Research Article

Thin Layer Chromatography and GC-MS Analysis of Bioactive Molecules of the *Acacia ferruginea* DC. Thorn Extract

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ABSTRACT

The aim of the current study is to investigate thethin layer chromatography (TLC) and gas chromatography and mass spectroscopy analysis (GC-MS) of methanolic extract of *Acacia ferruginea* thorns. The bioactive molecules were determined by qualitative TLC and GC-MS method. In TLC exhibited maximum 0.6 retention factor (RF) value of the plant extract in F_{254} wavelength in dark blue colour, F_{366} wave length and in visible light not shown any peaks and RF values. In the GC-MS analysis, 37 bioactive molecules were exhibited and in that 10 are in higher concentration by the retention time and their % of peak and area covered in the analysis compared to other chromatograms of the fractions. Important compounds identified as Methyl mannose (57.14), Phenol, 2-methoxy-3-(2-propenyl)- (4.85), stigmasterol (4.65), 1-Hexacosanol (3.83), Lupeol (3.60), gamma Sitosterol (3.52), Phenol, 4-[2-(dimethylamino)ethyl]-(3.17), Ergost-5-en-3-ol, (3.beta.)- (2.53), Oleic acid (2.44), Oleoyl chloride (1.53), Sucrose (1.82). The presence of these bioactive molecules in the plant extract may provide the scientific evidences for the cytotoxic effect, insecticidal and other biological properties.

Key words: *Acacia ferruginea*, GC-MS analysis, Bioactive molecules, Antiproliferative, Cytotoxic.

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INTRODUCTION

Medicinal plants are used for the many ailment benefits due to their rich source of therapeutic potential and active molecules. Since the ancient time, medicinal plants have been used to resolve, remedy, research to treat various diseases and disorders. In all the plant parts and products have their own medicinal properties.[1,2] In our Indian traditional medicine system Acacia species are recognized for the remedial measures of various diseases viz., itching, leukoderma, ulcers, stomatitis and diseases of the blood.[3] The genus 'acacia' name came from Greek and the meaning of word 'akis', is point. *Acacia ferruginea* belongs to Fabaceae is a small normally grown plant, highly drought resistant and deciduous in nature. Twigs are zigzag, wiry, hairless in the nodal region in greenish or reddish in colour. Leaves are bipinnate, alternate, stipulate, stipular spines in pairs, spine present on trunk to grow up to 15 cm, pale yellow flowers, bears pods strap shaped, dark brown colour and sweetish pulp. Seeds are flat ovate, oblong, distinctly stalked greenish in young and when dried it is brownish in colour. In India, flowers of *A. ferruginea* appears in March to May during the tree foliages are very little and ripening of pods start in November and ends in February.[4,5] Various Acacia plants species were identified for their biological action in the traditional medicinal system. Among them *A*cacia *ferruginea* also called Rusty acacia studied for the natural therapeutic action and reported for various pharmacological properties.[6,7] There are enormous literature available on the *Acacia* genus which was commonly used as folk medicine in traditional system for the pain management, anti-inflammatory, anticancer, types of hemorrhage, bowel syndrome and microbial

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infections[8,9] The leaves were used for the application to bad breath remedy, for hepatic issues and dysentery; the bark owned for strong antioxidant activities.[10,11] *A. ferruginea*, is identified as Nitrogen Fixing Tree (NFT), it has the potency of maintaining soil moisture in agro-horticulture or integrated with rainfed practices in rabi season with sorghum and significantly support for the uptake of nitrogen by stems, leaves and grain of cowpea crop and which are cultivated lower fertile soil called as alfisols crops can be practiced along with *A. ferruginea* as NFT species.[12,13] A comprehensive work on all the parts of the plants were done and reported, in the current study aimed to focus on thorns or spines of the plant for their active molecules by the chromatography approach to explore all the profile and to know the bioactive molecules of the methanolic extract of *A. ferruginea* thorns.

MATERIALS AND METHODS

Sample collection and extraction

The plant thorns were collected from Dhanvantri forest, Bangalore, authenticated from the Department of Botany, St. Joseph Autonomous College, Bangalore, India. The thorn to be investigated were dismantled from the collected plants, washed with tap water, wiped with tissue paper and allowed to shade dry. Around 20 grams of thorny plant material was weighed, powdered and dissolved with 125 ml of methanol, kept for 4h extraction on water bath at 50°C, filtered using Whatman filter paper no. 1. Methanol filtrate was reduced by evaporation and stored in refrigerator for further use.

Thin layer chromatography of the crude methanolic extract of *Accacia ferruginea* thorns

10mg/ml methanolic extract of *A. ferruginea* thorns were prepared in methanol solvent and in that 2.5 µl of samples were spotted on TLC plate and allowed to dry. A TLC plate is made up of a thin layer of Silica gel 0.25mm with fluorescent indicator F_{254} with solvent system chloroform: methanol (9.5:0.5) was used for TLC analysis. The strip or plate is then placed with this end dipping in to the solvent mixture, taking care that the sample spot/zone is not immersed in the solvent. As the solvent moves towards the other end of the strip, the test mixture separates into various components. This is called as the development of TLC plates. The separation depends on several factors, the plate is removed after an optimal development time and dried and the spots/zones are detected using UV chamber and R_f value is calculated using

R_f = Distance moved by compound /distance moved by solvent.

Gas chromatography mass spectroscopy analysis

Preparation of extract

10mg/ml methanolic extract of *A. ferruginea* thorns were prepared in methanol solvent and in that 1 µl extract was employed for GC-MS analysis.

Instruments and chromatographic conditions

GC-MS analysis of *Accacia ferruginea* extract was performed using a Thermo GC-MS Clarus 500 (Perkin Elmer). For MS detection, the MS DSQ II electron ionization mode with ionization energy of 70 eV was used, with a mass range at m/z 50-650. Restek RtxR-5M Scapillary column (30m x 0.25mm, film thickness=0.25) 5% diphenylamine/95% dimethyl polysiloxane) was used for the analysis. The initial column temperature was programmed at 60°C/5min, respectively. The GC injector and MS transfer line temperatures were set at 280°C and 290°C respectively. GC was performed in the splitless mode. Helium (at flow rate=1.0 ml/min) was used as the carrier gas. A 1.0 µL injection volume was used. The plant extract was dissolved in methanol and filtered with polymeric solid phase extraction (SPE) column and analysed in GCMS for different constituents. Using computer searches on a NIST REFPROP Version 9.1 database and comparing the spectrum obtained through GC-MS compounds present in the plants sample were identified.

Identification of bioactive constituents

Interpretation on Mass Spectrum GC-MS was carried out by using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular formula, weight and chemical structure of the components of the test materials were ascertained.

RESULTS

The results of thin layer chromatography of the methanolic extract of *Accacia ferruginea* thorns studied under 3 different lights to identify the elution of the fractions present in the plants (Figure 1). The obtained chromatograms of the fraction were identified under 254 wavelength, 366 wavelength and visible light, exhibited maximum 0.6 retention factor (RF) value of the plant extract in F_{254} wave length in dark blue colour, in F_{366} wavelength and in visible light not shown

any chromatograms and RF values. The identified wavelength can be used for the separation of fraction by preparative TLC to obtain the yield for further studies.

The GC-MS chromatogram of the extract is shown in Figure 2. GC-MS analysis resulted in identification of 37 different metabolites. Compound identification was done in comparison with the reference standards present in NIST and Wiley 9.1. Some of the bioactive were analysed with their respective % of area present and retention time. Among the obtained chromatograms, found maximum % of area by Methyl mannose (57.14), Phenol, 2-methoxy-3-(2-propenyl)- (4.85), stigmasterol (4.65), 1-Hexacosanol (3.83), Lupeol (3.60), gamma Sitosterol (3.52), Phenol, 4-[2-(dimethylamino)ethyl]- (3.17), Ergost-5-en-3-ol, (3.beta.)- (2.53), Oleic acid (2.44), Oleoyl chloride (1.53), Sucrose (1.82) and remaining 26 molecules exhibited below 1 % of area in respective retention time. It is found that some of the bioactive components are notably responsible for the cytotoxicity activities by these molecules and also reported for the other various biological activities by these molecules and these bioactive molecules listed with their biological action in Table 1.

GC-MS chromatogram of the methanolic extract of *Acacia ferruginea* (Figure 2) showed 37 peaks with different retention time and these are indicating the presence of thirty seven bioactive molecules. The total numbers of molecules identified in the methanolic extract were differentiated by retention time (RT) and their % of peak of the individual molecules in GC-MS studies. The active principles with their name of the molecules, retention time (RT), concentration (peak area

Table 1: Presence of bioactive molecules in methanolic extract of *Acacia ferruginea* thorns and their biological action.

Sl. No.	Bioactive molecules names	Percentage area	Biological action	References
01	Cyclopropane carboxylic acid	0.26	High insecticidal activity or acaricial properties, highly insecticidal activity	[14]
02	1,2-Benzenedicarboxylic acid	0.31	Cytotoxic Anti-microbial and Anti-fungal activity	[15]
03	2-Hydroxy-gamma-butyrolactone	0.38	Cytotoxic, may cause acute respiratory irritation, causes central nervous system (CNS) depression and Low acute toxicity	[16]
04	Gamma Sitosterol	3.52	Larvicidal to *Spodoptera litura* when treated along with endotoxin of *Bacillus thuringiensis*	171 [17]
05	Hexacosanol	3.83	Insect repellent, larvicidal and neurotoxic activity	[18]
06	Stigma sterol	4.65	Liver disease, Jaundice, Arthrosclerosis activity, Cytotoxic to *Spodoptera litura* insect repellent, larvicidal and neurotoxic activity	[18,19]
07	Phenol derivatives	8.02	Anti-herbivores, can inhibit the activity of enzyme by binding to the gut of herbivores, defensive compound against herbivores, and phenollc compound affect the development of larva *Spodoptera litura*.	[20-22]
08	4-O-Methyl mannose	57.14	Cytotoxic, antimicrobial and anti-larvicidal activity	[23]
09	Ergost-5-en-3-ol, (3.beta.)-	2.53	Liver disease, Jaundice, Arthrosclerosis activity	[19]
10	Oleic acid	2.44	Antibacterial and antifungal	[24,25]
11	Oleoyl chloride	1.53	Antimicrobial activity	[26]
12	Sucrose	1.82	Antimicrobial and cytotoxic activity	[27]
13	Lupeol	3.60	Antiinflammatory, antimicrobial, antiprotozoal, antiproliferative, antiinvasive, antiangiogenic and cholesterol lowering agent	[28]

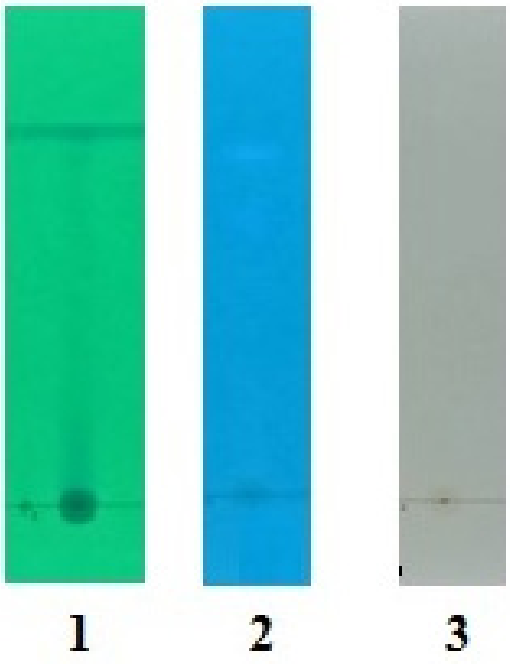


Figure 1: TLC chromatogram at 1) 254 wavelength, 2) 366 wavelength and 3) visible

%), molecular formula, molecular weight and molecular structures were presented in Table 2. The results revealed that methyl mannose (57.14), Phenol, 2-methoxy-3-(2-propenyl)- (4.85), stigmasterol (4.65), 1-Hexacosanol (3.83), Lupeol (3.60), gamma Sitosterol (3.52), Phenol, 4-[2-(dimethylamino)ethyl]-(3.17), Ergost-5-en-3-ol, (3.beta.)- (2.53), oleic acid (2.44), oleoyl chloride (1.53), sucrose (1.82) and remaining 26 molecules exhibited below 1 % of area in respective retention time.

DISCUSSION

The study of TLC chromatograms may helpful for the isolation of pure fractions, methanolic extract of *Accacia ferruginea* thorns obtained chromatograms of the fraction were identified under 254 wavelength exhibited maximum 0.6 retention factor (R_f) compared to other wavelength and used lights. The ultra violet (UV) analysis showed wavelength range from 220nm-750nm. Omodara *et al.*[29] described the UV spectroscopy importance in TLC studies for identification of fraction of the unsaturated bonds which was present in the plant extracts and it can be used to differentiate between the conjugated and nonconjugated structure. The principle of absorption mechanism to the structure of molecules can be derived. Similar studies observed by Alebiosu and Yusuf on the result of the UV analysis of the fractions eluted the absorption peaks at 220nm (n-hexane), 410nm (chloroform), 375nm (ethyl acetate), 390 (n-butanol) and 220nm (methanol).[30] Many fractions having the

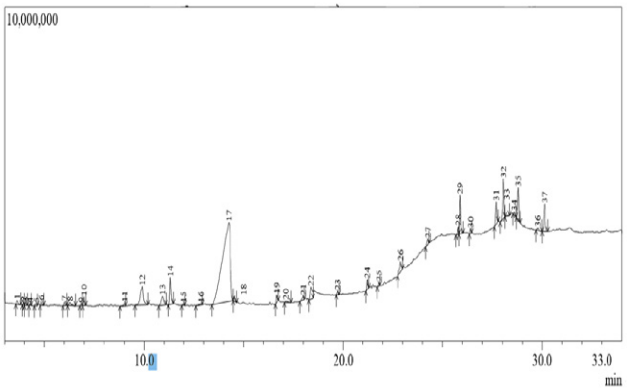
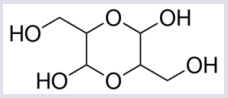
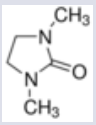
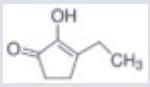
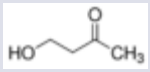
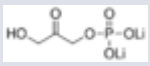
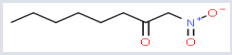
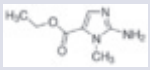
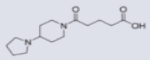
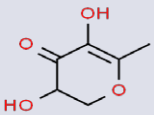
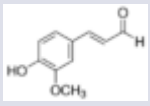
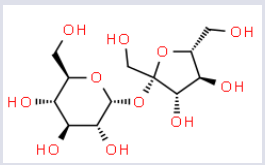
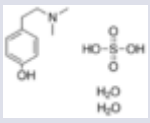
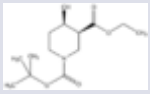
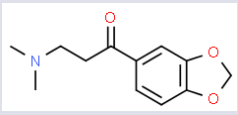


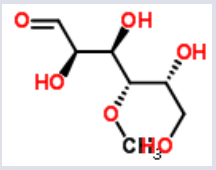
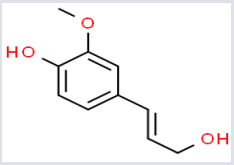
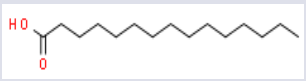
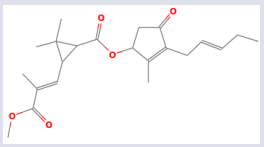
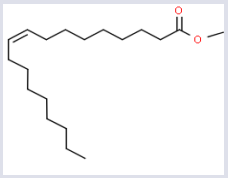
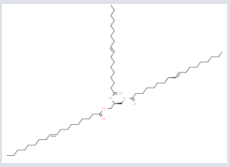
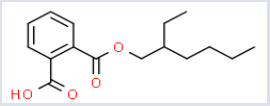
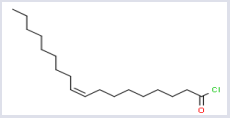
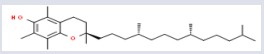
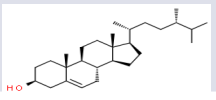
Figure 2: GC-MS analysis of methanolic extract of *Acacia ferruginea* thorns.

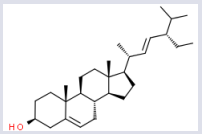
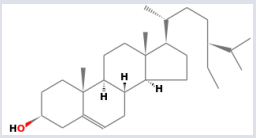
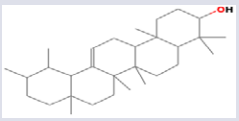
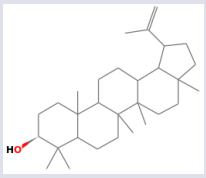
chromophores affinity and can be referred that the molecules present by the absorption takes place to let transit it as coloured peaks. The various solvent extracts of *Asparagus officinalis* is applied to TLC, separation of fraction spots were observed under day light in a lower wavelength at 254nm and higher wavelength 365nm with selected solvent systems, and their R_f values were calculated by comparing the standard drugs of rutin and quercitin. TLC and qualitative analysis of phytochemicals exhibited the presence of active constituents and secondary metabolites.[31]

The methanolic extract of *A. ferruginea* thorns further studied for GC-MS and found the data of bioactive molecules, in that presence all the molecules represented as carbohydrates and hydroxy fatty acid moieties. From the result data we can anticipate the purity of the molecule exhibited as only one maximum i.e., 4-O-Methyl mannose (57.14 % of area), other 10 molecules exhibited altogether (31.94 % area) and rest of them exhibited (10.92 % area) 26 molecules. Based on the literature of each molecules have their own biological action, listed in Table 1 with few of the molecules and in our studies also proven with cytotoxic and insecticidal activity.[32] Patil *et al.*[33] reported for the mixture of hexadeconic acid, 9,12-octa dedeconic acid, ß-Sitosterol and oleic acid in the structure of these molecule are in accordance with the proposal made further possible mixture of fatty acids were present in the isolated sample of *Citrus medica* seeds. Sowndhararajan *et al.*[34] revealed the GC-MS and liquid chromatography and mass spectroscopy (LC-MS) analysis of acetone extract of *A. ferruginea* bark and shown the presence of 12 bioactive molecules such as catechin, procyanidin B1, quercetin, ellagic acid, rosmanol, etc. The methanolic extract of *A. ferruginea* aerial parts constituents such as quinone (37.3%), quinoline (22.9%), imidazolidine (6.4%), pyrrolidine (4.5%) and cyclopentenone (3.5%) were

Table 2: GC-MS analysis of methanolic extract of *Acacia ferruginea* thorns and their name of the molecules, molecular weight, formula and structure.

Peak. No.	Name of the molecules	Retention time	Peak area (%)	Molecular formula	Molecular weight	Molecular structure
1	dl-Glyceraldehyde dimer	3.617	0.55	$C_6H_{12}O6$	180.16	
2	Urea, 1-methylcyclopropyl-	3.962	0.24	$C_5H_{10}N_2O$	114.15	
3	2-Cyclopenten-1-one, 2-hydroxy-	4.043	0.24	$C_7H_{10}O_2$	126.15	
4	2-Butanone, 4-hydroxy-3-methyl-	4.267	0.12	$C_5H_{10}O_2$	88.11	
5	2-Propanone, 1,3-dihydroxy-	4.589	0.27	$C_3H_6O_3$	181.92	
6	2-Hydroxy-gamma-butyrolactone	4.835	0.38	$C_4H_6O_3$	102.09	
7	2-Octanone, 1-nitro-	6.010	0.39	$C_8H_{15}NO_3$	173.21	
8	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	6.285	0.60	$C_6H_7N_3O_2$	153.14	
9	Pentanoic acid, 4-oxo-	6.834	0.18	$C_5H_8O_3$	116.11	
10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.982	0.83	$C_6H_8O_4$	144.12	
11	Hexadecane	9.020	0.32	$C_{16}H_{34}$	226.44	
12	Phenol, 2-methoxy-3-(2-propenyl)-	9020	4.85	$C_{10}H_{12}O_2$	164.2	
13	Sucrose	10.924	1.82	$C_{12}H_{22}O_{11}$	342.3	
14	Phenol, 4-[2-(dimethylamino) ethyl]-	11.322	3.17	$C_{10}H_{15}NO$	165.23	
15	N-Ethyl-4-hydroxypiperidine	11.892	0.19	$C_7H_{15}NO$	129.199	
16	1-Propanone, 1-(1,3-benzodioxol-5-yl)-3-(dimethylamino)-	12.862	0.25	$C_{12}H_{15}NO_3$	221.25	

17	4-O-Methyl mannose	14.266	57.14	$C_7H_{14}O_6$	194.18	
18	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	14.538	0.50	$C_{10}H_{12}O_3$	180.2	
19	Pentadecanoic acid	16.672	0.75	$C_{15}H_{30}O_2$	242.4	
20	Cyclopropanecarboxylic acid, 3-(3-methoxy-2-methyl-3-oxo-1-propenyl)-2,2-dimethyl-, 3	17.129	0,26	$C_{21}H_{28}O_5$	360.4	
21	9-Octadecenoic acid (Z)-, methyl ester	19.001	0.49	$C_{19}H_{36}O_2$	296.5	
22	Oleic Acid	19.736	2.44	$C_{18}H_{34}O_2$	282.5	
23	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	21.217	0.28	$C_{39}H_{76}O_5$	625	
24	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	21.811	0.92	$C_{57}H_{104}O_6$	885.4	
25	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	22.888	0.31	$C_{16}H_{22}O_4$	278.34	
26	Oleoyl chloride	24.261	1,53	$C_{18}H_{33}ClO$	300.9	
27	1-Pentacosanol	25.775	0.72	$C_{25}H_{52}O$	368.7	
28	Hexatriacontane	25.867	0.71	$C_{36}H_{74}$	507	
29	1-Hexacosanol	26.404	3.83	$C_{26}H_{54}O$	382.7	
30	Vitamin E	26.404	0.42	$C_{29}H_{50}O_2$	430.7	
31	Ergost-5-en-3-ol, (3.beta.)-	27.688	2.53	$C_{28}H_{48}O$	400.7	

32	Stigmasterol	28.049	4.65	$C_{29}H_{48}O$	412.7	
33	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl) oxy]propyl ester	28.219	0.25	$C_{37}H_{70}O_5$	594.9	
34	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl) oxy]propyl ester	28.594	0.36	$C_{37}H_{70}O_5$	594.9	
35	gamma.-Sitosterol	28.791	3.52	$C_{29}H_{52}O_2$	432.7	
36	alpha.-Amyrin	29.778	0.41	$C_{39}H_{56}O_2$	556.9	
37	Lupeol	30.124	3.60	$C_{30}H_{50}O$	426.7	

identified as major bioactives. Also identified, reported for the derivatives of the extract like Hexadecanoic acid, propanoic acid, pyridine, pyrazole and pyrimidine. In the LC-MS studies, identified carboxamidine, imidazole, thiazole, catechin and coumarin derivatives were observed.[35]

CONCLUSION

In the present study isolated pure fraction at 254 wavelength in defined solvent system by the TLC and 37 bioactive constituents have been identified from methanolic extract of *Acacia ferruginea* thorns by GC-MS analysis. The presence of various bioactive molecules justified their importance by the biological action in the past, present and future perspectives of various ailments by traditional to modern system of practitioners. The above findings and their observations emphasize the *A. ferruginea* thorns are having rich carbohydrates and fatty acid moieties and some of the bioactive molecules are said to be as antiherbivore agents. These are pharmacologically important active biomolecules for the various commercial applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

µg: micro gram; **CNS:** Central nervous system; **GC-MS:** Gas chromatography mass spectroscopy; **LC-MS:** Liquid chromatography and mass spectroscopy; **mg/ml:** mili gram/mili litre; **min:** minute; **NFT:** Nitrogen fixing tree; **NIST:** National institute standard and technology; **nm:** nanometer; **RF:** Retention factor; **RT:** Retention time; **SPE:** solid phase extraction; **TLC:** Thin layer chromatography; **UV:** Ultra violet.

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