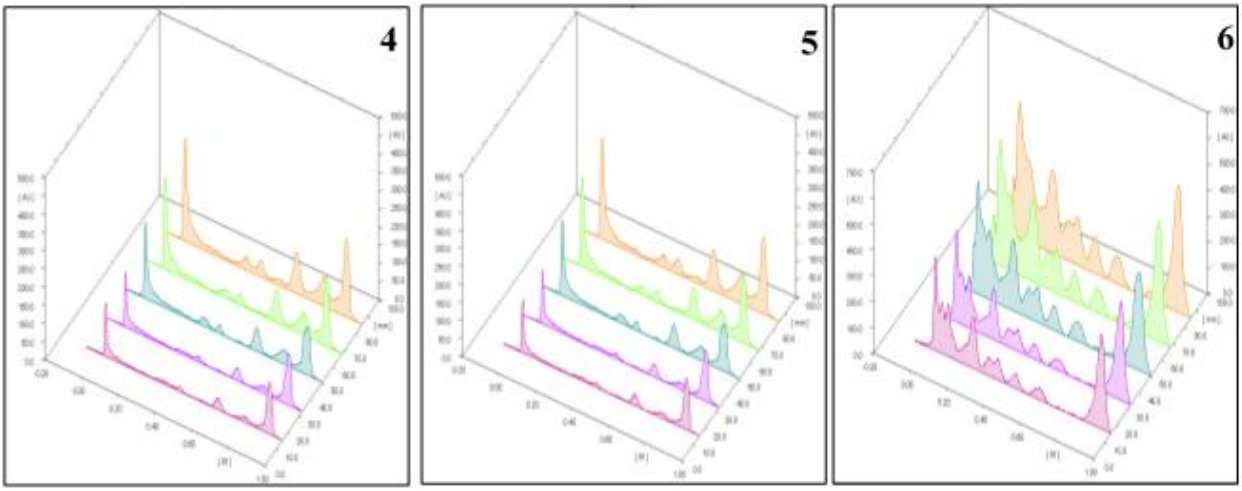

Figure 7: 3D overlay chromatogram of *Tamarindus indica* at 254 nm (4), 366 nm (5) and white light after derivatization (6)

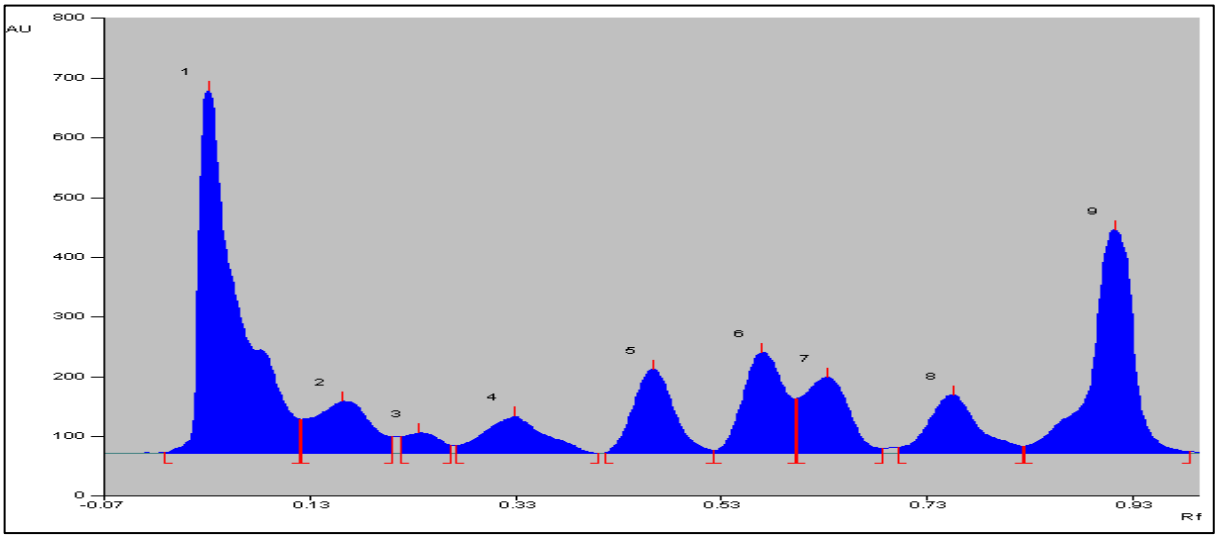

Figure 8: Densitometric scan of *Tamarindus indica* extract [track 4 (6 µL)] at 254 nm

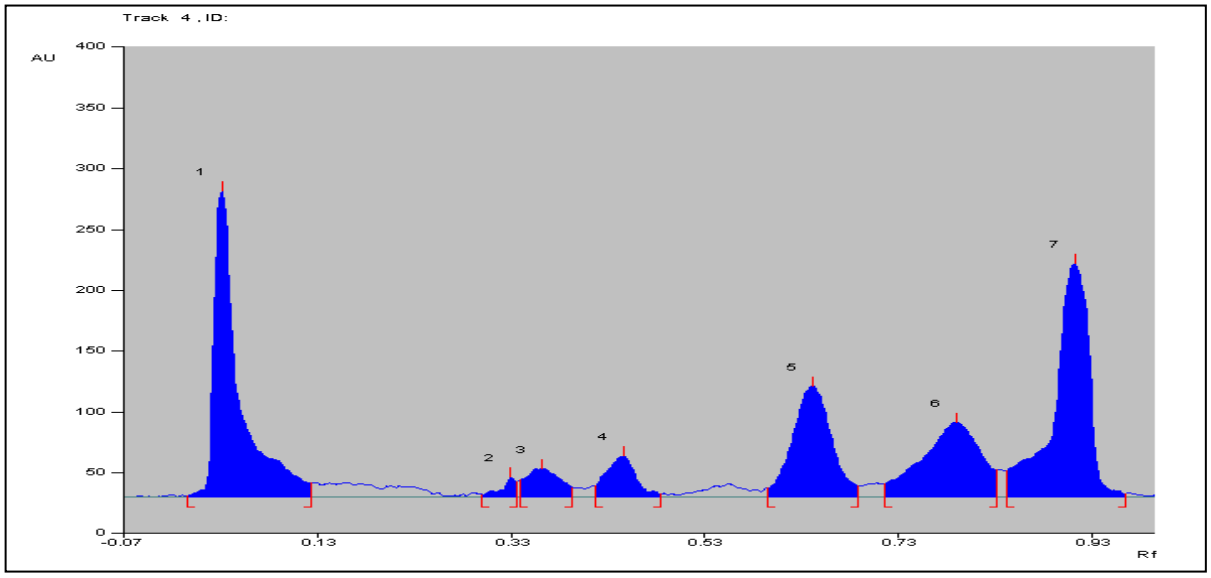

Figure 9: Densitometric scan of *Tamarindus indica* extract [track 4 (6 µL)] at 366 nm

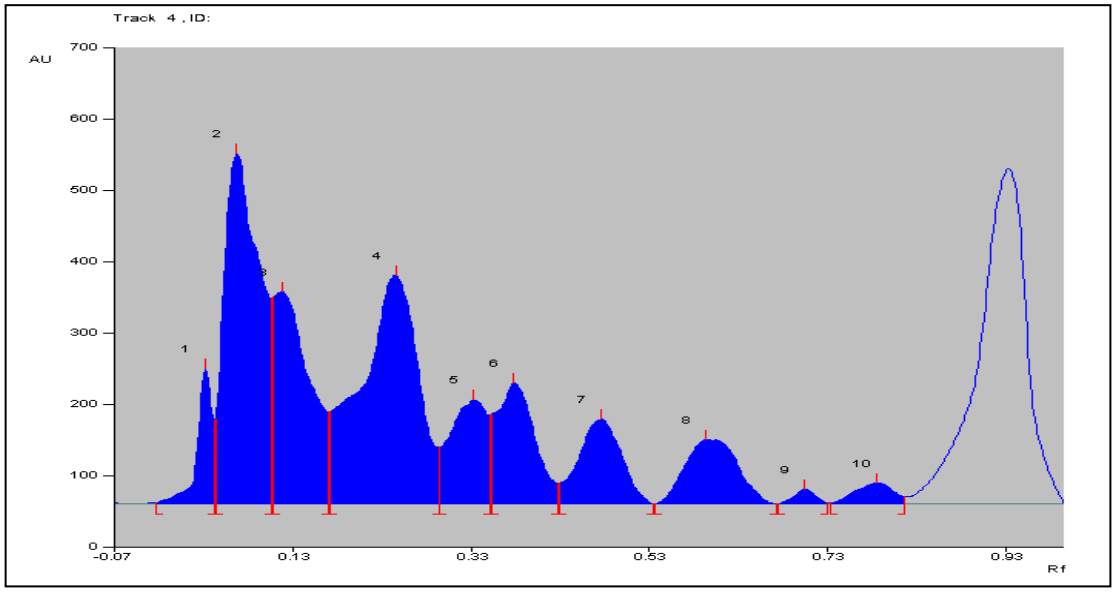

Figure 10: Densitometric scan of *Tamarindus indica* extract [track 4 (6 µL)] in white light after derivatization

GCMS Column Used: RTX5
Length: 30m
Film Thickness: 0.25 micron
GCMS Condition: Column Oven Initial 60 ^{0} C,
Injection mode: Split (The Sample is dissolved in solvent (methanol), filtered and injected to GCMS)
Split Ratio: 4.0

Oven Temp Ramp:

Rate (ml/min)	Temperature (^{0} C)	Hold Time (Min)
-	60	2.00
5.0	150	0.00
10	280	0.00

Ion Source: 200 ^{0} C
Interface temp: 280 ^{0} C
MS 40 - 500m/z.

Table 2. GC-MS analysis of Hydro alcoholic extract of *Aegle marmelos* leaves

Peak	**Retention Time (R.Time)**	**Area%**	**Name**	**Molecular formula**	**Molecular Weight (g/mole)**
1	3.214	1.51	N-Methylglycine	$C_3H_7NO_2$	89.09
2	4.634	11.47	Glycerin	$C_3H_8O_3$	92.09
3	6.373	9.37	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130.18
4	7.783	0.85	Nonane, 3-methyl-5-propyl-	$C_{13}H_{28}$	184.36
5	8.1	0.66	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15
6	9.157	1.55	Nonane, 3-methyl-5-propyl-	$C_{13}H_{28}$	184.36
7	9.258	0.34	6-Methyl-cyclodec-5-enol	$C_{11}H_{20}O$	168.28
8	9.5	1	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl	$C_{30}H_{50}O_2$	442.7
9	10.445	0.45	Arabino-heptitol, 2,3:5,6-dianhydro-1,7-dideoxy-2,6-di-C-methyl	$C_9H_{16}O_3$	172.22
10	10.888	0.92	2-Propenoic acid, 3-phenyl-	$C_9H_8O_2$	148.16
11	10.997	0.62	alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofura	$C_{18}H_{32}O_{16}$	504

12	11.274	0.46	2-Cyclohexen-1-one, 3-(hydroxymethyl)-6-(1-methylethyl)-	$C_{10}H_{16}O_2$	168.23
13	11.711	0.72	Octyl-.beta.-D-glucopyranoside	$C_{14}H_{28}O_6$	292.37
14	12.418	1.42	trans-1-Cinnamoylimidazole	$C_{12}H_{10}N_{2O}$	198.22
15	13.157	7.39	1,2,3,5-Cyclohexanetetrol, (1.alpha.,2.beta.,3.alpha.,5.beta.)-	$C_6H_{12}O_4$	148.16
16	13.307	1.24	beta.-D-Glucopyranoside, methyl	$C_{32}H_{34}O_{14}$	642.6
17	13.424	5.56	Ethyl .alpha.-d-glucopyranoside	$C_8H_{16}O_6$	208.21
18	13.605	0.65	2-Diisopropylsilyloxymethyltetrahydrofurane	$C_{11}H_{23}O_2Si$	215.38
19	13.696	1.85	Ethyl .alpha.-d-glucopyranoside	$C_8H_{16}O_6$	208.21
20	13.817	0.53	4-Methylmannitol	$C_7H_{16}O_6$	196.2
21	14.708	0.56	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.2
22	15.132	1.13	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7.alpha.,9.alpha.)	$C_{15}H_{26}$	206.37
23	15393	1.73	Muco-Inositol	$C_6H_{12}O_6$	180.16
24	16.485	4.81	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
25	16.82	0.97	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.9
26	17.145	1.28	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5
27	18.21	7.23	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.5
28	18.314	0.34	Phytol	$C_{20}H_{40}O$	296.5
29	18.552	1.88	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234.38
30	18.814	2.43	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.5
31	19.631	0.34	cis-1-Chloro-9-octadecene	$C_{18}H_{35}Cl$	286.9
32	19.816	0.5	1,8-Diazacyclotetradecane-2,7-dione	$C_{12}H_{22}N_{2O}2$	226.32
33	20.488	0.41	Oxazole, 2-(3-methoxyphenyl)-5-phenyl-	$C_{16}H_{13}NO_2$	251.28
34	20.977	0.45	Anisindione	$C_{16}H_{12}O_3$	252.26
35	22.024	0.4	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.34
36	22.527	2.49	1-Phenyl-1-cyclopropanecarbonitrile	$C_{10}H_9N$	143.18
37	23.727	2.96	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.6
38	24.283	0.52	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180.24
39	25.285	19.22	trans-4-Ethoxy-.beta.-methyl-.beta.-nitrostyrene	$C_{11}H_{13}NO_3$	207.23
40	25.79	0.37	Acrylamide, N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)-3-phenyl-	$C_{17}H_{13}NO_3$	279.29
41	29.373	0.96	beta.-Sitosterol	$C_{29}H_{50}O$	414.7
42	30.125	0.45	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296.58

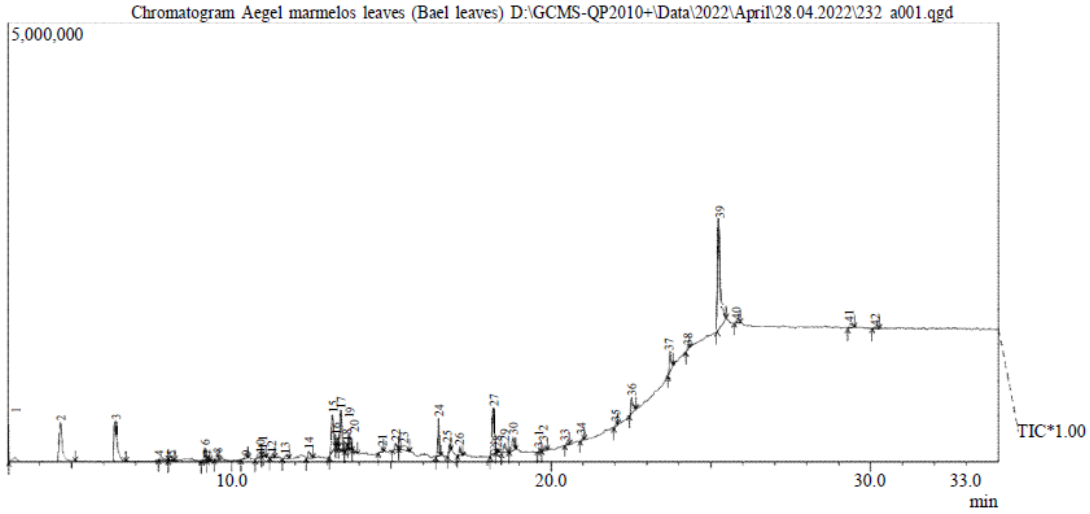

Figure 11. GCMS Total Ion Chromatogram of hydro alcoholic extracts of *Aegle marmelos*

4.4. GC-MS analysis of hydroalcoholic extracts of *Tamarindus indica* seeds:

Table 3. GCMS analysis of Hydroalcoholic extract of *Tamarindus indica* seeds

Peak	Retention Time (R.Time)	Area%	Name	Molecular formula	Molecular weight (g/mole)
1	3.215	0.13	2-Oxoacetic acid, ethyl ester, oxime	$C_4H_7NO_3$	117.10
2	4.071	0.13	Hexanoic acid, methyl ester	$C_7H_{14}O_2$	130.18
3	4.652	4.59	Hexanoic acid	$C_6H_{12}O_2$	116.16
4	4.746	5.53	Glycerin	$C_3H_8O_3$	92.09
5	5.948	0.1	Heptanoic acid	$C_7H_{14}O_2$	130.18
6	7.667	0.11	1-Methyl-pyrrolidine-2-carboxylic acid	$C_6H_{11}NO_2$	129.16
7	7.79	0.36	Dodecane, 2,6,10-trimethyldodecane	$C_{15}H_{32}$	212.41
8	8.512	0.1	Hexadecane	$C_{16}H_{34}$	226.44
9	9.164	0.67	Nonane, 3-methyl-5-propylnonane	$C_{13}H_{28}$	184.36
10	9.658	0.1	Methyl 8-oxooctanoate	$C_9H_{16}O_3$	172.22
11	9.888	0.43	3-Nonen-2-one	$C_9H_{16}O$	140.22
12	10.999	0.88	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186.25
13	12.056	0.31	2(1H)-Quinolinone	C_9H_7NO	145.16
14	12.384	0.61	Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	216.27
15	12.817	0.14	Butanedioic acid, ethyl-, dimethyl ester	$C_8H_{14}O_4$	174.19
16	13.417	0.89	Nonanedioic acid, monomethyl ester	$C_{10}H_{18}O_4$	202.25
17	13.66	0.28	.alpha.-D-Glucopyranoside, methyl , (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-	$C_7H_{14}O_6$	194.18

			methoxyoxane-3,4,5-triol		
18	14.237	2.06	alpha.-d-Mannofuranoside, methyl	$C_{24}H_{36}O_{14}S$	580.6
19	14.701	0.18	Dotriacontane	$C_{32}H_{66}$	450.9
20	14.701	0.12	Hexadecane, 1-iodohexadecane	$C_{24}H_{36}O_{14}S$	580.6
21	14.812	0.14	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)-	$C_{15}H_{22}O$	218.33
22	16.262	0.28	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.4
23	16.487	2.69	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
24	16.603	0.11	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.4
25	16.843	10.72	l-(+)-Ascorbic acid 2,6 dihexadecanoate	$C_{38}H_{68}O_8$	652.9
26	17.147	0.26	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5
27	18.189	5.68	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5
28	18.402	1.67	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.5
29	18.558	33.48	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.5
30	18.732	10.92	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5
31	18.998	0.09	Heptadecanoic acid, 15-methyl-, ethyl ester	$C_{20}H_{40}O_2$	312.5
32	19.55	0.66	2-Heptanone, 6-(3-acetyl-1-cyclopropen-1-yl)-3-hydroxy-6-methyl-, (R*,R*)-	$C_{13}H_{20}O_3$	224.30
33	19.779	1.04	1-Methylbutyl hexadecanoate	$C_{21}H_{42}O_2$	326.6
34	19.97	1.07	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5
35	20.157	1.24	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.6
36	20.293	0.45	18-Nonadecenoic acid	$C_{19}H_{36}O_2$	296.5
37	20.46	1.94	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.9
38	20.965	0.23	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	236.35
39	21.233	1.81	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.5
40	21.415	0.33	Octadecanoic acid, docosyl ester	$C_{40}H_{80}O_2$	593.1
41	21.692	0.33	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5

42	21.775	1.63	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.6
43	22.077	0.81	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.9
44	22.543	0.12	Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	368.6
45	22.934	0.37	8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252.4
46	23.094	0.5	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294.5
47	23.273	1.32	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.7
48	23.732	0.92	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.6
49	24.034	0.15	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	$C_{30}H_{50}$	410.7
50	24.522	0.15	Docosanoic acid, 8,9-dihydroxy-, methyl ester	$C_{23}H_{46}O_4$	386.6
51	24.721	0.12	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	410.7
52	26.043	0.34	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	$C_{57}H_{104}O_6$	885.4
53	28.193	0.09	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	$C_{11}H_{32}O_4Si_4$	340.71
54	29.377	0.22	Cholest-5-en-3-ol (3.beta.)-$$ (-)-Cholesterol	$C_{37}H_{64}O_2$	540.9
55	30.936	0.45	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410.7

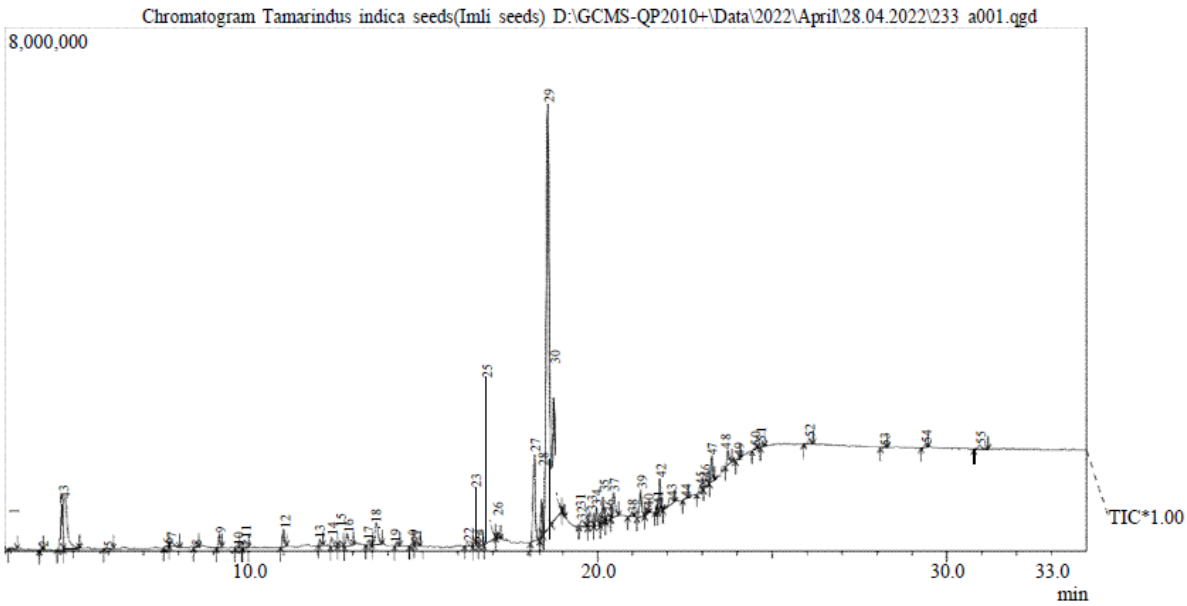

Figure 12. GCMS Total Ion Chromatogram of hydro alcoholic extracts of *Tamarindus indica* seeds

5. CONCLUSION

Herbal drugs comprise a plethora of chemicals in complex matrices in which no one active constituent is responsible for the total efficacy. This creates a challenge in establishing quality control standards for raw materials and standardization. Chromatographic fingerprinting of herbal drugs helps to understand the presence of active constituents in the extracts. The mobile phase for *Aegle marmelos* extract was Butanol: Acetic acid: Water (2:1:7) and Chloroform: Glacial acetic acid: Methanol: Water (6.4:3.4:1.2:0.8) was used for *Tamarindus indica*. The develoed chromatogram shown many peaks at 254 nm, 366 nm and after derivatization, indicating the presence of a wide range of chemical components in the extract. The GC-MS profile of *Aegle marmelos* leaf extract revealed around 42 chemical constituents, whereas *Tamarindus indica* seed extract revealed approximately 55 chemical constituents.

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