

Phytochemical Investigations of Methanol Leaf Extracts of *Randia spinosa* Using Column Chromatography, HPTLC and GC-MS

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Abstract

The present study was aimed to identify phytochemical presence in leaves of *Randia spinosa* using hexane, chloroform, ethanol, methanol and distilled water for extraction. Among these solvents, methanol have shown important phytochemicals in qualitative method. Further, column chromatography has been carried out for the partial purification of methanol extract by sequential elution with hexane, ethyl acetate and methanol as eluents. The methanol extract of HPTLC study shows the significant phytochemicals. GC-MS result of ethyl acetate extraction of leaves shown the presence of triterpenoid esters, cinnamate, naphthalene, phenolic compounds mainly BHT and methanol fraction has shown the presence of triterpenoid esters, amines and coumarins mainly, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-pyrano[4,3-b]benzopyran-1,9- dione. Future studies is needed to characterize each individual compounds and their role in biological activity.

Keywords: GC-MS; HPTLC; Phytochemicals; *Randia spinosa*

Introduction

Plants are the treasures of phytochemicals are useful to treat most of all the human diseases and ailments with proper guidance. Because of the lack of knowledge in identifying the exact plants and plant parts and their proper applications for particular ailments, the herbal medicines are still not yet fully exploited. Herbal medicines have been used for long years. The World Health Organization (WHO) reveals that, there are around 2,50,000 number of higher plant species on earth and among them around 35,000 to 70,000 species were used at one time or another for their medicinal properties [1].

The herbals of *Randia spinosa* (Rubiaceae family) are well known for their medicinal values of traditional and modern studies. The plants of *Randia* species viz., *Randia dumetorum* contains saponins, terpenoids, coumarins, iridoid glycoside [2], triterpenoid glycosides and saponins [3], hemolytic triterpenoid saponin [4] and used as an emetic in skin diseases and as an antibacterial [5], anti-inflammatory [6,7] and immunomodulatory agent [8]. *Randia uliginosa* contains alkaloids, saponins phenols, flavonoids, tannins and possesses antimicrobial, antioxidant activities [9].

Investigation of herbal constituents of plants with different analytical measures is important to authenticate the plant and its phytoconstituents in distinguishing from other plant species and phytochemicals. The present research was aimed to identify different phytochemicals using different solvents in preliminary phytochemical and column chromatography, HPTLC and GC-MS studies of *Randia spinosa*. The present study will help to know the presence of phytochemicals and useful in making data base or they can used for any biological activities.

Materials and Methods

Collection and processing of plant

On the basis of present literature review with slight experimental modifications, the fresh leaves of *Randia spinosa* were collected from hill station of Devarayanadurga, Tumkur district, Karnataka, India in the month of June, 2014 and identified based on the characteristics. The plant material was washed with distilled water, shade-air dried (26 ± 2°C) and pulverized to a coarse powder in a mechanical grinder, passed through a 40 mesh sieve and stored in air tight container for further work.

Preliminary solvent extraction

Dried leaves of *Randia spinosa* extraction was done with different solvents, n-hexane, chloroform, ethanol, methanol and distilled water individually by increase in their polarity. The solvent extracts were dried and preliminary phytochemical analysis was carried in selection of suitable solvent for extraction.

Preliminary phytochemical analysis

Preliminary characterization of phytochemicals was carried out according to standard detection procedures for identification of general phytochemicals viz., carbohydrate, cardiac glycosides, fixed oils and fats, proteins and amino acids, saponins, phytosterol, alkaloids, flavonoids, phenol, tannins, gums and mucilages, anthraquinones and reducing sugars [10-12].

Preparation of crude extracts for column chromatography

Based on the significant results of preliminary phytochemical analysis, only methanol extracts concentrated for further studies. Crude leaf extraction of *Randia spinosa* was carried out by the sequential extraction at different temperatures using methanol as a solvent. First extraction was carried out with 10 g of dried leaf powder in 50 ml of methanol was extracted at 50°C for 2 h. Second extraction was done by using residue for first extraction with the addition of 50 ml of methanol and extracted for 60 min at 60°C. Third extraction was done using residue for second filtration and with the addition of another 50 ml of methanol to it and heated for 60 min at 70°C. All the extracts at different temperature were mixed. Hexane was added in equal volume, mixed vigorously and hexane layer was separated to remove the trace

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Received January 20, 2016; **Accepted** February 07, 2016; **Published** February 15, 2016

Citation: Mahabaleshwara K, Chandrasekhar N, Govindappa M (2016) Phytochemical Investigations of Methanol Leaf Extracts of *Randia spinosa* Using Column Chromatography, HPTLC and GC-MS. Nat Prod Chem Res 4: 202. doi:10.4172/2329-6836.1000202

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of plant pigments. The methanol extract was dried and preserved for further experiments.

Column chromatography

The partial purification of methanol extract was carried out by the sequential purification through column chromatography. Activated silica gel (pore size 100-200) was used as a stationary phase and n-hexane, ethyl acetate and methanol in sequence were used as a mobile phase. The crude methanol residue partially dissolved in n-hexane and was triturated with silica and further column chromatography was carried out. First n-hexane fraction was collected followed by ethyl acetate fractions and finally the methanol fraction. Further HPTLC was carried out for each fraction to confirm the separation and presence of distinguishable phytochemicals.

Analysis of eluted fractions by HPTLC

High-Performance Thin-Layer Chromatography (HPTLC) is the most advanced form of TLC and comprises the use of chromatographic layers of utmost separation efficiency and the employment of state-of-the-art instrumentation for all steps in the procedure: precise sample application, standardized reproducible chromatogram development and software controlled evaluation. HPTLC is an entire concept that includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis. HPTLC was carried out with different injection volumes in duplicates.

Instruments used

Sampler: CAMAG® Linomat 5 sampler

Scanner: CAMAG® TLC Scanner 4

Software: winCATS148

TLC Plates: Aluminium sheets Silica Gel 60F254 Coated

Supplier: EMD MILLIPORE - AA – CHROM

Dimension: 10 × 20 cm

Mobile phase: N-6-Hexane: Ethyl acetate (8:2)

Sample preparation: Eluted fractions were dried at 60°C in water bath to get residue and residue was dissolved in methanol used for HPTLC.

GC-MS analysis: GC-MS analysis of column fractions of ethyl acetate and methanol fractions of *Randia spinosa* carried out with Agilent 7890-A having an MS detector 5975-C, ionization for MS is electron impact ionization. Mass analyzer was Quadrupole. The peaks were analyzed using data analysis software NIST-2008. Experiments were carried in column HP- 5 ms, dimensions- 30 m × 0.25 mm ID × 0.25 um film thickness. The initial temperature ramp was maintained 40°C, hold time 2 min. At the end the temperature ramp was 310°C and hold time was 10 min. The rate of temperature ramp was 10°C/min. The experiment was programmed with total run time 39 min, helium was used as a carrier gas at a constant flow rate of 1.0 ml/min, split less flow 1 ml/min. Injection volume was 1 µl with scan mass range 30 m/z- 600 m/z having positive polarity (+ve).

Identification of phytoconstituents

Interpretation of GC-MS mass-spectra were carried out using the database of National Institute Standard and Technology- 2008 (NIST-2008) having more than 62,000 patterns. The spectrums of the unknown components were compared with the spectrum of known components of NIST library and the parameters *viz.*, molecular weight, structure of the components, total ionic chromatograms and ionization chromatograms were ascertained in naming of each compound.

Results and Discussion

Preliminary phytochemical studies

Among n-hexane, chloroform, ethanol, methanol and distilled water leaf extracts of *Randia spinosa*, methanol was considered as the effective solvent in exhibiting important phytochemicals and was concentrated further studies in detail. Methanol extract has given good results in yield and number of different phytoconstituents compared to other solvent extracts. Phenolic compounds, flavonoids, alkaloids, tannins, cardiac glycosides and phytosterols were observed in significant quality in methanol extracts (Table 1).

Column chromatography and HPTLC

Partially purified column fractions of methanol extract further screened to identify different phytochemicals with different aliquots. The HPTLC studies have shown significant results. The methanol and ethyl acetate fractions have shown different phytoconstituents compared to n-hexane fraction. The duplicates of different concentrations of each fraction spotted were visualized under UV-366 nm. The results confirm that, there was a single major band in

Phytochemicals	Solvent extracts				
	Hexane	Chloroform	Ethanol	Methanol	Aqueous
Carbohydrate	+	+	+++	++	+
Cardiac Glycosides	+	+	++	++	+
Fixed oils and Fats	-	-	-	-	-
Proteins and Amino acids	-	-	-	+	++
Saponins	-	-	-	-	++
Phytosterol	+	+	++	+++	+
Alkaloids	-	-	++	++	+
Flavonoids	-	-	+	+++	++
Phenol	+	+	+	+++	+
Tannins	+	+	+	++	++
Gums and mucilages	-	-	-	-	++
Anthraquinones	++	++	-	-	-
Reducing sugars	-	-	++	+	+

Note: +: Indicates; ++: Presence; +++: Extensively present; -: Absence/not clear; Repeated each experiment thrice.

Table 1: Preliminary phytochemical studies of different solvent extracts of leaf of *Randia* species.

n-hexane fraction. Whereas, methanol and ethyl acetate were efficient in extracting different phytoconstituents and it has been confirmed by HPTLC bands (Figures 1 and 2; Tables 2 and 3).

GC-MS studies

Based on the HPTLC results of hexane, ethyl acetate and methanol fractions, only ethyl acetate and methanol fractions were selected for GC-MS studies due to presence of varied phytochemicals. The GC-MS study has depicted significant results with the presence of twelve different identifiable compounds, Ethanol,2-phenoxy-; 3-Heptanone,2,6-dimethyl-; Butylated Hydroxytoluene; 2-[4a,8-Dimethyl -6-oxo-1,2,3,4,4a,5,6,8a - octahydro - naphthalene -2-yl] - propionaldehyde; 1-Hexadecene; Hexadecanoic acid,methyl ester; Isopropyl Palmitate; Ethyl Oleate; 2-Ethylhexyl trans-4-methoxycinnamate; Dasycarpidan-1-methanol,acetate (ester); Hexadecanoic acid,2-methylpropyl ester; 9-Octadecenoic acid [z]-,2,3-dihydroxypropyl ester in ethyl acetate and nine, 3-Eicosyne; Isopropyl linoleate; Pentadecanoic acid,14-methyl-,methyl ester; n-Butyl myristate; 16-Octadecenoic acid,methyl ester; Heptadecanoic acid,9-methyl-,methyl ester; 9,12,15-Octadecatrienoic acid,ethyl ester,[z,z,z]-; Benzoic acid,2-[2-methoxyethoxy)-5-(2,2-dimethylpropanoylamino); Pyrano[4,3-b]benzopyran-1,9- dione,5a-methoxy-9a-methyl-3-(1-propenyl)perhydro- in methanol fraction. Among these phenolic compounds, terpenoid esters, cinnamate, naphthalene compounds are in predominant. Ethyl acetate has shown a familiar antioxidant compound *i.e.*, Butylated Hydroxy Toluene (BHT) and methanol fraction has shown one of the important pyrano coumarin *i.e.*, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-pyrano [4,3-b]benzopyran-1,9- dione (Figures 3-6; Tables 4 and 5).

Phytochemical investigations of *Randia spinosa* have given significant results in all tested analytical procedures. The plants of *Randia* species are well known in having the presence of terpenoid and phenolic compounds. GC-MS study reveals the presence of terpenoid esters, phenolic compounds and naphthalene derivatives. These studies have been confirmed by the earlier studies carried out in different *Randia* species [3,4,9].

We found the presence of an effective antioxidant, Butylated Hydroxy Toluene (BHT) in the leaf methanol extract and same BHT have reported in green algae *Botryococcus braunii* and in cyanobacteria like *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa* and *Oscillatoria* with GC-MS studies [13]. BHT have anticancer activity in human promyelocytic leukemia cell lines (HL-60) and human squamous cell carcinoma cell lines (HSC-2) [14]. So, our BHT can work as antoproperty also. Phenols and flavonoids have noticed the extract and similar results was bserved in *Olea europaea* and have been characterized by HPTLC. Hence, our results to these findings. These phenol and flavonoids have used to treat different cancers such as breast, colon, hepatocellular and cervical carcinoma cells [15]. Possibly our extract can also use for isolation, characterization and used for treatment of cancer. Many plant phenolic compounds showed the properties of antioxidant and anticancer activities [16]. Hence, our methanol extract of *Randia spinosa* yielded very important phytochemicals such BHT, phenols, flavonoids, alkaloids and tannins. Further research is needed to identify potent antioxidant and anticancer gents from the extract after purification in *in vitro* and *in vivo*.

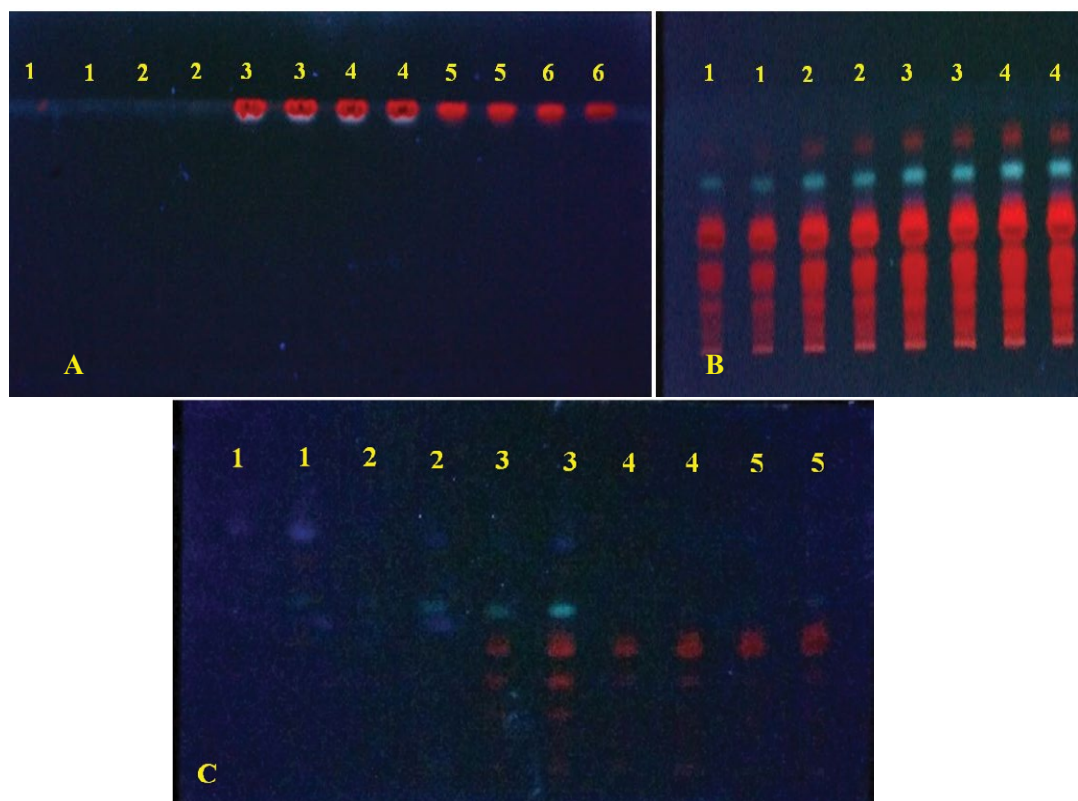


Figure 1: HPTLC chromatograms of column fractions, A) Hexane, B) Ethyl acetate and C) Methanol of leaves of *Randia* species. Mobile phase: n-Hexane and ethyl acetate (8:2). Visualization: Under UV 366 nm.

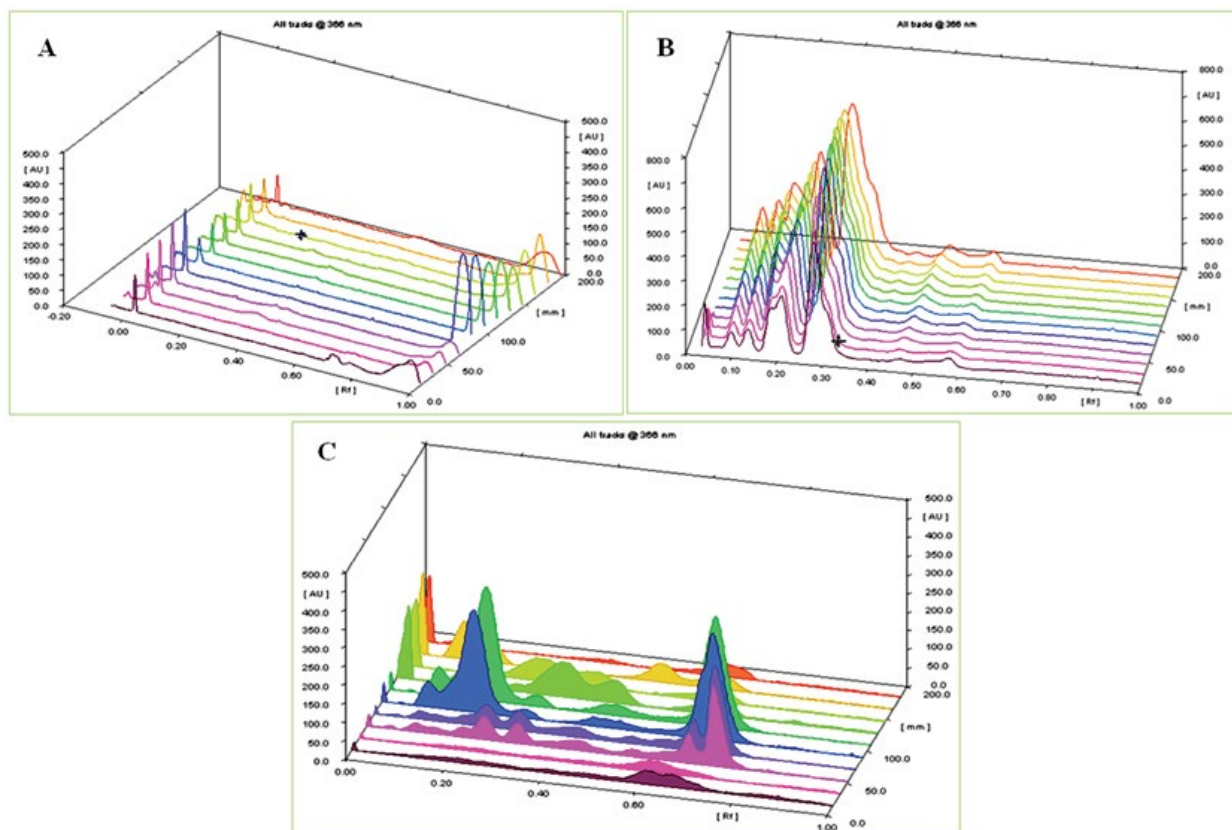


Figure 2: HPTLC-spectra/chromatograms of column fractions, A) Hexane, B) Ethyl acetate and C) Methanol fraction of leaves of *Randia* species at UV-366 nm.

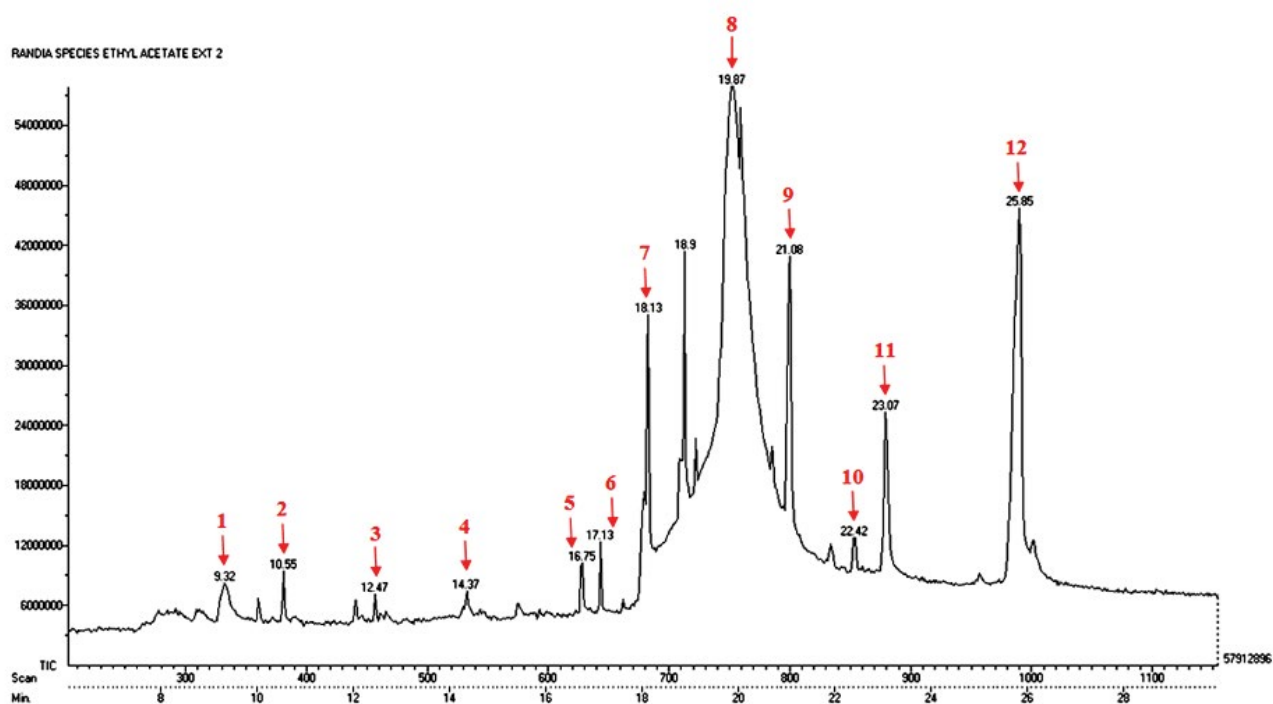
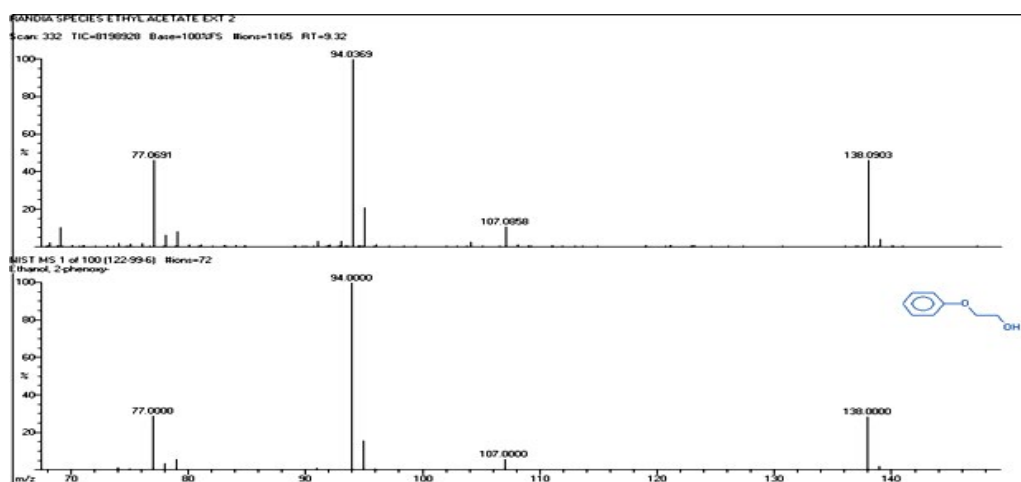
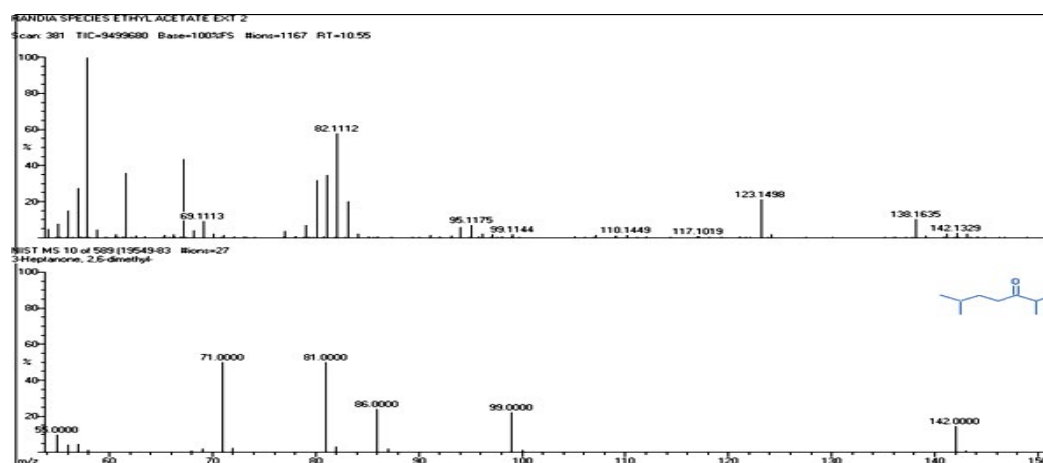


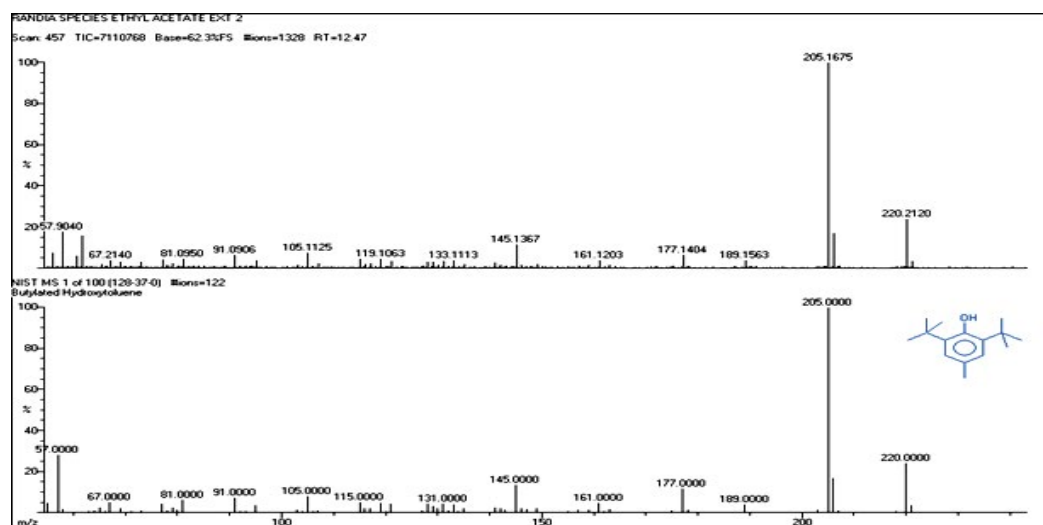
Figure 3: Total ionic chromatogram of ethyl acetate fraction from *Randia* species leaf extract.



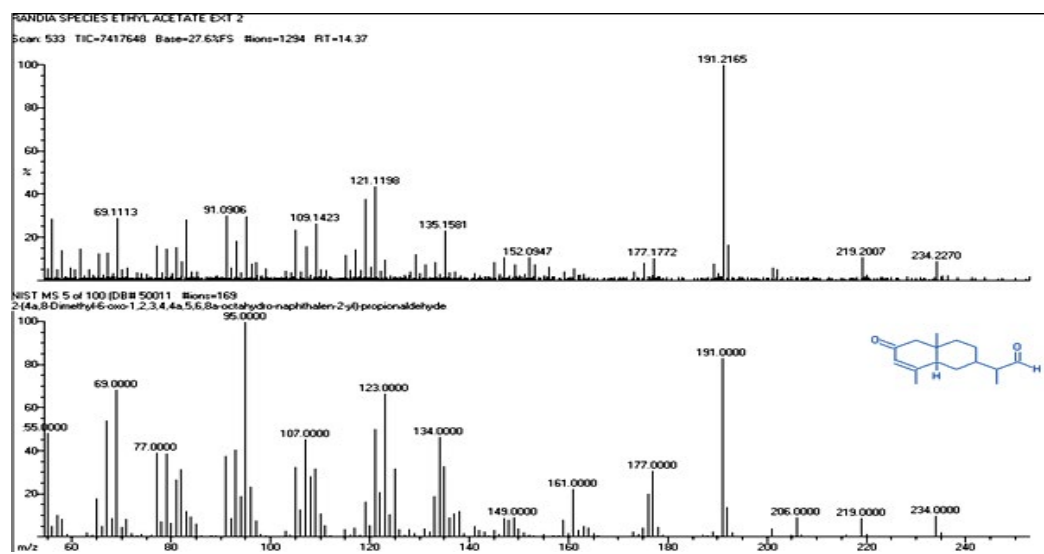
Ethyl acetate Fraction, RT 9.32 minutes: Ethanol, 2-phenoxy-
Figure 4: Extracted ionic chromatograms of ethyl acetate fraction from *Randia* species leaf extract.



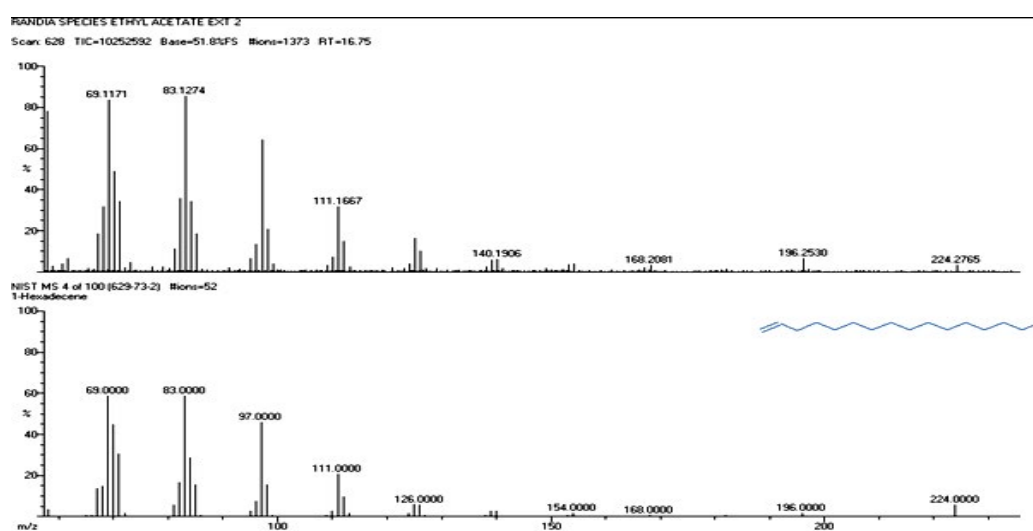
Ethyl acetate Fraction, RT 10.55 minutes: 3-Heptanone, 2,6-dimethyl-



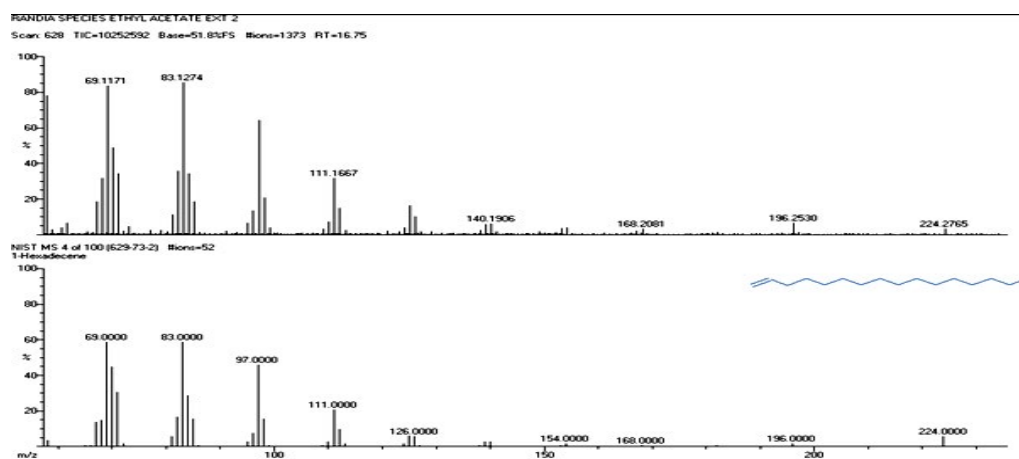
Ethyl acetate Fraction, RT 12.47 minutes: Butylated Hydroxytoluene.



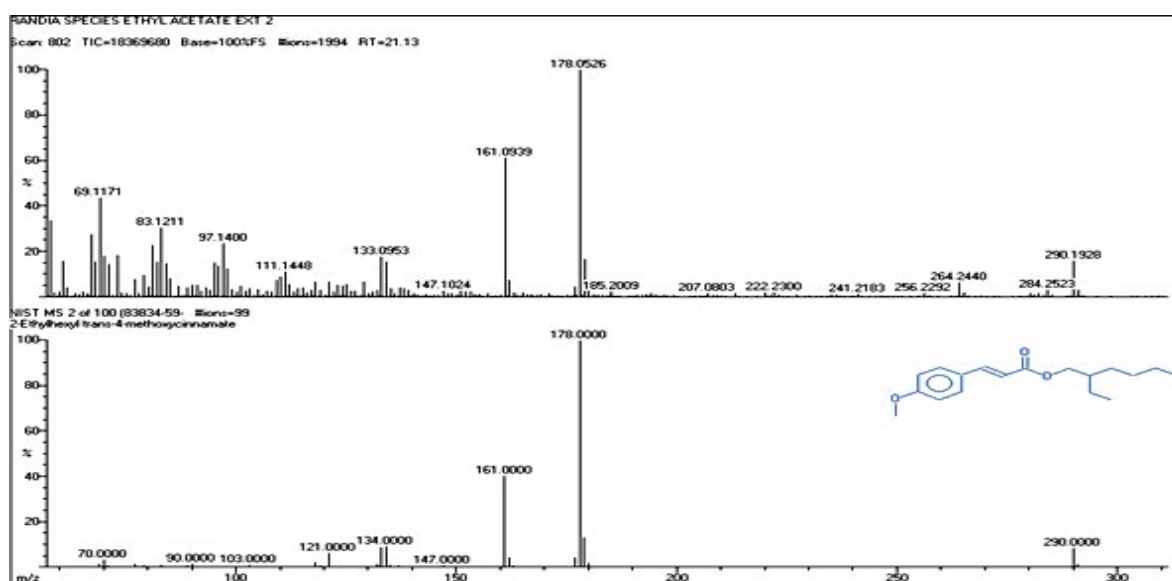
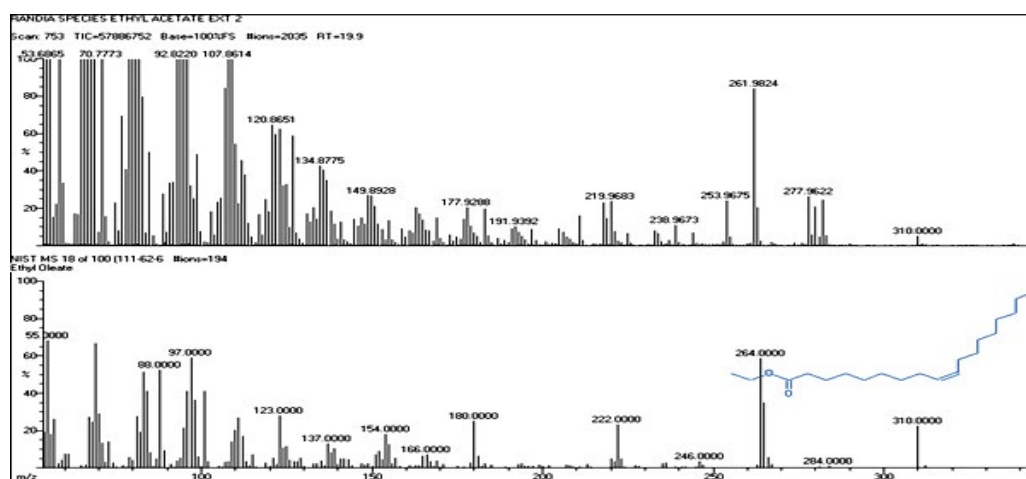
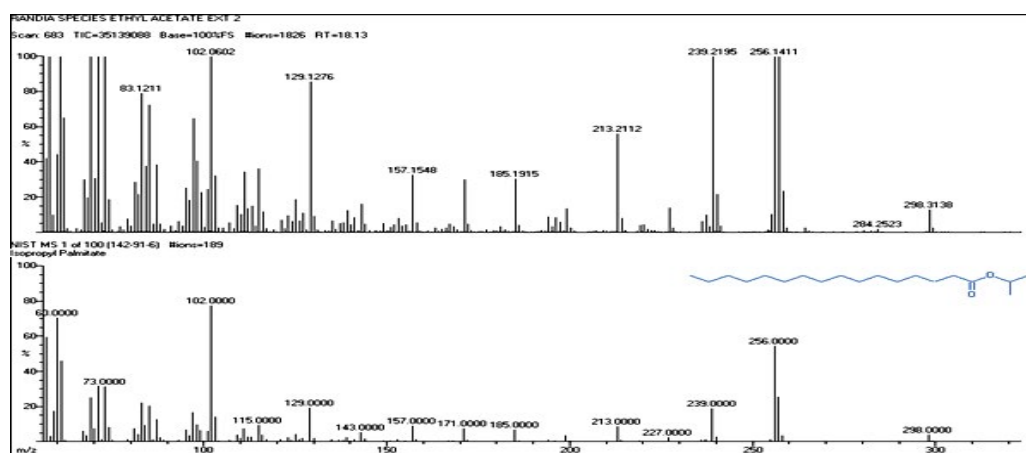
Ethyl acetate Fraction, RT 14.37 minutes: 2-[4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,5,6a-octahydro-naphthalen-2-yl]-propionaldehyde.

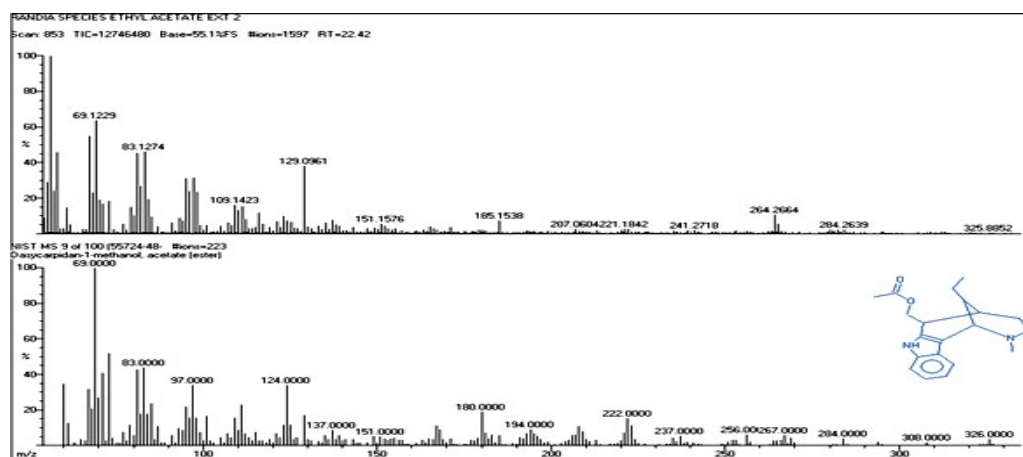


Ethyl acetate Fraction, RT 16.75 minutes: 1-Hexadecene.

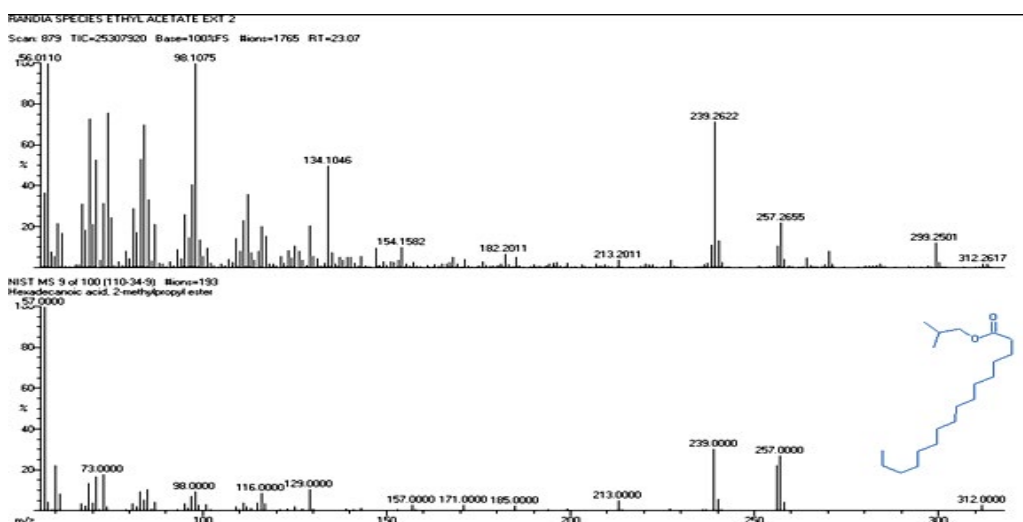


Ethyl acetate Fraction, RT 17.13 minutes: Hexadecanoic acid, methyl ester.

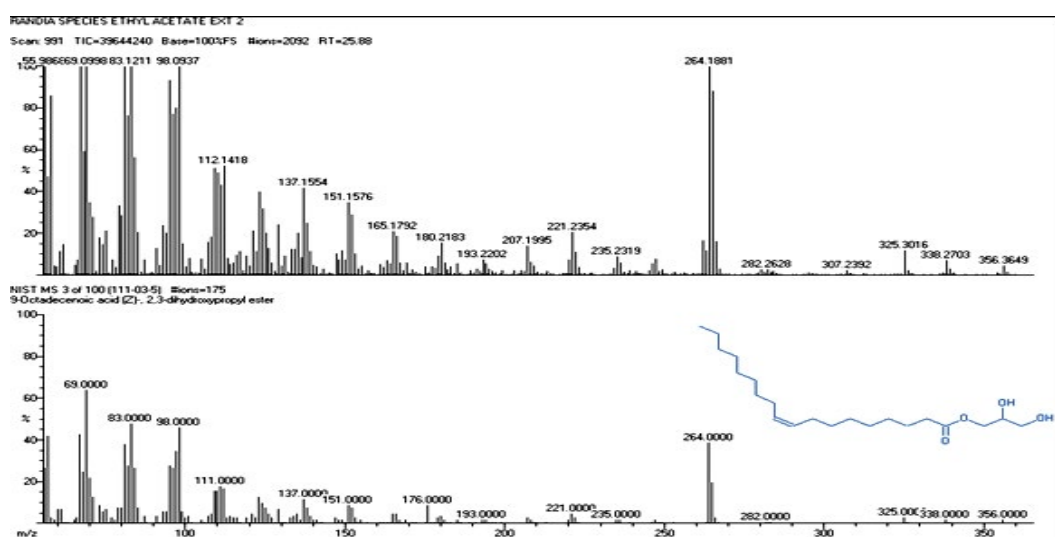




Ethyl acetate Fraction, RT 22.42 minutes: Dasycarpidan-1-methanol, acetate (ester).



Ethyl acetate Fraction, RT 23.07 minutes: Hexadecanoic acid, 2-methylpropyl ester.



Ethyl acetate Fraction, RT 25.85 minutes: 9-Octadecenoic acid [z]-2,3-dihydroxypropyl ester.

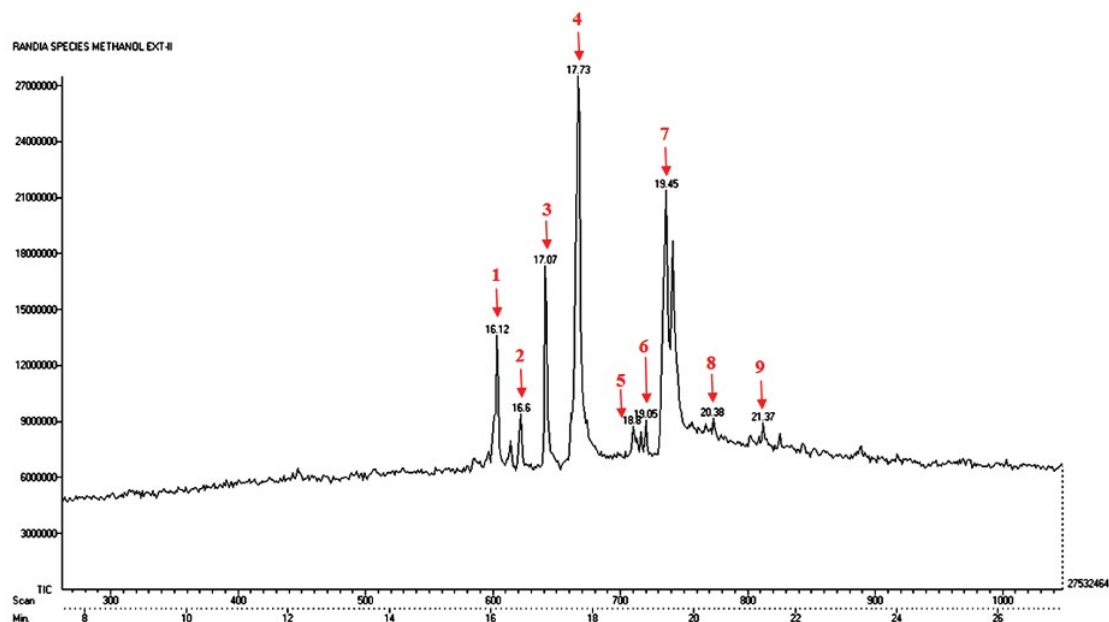
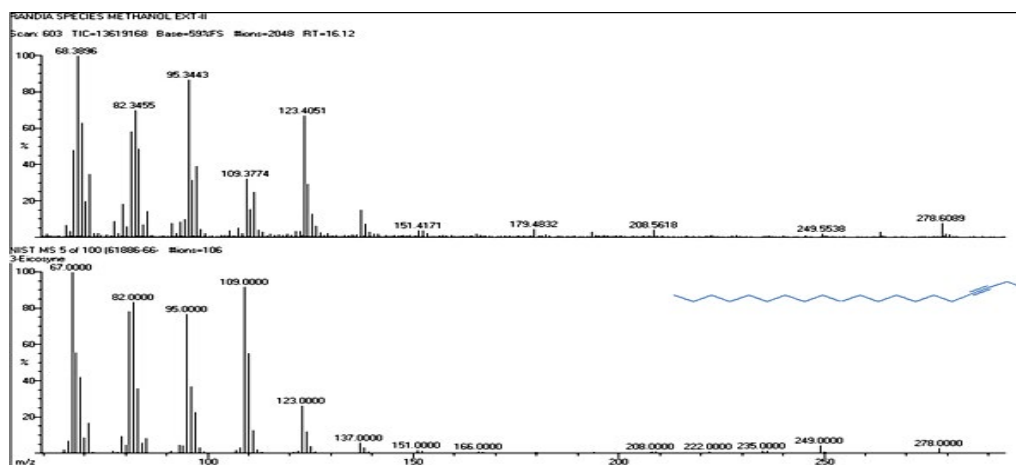
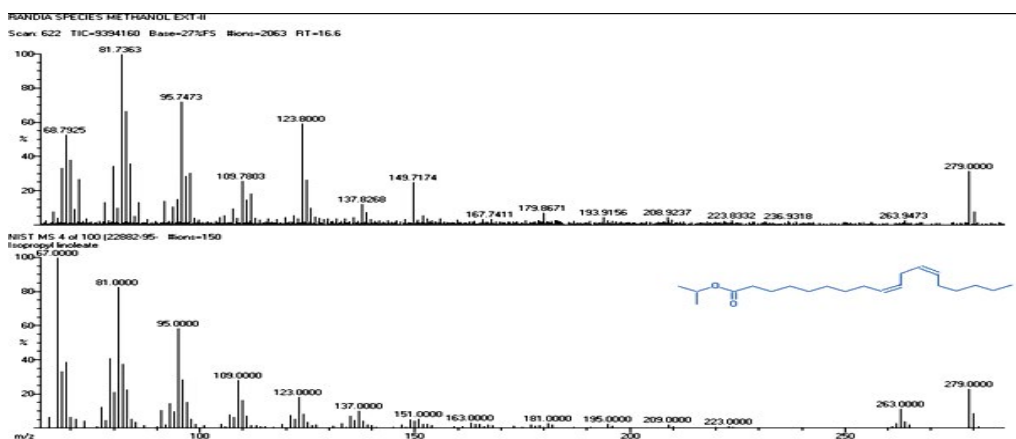


Figure 5: Total ionic chromatogram of methanol fraction from *Randia* species leaf extract.

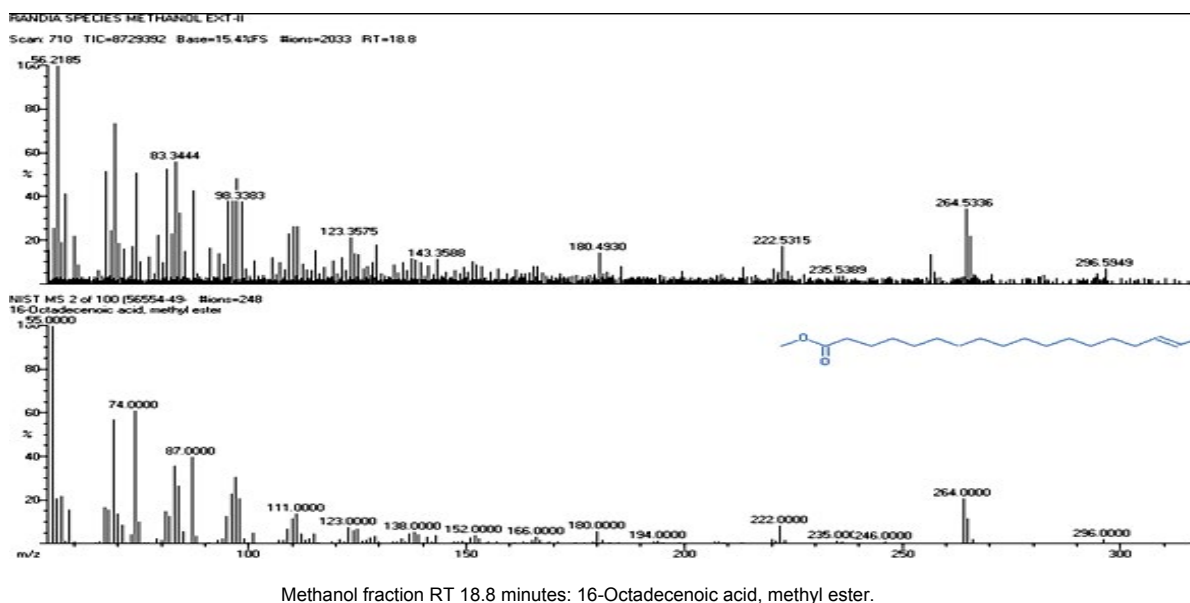
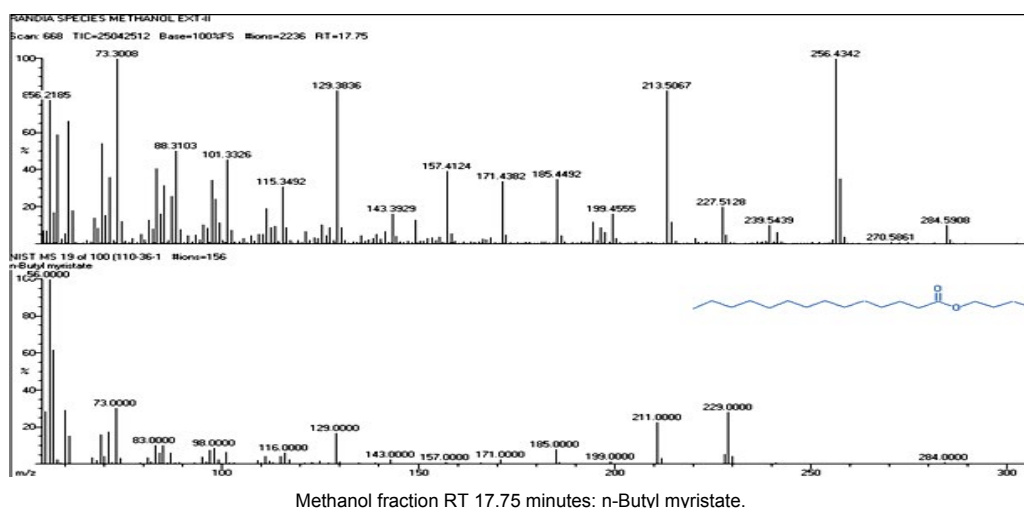
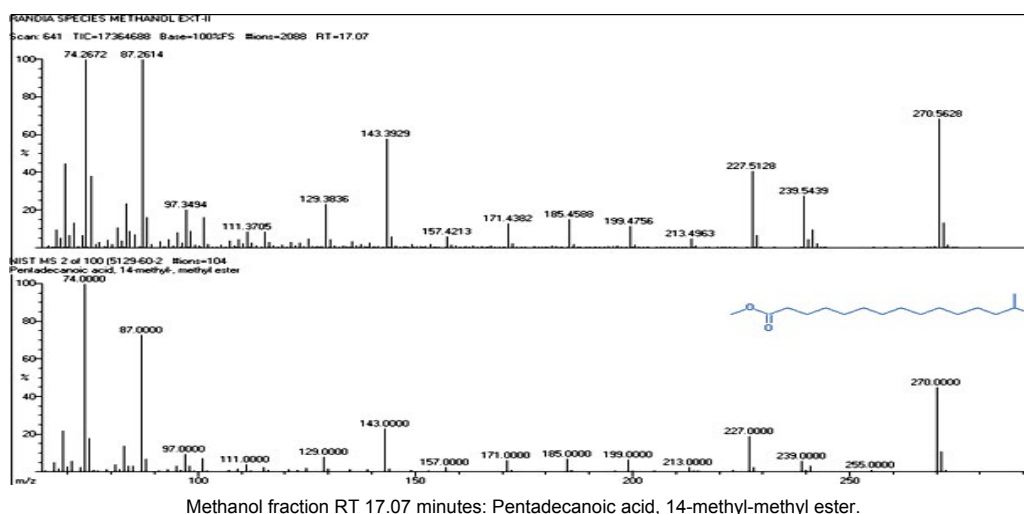


Methanol fraction RT 16.12 minutes: 3-Eicosyne.

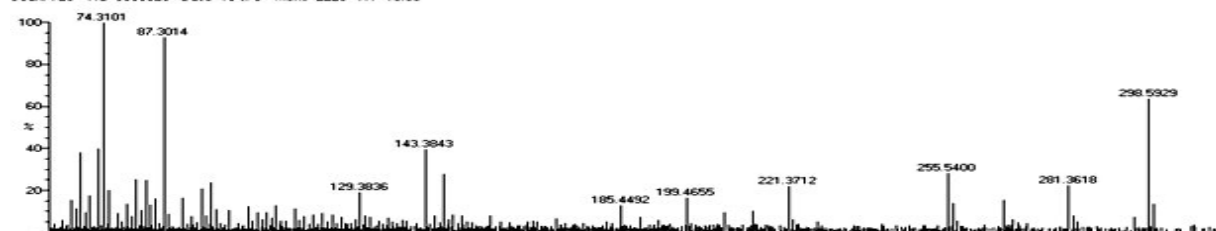
Figure 6: Extracted ionic chromatograms of methanol fraction from *Randia* species leaf extract.



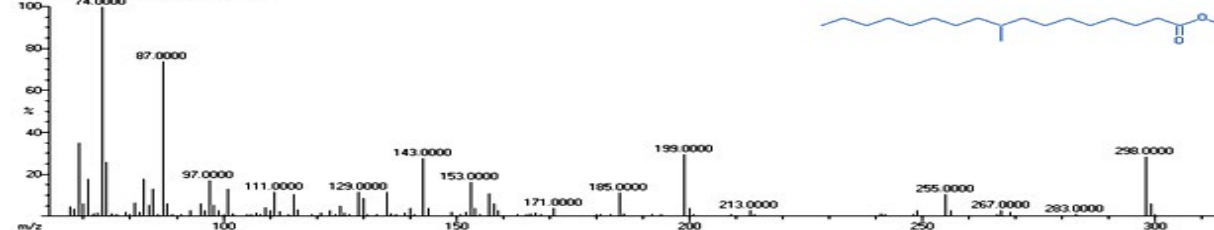
Methanol fraction RT 16.6 minutes: Isopropyl linoleate.



RANDIA SPECIES METHANOL EXT-II
Scan: 720 TIC=9060320 Base=15%FS Ions=2228 RT=19.05

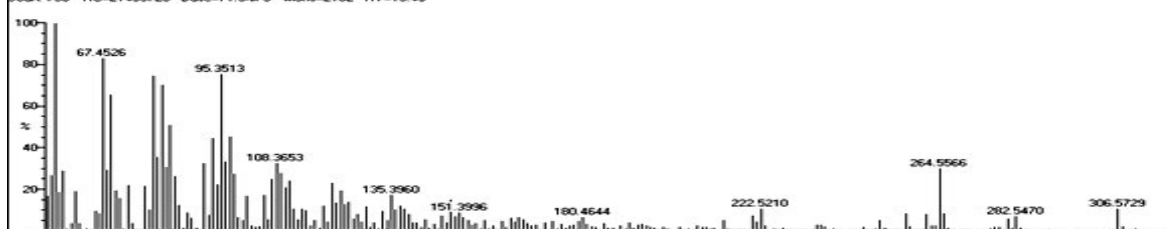


NIST MS 30 of 100 (54934-57) Ions=170
Heptadecanoic acid, 9-methyl-, methyl ester

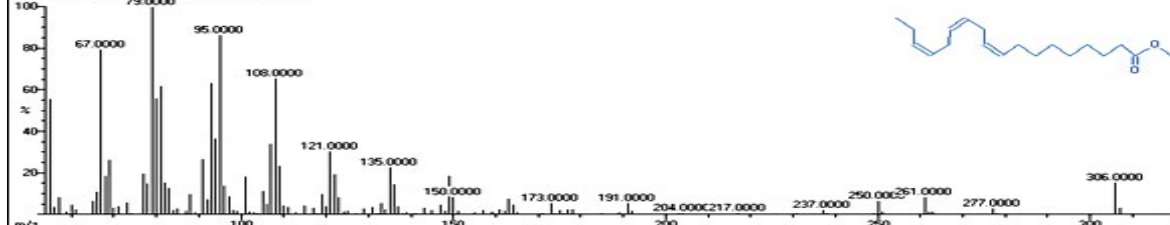


Methanol fraction RT 19.05 minutes: Heptadecanoic acid, 9-methyl-methyl ester.

RANDIA SPECIES METHANOL EXT-II
Scan: 736 TIC=21433728 Base=71.5%FS Ions=2182 RT=19.45

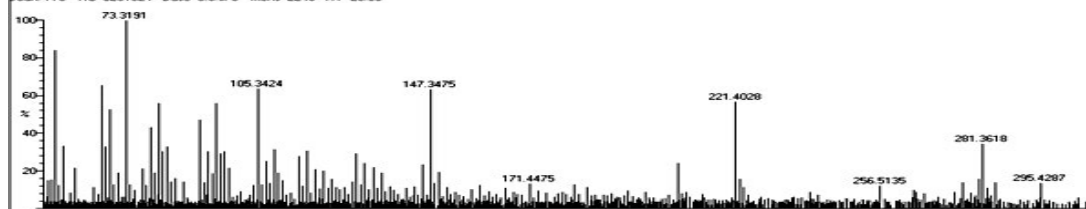


NIST MS 35 of 100 (1191-41) Ions=230
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-

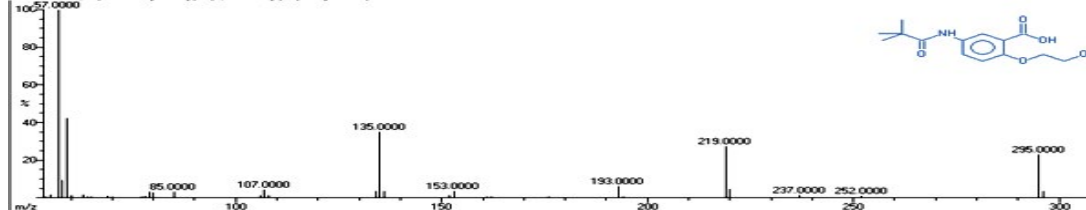


Methanol fraction RT 19.45 minutes: 9,12,15-Octadecatrienoic acid, ethyl ester[z,z,z]-

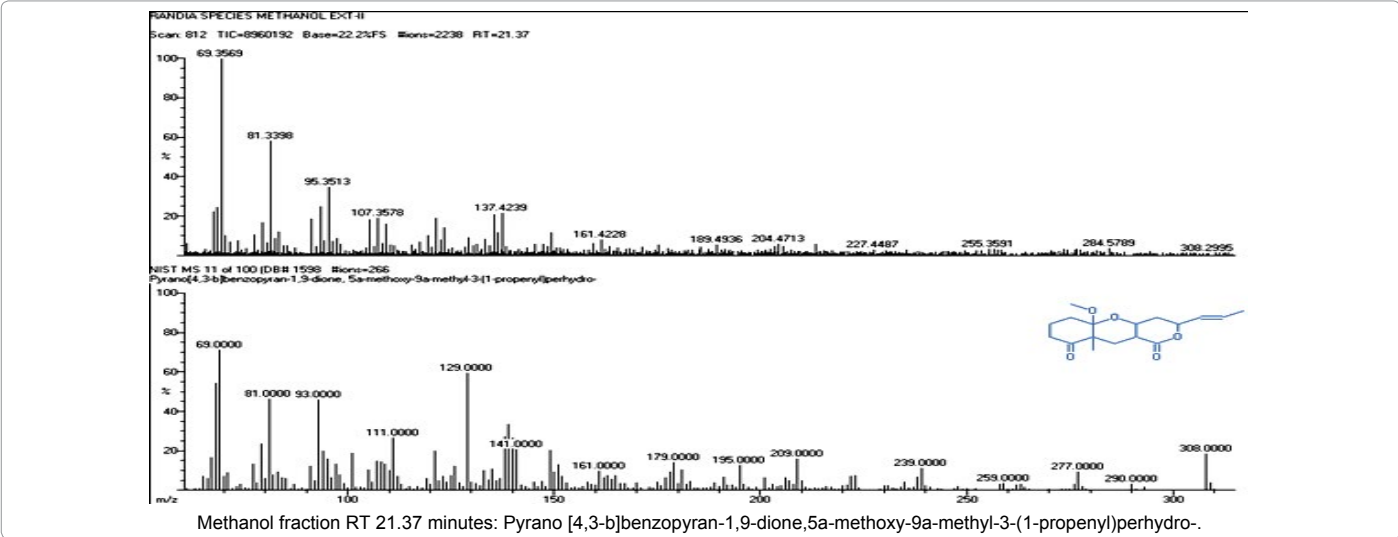
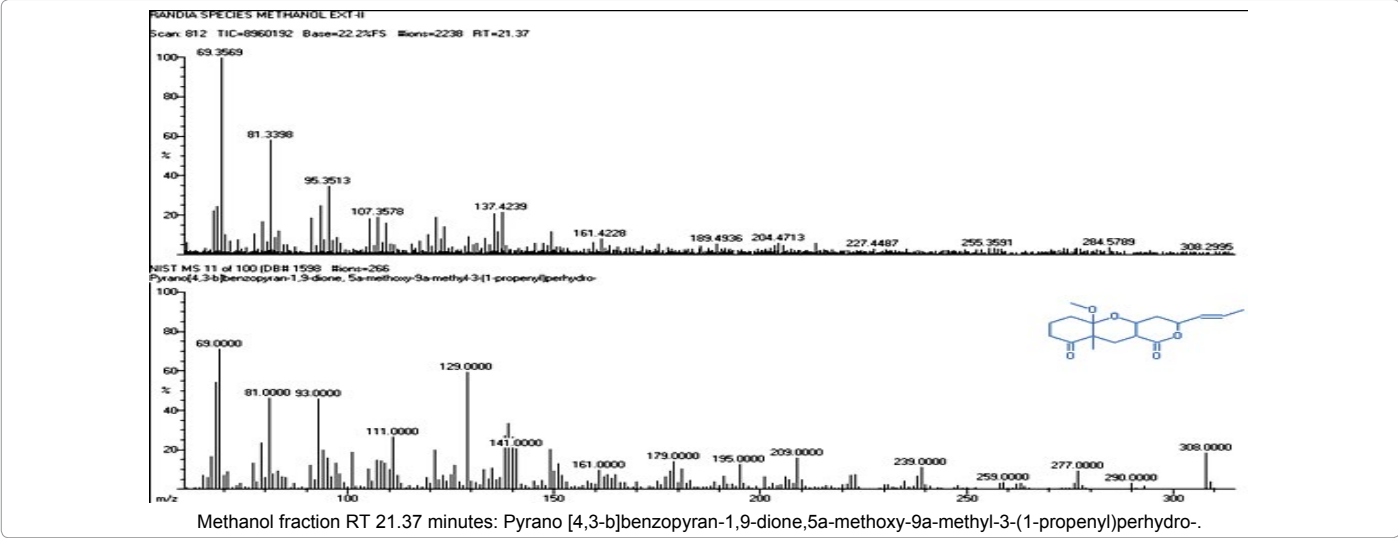
RANDIA SPECIES METHANOL EXT-II
Scan: 773 TIC=9201024 Base=9.6%FS Ions=2245 RT=20.38



NIST MS 15 of 368 (284680-1) Ions=151
Benzoic acid, 2-[2-methoxyethoxy]-5-(2,2-dimethylpropanoylamino)-



Methanol fraction RT 20.38 minutes: Benzoic acid, 2-[2-methoxyethoxy]-5-(2,2-dimethylpropanoylamino).

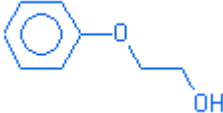
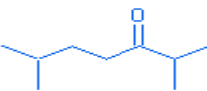
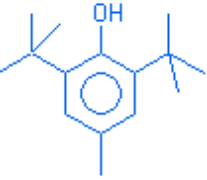
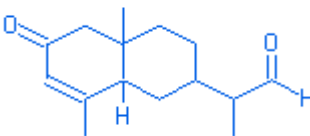



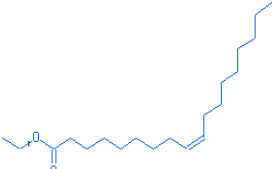
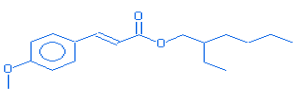
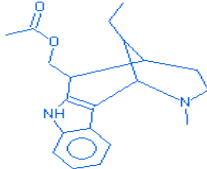


S No	Track number	Injection volume (µl)
1	1 and 2	2
2	3 and 4	4
3	5 and 6	6
4	7 and 8	8
5	9 and 10	10
6	11 and 12	12

Table 2: Injection volumes and track numbers of spectra/chromatograms for hexane and ethyl acetate fractions of *Randia* species.

S No	Track number	Injection volume (µl)
1	1 and 2	2
2	3 and 4	4
3	5 and 6	10
4	7 and 8	8
5	9 and 10	6

Table 3: Injection volumes and track numbers of spectra/chromatograms for methanol fractions of *Randia* species.

Peak No	RT (min)	Identified compound name	Structure	Synonyms
1	9.32	Ethanol,2-phenoxy-		β -Hydroxyethyl phenyl ether; β -Phenoxyethyl alcohol; Aerosol; Ethylene glycol monophenyl ether; Glycol monophenyl ether; Phenoxethol; Phenoxetol; Phenoxyethanol; Phenoxyethyl alcohol; Phenyl cellosolve; 1-Hydroxy-2-phenoxyethane
2	10.55	3-Heptanone,2,6-dimethyl-		2,6-Dimethyl-3-heptanone; 2,6-dimethylheptan-3-one
3	12.47	Butylated Hydroxytoluene		2,6-Di- <i>tert</i> -butyl-4-methylphenol, 2,6-Di- <i>tert</i> -butyl- <i>p</i> -cresol (DBPC), 3,5-Di- <i>tert</i> -butyl-4-hydroxytoluene, BHT, Dibutylated hydroxytoluene, 4-Methyl-2,6-di- <i>tert</i> -butyl phenol
4	14.37	2-[4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydronaphthalen-2-yl]-propionaldehyde		2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydronaphthalen-2-yl)-propionaldehyde, 2-(4a,8-dimethyl-6-oxo-1,2,3,4,5,8a-hexahydronaphthalen-2-yl)propanal, 2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)propanal
5	16.75	1-Hexadecene		1-Hexadecene; Cetene; Hexadec-1-ene; Hexadecylene-1; 1-Cetene
6	17.13	Hexadecanoic acid, methyl ester		Palmitic acid, methyl ester; n-Hexadecanoic acid methyl ester; Methyl hexadecanoate; Methyl n-hexadecanoate; Methyl Palmitate;
7	18.13	Isopropyl Palmitate		Hexadecanoic acid, 1-methylethyl ester; Palmitic acid, isopropyl ester; Deltyl; Deltyl Prime; Hexadecanoic acid, isopropyl ester; Isopal; Isopropyl n-hexadecanoate
8	19.87	Ethyl Oleate		9-Octadecenoic acid (Z)-, ethyl ester; Oleic acid, ethyl ester; (Z)-9-Octadecenoic acid ethyl ester; Ethyl cis-9-octadecenoate; Ethyl Z-9-octadecenoate
9	21.08/ 21.13	2-Ethylhexyl trans-4-methoxycinnamate		4-Methoxycinnamic acid 2-ethylhexyl ester, OMC, Octyl methoxycinnamate
10	22.42	Dasycarpidan-1-methanol,acetate (ester)		Dasycarpidan-1-methanol,acetate

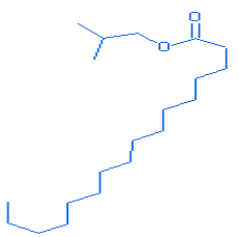
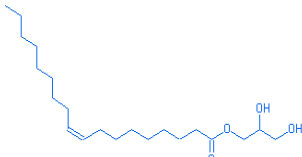
11	23.07	Hexadecanoic acid,2-methylpropyl ester		2-methylpropyl hexadecanoate; isobutyl hexadecanoate; Isobutyl Palmitate
12	25.85	9-Octadecenoic acid [z]-,2,3-dihydroxypropyl ester		Glycerol Monooleate; Olein, 1-mono-; α-Monoolein; Glycerin 1-monooleate; Glycerol α-cis-9-octadecenoate; Glycerol α-monooleate; Glycerol 1-monooleate; Monoolein; 1-Glycerol oleate; 1-Oleoylglycerol; 1-Oleoylglycerol; dl-α-Monoolein;

Table 4: GC-MS detection of phyto constituents of ethyl acetate fraction from *Randia* species leaf extract.








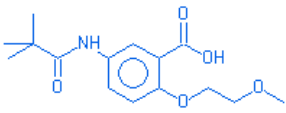
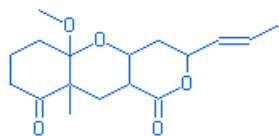
Peak No	RT (min)	Identified compound name	Structure	Synonyms
1	16.12	3-Eicosyne		3-Icosyne; 3-Eicosyne; Icos-3-yne; 3-Icosyne
2	16.6	Isopropyl linoleate		Isopropyl linoleate; Ceraphyl IPL; Isopropyl linolate; Linoleic acid, isopropyl ester; 9,12-Octadecadienoic acid (Z,Z)-, 1-methylethyl ester
3	17.07	Pentadecanoic acid,14-methyl-,methyl ester		Methyl isohexadecanoate
4	17.73/ 17.75	n-Butyl myristate		n-Butyl myristate; Myristic acid n-butyl ester; Tetradecanoic acid, butyl ester; Bumyr; Myristic acid, butyl ester; Butyl n-tetradecanoate; Butyl tetradecanoate; Crodamol BM; n-Butyl tetradecanoate; Tetradecanoic acid, n-butyl ester
5	18.8	16-Octadecenoic acid, methyl ester		Methyl (E)-octadec-16-enoate; • Methyl (16E)-16-octadecenoate
6	19.05	Heptadecanoic acid,9-methyl-,methyl ester		Methyl isostearate; Methyl 16-methylheptadecanoate
7	19.45	9,12,15-Octadecatrienoic acid, ethyl ester,[z,z,z]-		Linolenic acid, ethyl ester; Ethyl cis,cis,cis-9,12,15-octadecatrienoate; Ethyl linolenate; Ethyl α-linolenate; Ethyl (Z,Z,Z)-9,12,15-octadecatrienoate; 9,12,15-Octadecatrienoic acid, ethyl ester, (9Z,12Z,15Z)-; ethyl (9Z,12Z,15Z)-9,12,15-octadecatrienoate
8	20.38	Benzoic acid,2-[2-methoxyethoxy]-5-(2,2-dimethylpropanoylamino]		----
9	21.37	Pyrano[4,3-b]benzopyran-1,9-dione,5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-		5a-Methoxy-9a-methyl-3-[(1E)-1-propenyl] decahydro-1H,9H-pyrano[4,3-b]chromene-1,9-dione; 5a-methoxy-9a-methyl-3-[(E)-prop-1-enyl]-3,4,4a,6,7,8,10,10a-octahydropyrano[4,3-b]chromene-1,9-dione; 5a-methoxy-9a-methyl-3-[(E)-prop-1-enyl]-3,4,4a,6,7,8,10,10a-octahydropyrano[4,3-b]chromene-1,9-quinone

Table 5: Bioactive compounds of methanol fraction from *Randia* species leaf extract.

Conclusion

Among five different solvent, the methanol leaf extract of *Randia spinosa* have shown important phytochemicals identified from analytical, column chromatography, HPTLC and GC-MS followed by ethyl acetate. The ethyl acetate extraction yielded 12 different phytochemicals whereas the methanol extract exhibited nine phytochemicals. The methanol extract yielded BHT, phenol, flavonoids at high amount and which have already role of antioxidant and anticancer role. The phytosterols, alkaloids and tannins were also found to presence in the extract at less amount. Future studies need to isolate and characterize individual phytochemicals and test for their biological activities such as antioxidant and anticancer.

Acknowledgements

We thank Dr MR Hulinaykar, Managing Trustee, Sri Shridevi Charitable Trust (R) and Dr MN Eshwarappa, Principal, SIET, Tumkur, India for encouragement and suggestions during the research.

References

1. World Health Organization (2015) Media Center: Cancer. Fact sheets.
2. Sati OP, Chaukiyal DC, Miyahara K, Kawasaki T (1986) An iridoid from *Randia dumetorum*. *Phytochem* 25: 2658-2660.
3. Agrawal SS, Singh VK (1999) Immuno modulators-A review of studies on Indian medicinal plants and synthetic peptides. Part 1: Medicinal plants. *Proc Indian Natl Sci Aca* 65: 179-204.
4. Subramaniam S, Bokel M, Wolfgang K (1989) A hemolytic saponin Randianin from *Randia dumetorum*. *Phytochem* 28: 1544-1546.
5. Dharmishtha M, Falguni G (2009) Antibacterial activity of methanolic fruit extract of *Randia dumetorum* Lamk. *International Journal of PharmTech Research* 1: 679-681.
6. Kumar D, Satish C, Bhat ZA, Santosh S, Bhujbal R (2011) Antiallergic and anti-inflammatory effects of the fruits of *Randia dumetorum* Lamk. *Orient Pharm Exp Med* 11: 161-167.
7. Ghosh D, Thejomoorthy P, Veluchamy (1983) Anti-inflammatory and analgesic activities of oleanolic acid 3-/3- Glucoside (RDG-1) from *Randia dumetorum* (Rubiaceae). *Indian J Pharmacol* 4: 31-340.
8. Satpute KL, Jadhav MM, Karodi RS, Katare YS, Patil MJ, et al. (2009) Immunomodulatory activity of fruits of *Randia dumetorum* Lamk. *Journal of Pharmacognosy and Phytotherapy* 1: 36-40.
9. Gulnaz AR, AbdElrhim M, ELrahman MA, Chauhan JB (2014) Antimicrobial, antioxidant activities and phytochemical analysis of ethanolic extract of *Randia uliginosa* leaves. *Int J Curr Microbiol App Sci* 3: 200-210.
10. Sofowora I (1993) Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan. p: 150.
11. Trease E, Evans WC (1989) *Pharmacognosy*. 13th edn. Bailliere Tindall, London. pp: 176-180.
12. Siddiqui A, Ali M (1997) *Practical pharmaceutical chemistry*. 1st edn. CBS Publishers and Distributors, New Delhi. pp: 126-131.
13. Babu B, Wu JT (2008) Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton. *Journal of Phycology* 44: 1447-1454.
14. Saito M, Sakagami H, Fujisawa S (2003) Cytotoxicity and apoptosis induction by butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). *Anticancer Res* 23: 4693-4701.
15. Nashwa FS, Morsy ME, Aziz A (2014) Efficiency of olive (*Olea europaea* L.) leaf extract as antioxidant and anticancer agents. *Journal of Agroalimentary Processes and Technologies* 20: 46-53.
16. Elella AFM, Ali RFM (2014) Antioxidant and anticancer activities of different constituents extracted from Egyptian prickly pear Cactus (*Opuntia Ficus-Indica*) Peel. *Biochem Anal Biochem* 3: 158.