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Original Article

Evaluation of Anthelmintic Activity and GC-MS Characterization of *Urochloa distachya* (L.)

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ABSTRACT

Background: The helminthiasis parasitic worm present in the gastrointestinal tract of the animal cause infection and physiological damage. This occurs due to poor sanitation, impecunious personal hygiene, low socioeconomic position, and lack of education. **Aim:** *Urochloa distachya* (Poaceae) commonly called as signal grass and used as fodder for the animal. The present investigation was undertaken to investigate the phytochemicals and to study the anthelmintic activity. **Materials and Methods:** The plant material was extracted by using petroleum ether and methanol in Soxhlet apparatus and the obtained extract was subjected to GC-MS analysis for the identification of the bioactive components responsible for anthelmintic activity. The anthelmintic activity was performed against earthworms (*Eisenia fetida*) at concentrations 12.5, 25, and 50 mg/mL using piperazine citrate as standard. **Results:** The GC-MS analysis of the petroleum ether extract showed five major and ten minor compounds. The major compounds were identified as Stigmasterol (14.76%); α-Amyrin (12.47%); γ-Sitosterol (10.31%); 17-(1,5-Dimethyl-3-phen ylthiohex-4-enyl) -4,4,10,13,14-pentamethyl 2,3,4,5,6,7,10,11,12-(9.12%) and β-Amyrin (8.83%). The minor compounds were D:C-Friedo-B': A'-neogammacer-9(11)-ene, 3-methoxy-, (3β)-(8.28%); Tritetracontane (7.23%); Campesterol (4.68%); Phytol (4.05%); Cholesterol (3.39%); Hexadecanoic acid, methyl ester (2.20%); 2-Pyrrolidinone, 1-methyl-(1.46%); Oleic acid, 3-(octadecyloxy) propyl ester (1.27%); Squalene (0.84%) and 9,19-Cyclolanostane-3,7-diol (0.68%). The time taken for paralysis and death of *Eisenia fetida* parasite was noticed by methanolic and petroleum ether extract at a concentration of 50 mg/kg as compared to the standard drug. **Conclusion:** The anthelmintic activity of the plant was most achieved by methanolic extract which could be attributed to the presence of the Octadecanoic acid. Also, the anthelmintic active of the petroleum ether extract could be attributed to the antimicrobial, antibacterial, antifungal, and antiprotozoal activities of the Hexadecanoic acid, methyl ester; Phytol; Squalene; Cholesterol, and γ-Sitosterol.

Keywords: *Urochloa distachya*, GC-MS analysis, Anthelmintic activity.

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INTRODUCTION

Helminthiasis is an infectious disease of humans and other animals, in which parasitic worms live in the gastrointestinal tract and also other organs to induce physiological damage.[1] It is the most common infectious disease around the world's population infecting about 2 billion people. Children in developing countries are one of the most major burdens. Helminth parasites are classified into two types such as Heirlooms and Souvenirs that infect humans. Heirlooms are inherited from ancestors in Africa while Souvenirs are acquired from animals during contact through migration, evolution, and agricultural practices. Helminthes are divided into two major phyla which are nematodes and platyhelminths. Nematodes are roundworms that are soil-transmitted helminths and also include filarial worms which cause lymphatic filariasis and onchocerciasis. Platyhelminths which are also known as flatworms involve Fluke Schistosomes and pork tapeworm which causes cysticercosis. The soil-transmitted helminths such as *Ascaris lumbricoides* (Roundworm), *Trichuris trichuria* (Whipworm) and *Ancylostoma duodenale*, and *Necator americanus* (Hookworm) are arrived in the human body through contaminated soil that contains eggs of roundworm and hookworm. The larvae of hookworm could penetrate the skin directly.[2] The key risk factors of helminthiasis are poor sanitation, sub-standard personal hygiene, low socioeconomic position, lack of nail clipping, lack of access to health care, lack of education, etc. Many complications occur in helminthic infection which includes anemia, malnutrition, intestinal obstruction, growth impediment, developmental

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impediment, urinary bladder carcinoma, gastrointestinal hemorrhage, cholecystitis, pancreatitis, and blindness.[2] Evidence displays that there is synergism and antagonism in intestinal nematode and schistosome infection as well as filarial nematode infection and soil-transmitted helminth infection. A large number of epidemiological information designate that individual infected with a variety of species harbor heavier infection than individual infected with a single species.[3] The conventional drugs albendazole, mebendazole, and levamisole are used to both kill and expel the worms from the body. The commercially available anthelmintic drugs have a serious problem (GI disturbance, dizziness, headache, drowsiness, diarrhoea, nausea, vomiting, fever, and allergic reactions etc.) observed over the world. In this regard, medicinal plants have been effectively reducing parasite invasion in animals and making them propitious alternatives to common anthelmintics drugs.[4,5]

Urochloa distachya (Poaceae) commonly known as Signal grass. It is an annual grass with lance-shaped leaves, 2-3 racemes, and native to tropical Asia, Africa, and other parts of the world. This plant thrives in hot, humid climates, especially during monsoons. The flowers of this plant bloom all year round.[6]

Phyto-constituents present in the plant

The GC-MS analysis of methanolic extract of *U. distachya* exhibited valuable phytocomponents which have been reported in our previous study.[7,8] The methanolic extract obtained by hot method of extraction revealed 26 compounds. The major compounds identified were Z-7-Pentadecenol (31.37%); t-Butyl hydrogen phthalate (13.90%); Ricinoleic acid (11.88%); Methyl -12-hydroxy-9-octadecenoate (9.72%); Octadecanoic acid (3.14%); 4,6-Diphenyl-2-(2-hydroxyphenyl) pyrimidine (2.82%); Glycerin (2.69%); Eicosanoic acid, methyl ester (2.62%); 2-Naphthalenol, 2,3,4,4a,5,6,7-octahydro1,4adimethyl-7-(2-hydroxy-1-methylethyl) (2.34%); Cholest-5-en-3-ol (3á), tetradecanoate (2.26%); 6-Octadecenoic acid (2.10%).[7,8] Gas chromatography and mass spectrometry is the best accurate analytical technique which requires a trace amount of sample to identify various group of compounds like alkaloid, steroid, alcohol, hydrocarbons, nitro compounds, organic acid, ester, amino acid, etc.[9] The literature study revealed that many fodder plant are used as anthelmintic activity.[10] Therefore, the present study was undertaken to investigate the anthelmintic activity of *U. distachya* and the GC-MS analysis was carried to investigate the compounds responsible for this activity.

MATERIALS AND METHODS

Collection of plant material

The whole plants were collected from the local area of Hatgaon, Bargarh, Odisha, India, and authenticated by the Botanical Survey of India, Kolkata (BSI, Kolkata) bearing registration number CNH/Tech. II/2021/18/686.

Preparation of Plant Extracts

The collected plant materials (the entire part) were dried under the shade at room temperature. The dried plant materials were ground to a coarse powder and sieved to make the particle uniform. About 70 g of coarsely powdered drugs were loaded into the Soxhlet apparatus and extracted successively with petroleum ether and methanol. The extraction procedure was continued according to the boiling point of the solvents until all dissolved components were eluted. The extracts were concentrated with the help of a rotary evaporator and stored in a cool place in a desiccator.

Chemicals used

The different solvents petroleum ether and methanol were purchased from Central Drug House Pvt. Ltd., (New Delhi), piperazine citrate was procured from Harihar Pharmaceutical Pvt. Ltd., (SSP, Road, khamarmunda-767 065, Balangir, Odisha).

Collection of earthworms

The earthworms were collected from the department of horticulture Sonepur, Odisha, India. The species of earthworms are *Eisenia fetida* belonging to the family Lumbricidae.

Evaluation of anthelmintic activity

The anthelmintic activity was carried out as per the procedure described by Ishnava and Konar *et al.*[5] and Das, *et al.*[11] with some modifications. *Eisenia fetida* earthworms were taken for the test, because of their similar anatomical and physiological characteristic to the human intestinal parasite. The earthworms were cleaned with normal saline water. The methanolic extract of *U. distachya* (MUD) and petroleum ether extract of *U. distachya* (PUD) at concentrations 12.5, 25, and 50 mg/mL were dissolved in 2% DMSO solution. The standard drug piperazine citrate at concentration of 15 mg/mL was dissolved in 2% DMSO solution and used as positive control. The normal control group received 2% DMSO. The time taken for paralysis and death was determined whether the worms were neither moved when vigorously shaken nor when pricked by the help of syringe.

Gas chromatography-mass spectrometry (GC-MS) analysis

The petroleum ether and methanolic extract of *U. distachya* were subjected to GC-MS analysis which was carried out in Agilent 5977 MSD technology. The mass-spectrophotometer, fitted with a HP-5 MS fused silica column (Dimensions 30 m x 250 µm x 0.25 µm), interfaced with MSD. The carrier gas was used as Helium and the velocity flow of the column was adjusted 1.2mL/min. The temperature of the column ranges from 60°C-325°C (350°C) and the pressure was 11.367psi. The total run time of GC was 50.643 min. 1µl of the sample was injected through the injector and the mass was taken at 70eV. The quad temperature was set at 150°C and the source temperature was set at 230°C up to 10 min holding

Table 1: Anthelmintic activity with different extracts of *U. distachya* and standard drug.

Group	Treatments	Concentrations (mg/mL)	Time taken for paralysis (Mean ± SD) min	Time taken for death (Mean ± SD) min
Group-1	Control (2% DMSO)	-	-	-
Group-2	Piperazine Citrate	15	50.76 ± 0.40	66.93 ± 0.20
Group-3	MUD	12.5	59.86 ± 0.41	97 ± 0.20
Group-4	MUD	25	48.56 ± 0.85	89.50 ± 0.55
Group-5	MUD	50	33.76 ± 0.70	78.10 ± 0.55
Group-6	PUD	12.5	72 ± 0.30	85.93 ± 0.40
Group-7	PUD	25	62.36 ± 0.60	74.06 ± 0.20
Group-8	PUD	50	54.03 ± 0.05	66.93 ± 0.30

MUD: Methanolic extract of *U. distachya,* **PUD:** Petroleum ether extract of *U. distachya* Values are expressed as Mean ± Standard deviation (SD). Statistical significance and standard error were found by one-way ANOVA.

time. The quantity of the compound present in the petroleum ether and methanolic extract of *U. distachya* was expressed in the chromatogram as the percentage based.

Statistical analysis

The data were analyzed by both MS EXCEL (version 2019) and SPSS (version 21, IBM corporation). The data of anthelmintic activity were reported as mean ± standard deviation and the significant level *P* values less than 0.01, 0.05, and 0.001 were considered statistically significant.

RESULTS

In-vitro Anthelmintic Activity of different extract

The methanolic and petroleum ether extract of *U. distachya* showed paralysis as well as the death of worms at all the concentrations of the sample. It was observed that the methanolic extract and petroleum ether extract of *U. distachya* showed potent anthelmintic activity at different concentrations, when compared with the standard drug (piperazine citrate 15 mg/mL) in a dose-dependent manner. The time is taken for paralysis of methanolic extract at the concentration of 12.5, 25, and 50 mg/mL were 59.86 ± 0.41 min, 48.56 ± 0.85 min, 33.76 ± 0.70 min and the death time were 97 ± 0.20, 89.50 ± 0.55, 78.10 ± 0.55. The paralysis time taken of petroleum extract were 72 ± 0.30, 62.36 ± 0.60, 54.03 ± 0.05 and the death time were 85.93 ± 0.40, 74.06 ± 0.20, 66.93 ± 0.30 (Table 1). The standard drug, Piperazine citrate showed paralysis at 50-51 min and death time at 66-67 min in the concentration of 15 mg/mL (Table 1).

GC-MS analysis of petroleum ether extract of *U. distachya* plant

The petroleum ether extract of *U. distachya* were showed 15 compounds exhibiting various biological activities. The chromatogram of petroleum ether extract of *U. distachya* has depicted in Figure 1. Among 15 compounds, five major

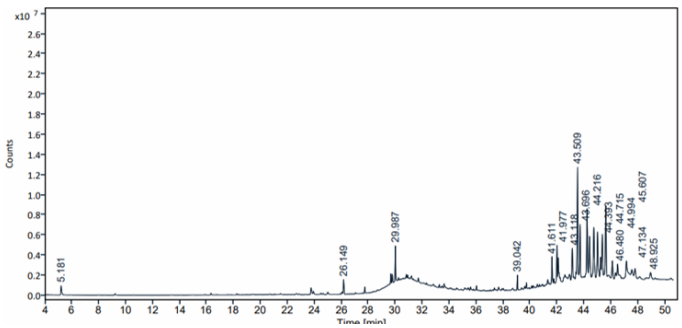


Figure 1: GC-MS chromatogram of petroleum ether extract of *U. distachya* F.

compounds were determined (Table 2). They are Stigmasterol (14.76) Figure 2B, α-Amyrin (12.47) Figure 3A, γ-Sitosterol (10.31) Figure 2D, 17-(1,5-Dimethyl-3-phenylthiohex-4-enyl) -4,4,10,13,14-pentamethyl 2,3,4,5,6,7,10,11,12-(9.12) Figure 3D, β-Amyrin (8.83) Figure 2E. The remaining 10 minor compounds were D:C-Friedo-B': A'-neogammacer-9(11)-ene, 3-methoxy-, (3β)-(8.28) Figure 3B, Tritetracontane (7.23) Figure 2C, Campesterol (4.68) Figure 2A, Phytol (4.05) Figure 4C, Cholesterol (3.39) Figure 4E, Hexadecanoic acid, methyl ester (2.20) Figure 4B, 2-Pyrrolidinone, 1-methyl-(1.46) Figure 4A, Oleic acid, 3-(octadecyloxy)propyl ester (1.27) Figure 3C, Squalene (0.84) Figure 4D, 9, 19-Cyclolanostane-3,7-diol (0.68) Figure 3E. The methanolic extract revealed 26 compounds which have been reported earlier.

DISCUSSION

The petroleum ether and methanolic extract of *U. distachya* showed anthelmintic activity in concentration dependent manner. The time taken for paralysis and death was best achieved by methanolic extract at a 50 mg/kg concentration as compared to Piperazine citrate. Many plants with nematicide activity have been reported as anthelmintic properties (Gad *et al.*, 2014). The compound 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z) in methanolic extract have been reported

Table 2: GC-MS analysis of petroleum ether extract of *U. distachya*.

Sl. No	R_t (min)	Name of the compound	Molecular formula	Molecular weight	Peak area%
01	5.181	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99.1311	1.46
02	26.148	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	2.20
03	29.986	Phytol	$C_{20}H_{40}O$	296.5	4.05
04	39.044	Squalene	$C_{30}H_{50}$	410.7	0.84
05	41.975	Cholesterol	$C_{27}H_{46}O$	386.7	3.39
06	43.119	Campesterol	$C_{28}H_{48}O$	400.7	4.68
07	43.513	Stigmasterol	$C_{29}H_{48}O$	412.7	14.76
08	43.701	Tritetracontane	$C_{43}H_{88}$	605.2	7.23
09	44.220	γ-Sitosterol	$C_{29}H_{50}O$	414.7067	10.31
10	44.720	β-Amyrin	$C_{30}H_{50}O$	426.7174	8.83
11	44.988	α-Amyrin	$C_{30}H_{50}O$	426.7174	12.47
12	45.601	D:C-Friedo-B': A'-neogammacer-9(11)-ene, 3-methoxy-, (3β)-	$C_{31}H_{52}O$	440.7	8.28
13	46.476	Oleic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	593.0	1.27
14	47.133	17-(1,5-Dimethyl-3-phenylthiohex-4-enyl) -4,4,10,13,14-pentamethyl 2,3,4,5,6,7,10,11,12-	$C_{36}H_{54}OS$	534.9	9.12
15	48.927	9,19-Cyclolanostane-3,7-diol	$C_{30}H_{52}O_2$	444	0.68

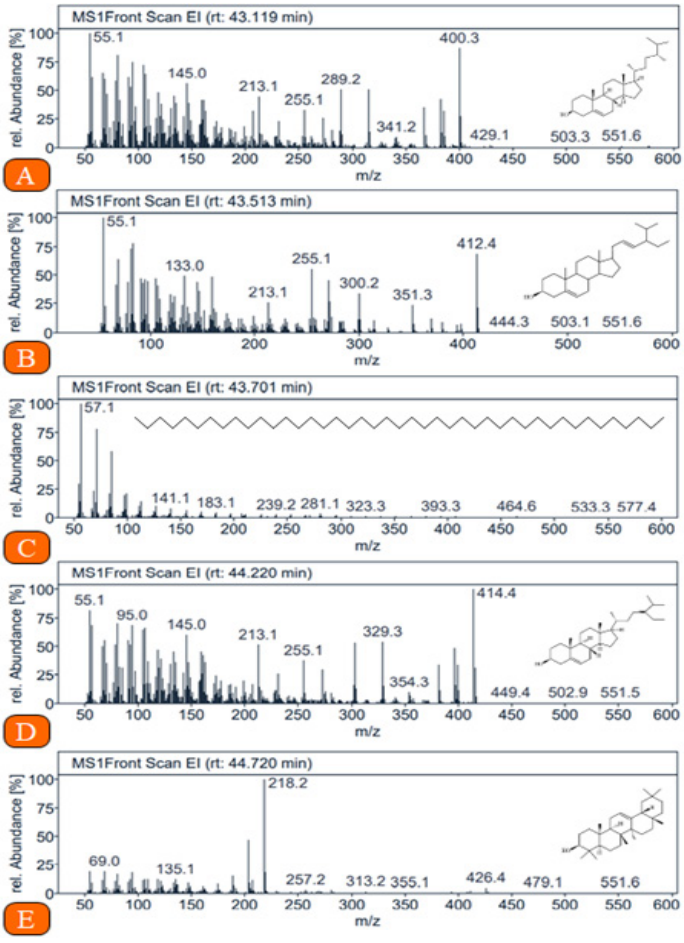


Figure 2: (A) Campesterol; (B) Stigmasterol; (C) Tritetracontane; (D) γ-Sitosterol (E) β-Amyrin.

as nematicide activity. Thus, the nematicide activity of the methanolic extract could be attributed the presence of these compounds.[12]

The GC-MS analysis of petroleum ether extract of the whole plant part of *U. distachya* (L.) have shown various therapeutic activities. The compound 2-Pyrrolidinone, 1-methyl-compound belonging to ketone group has shown anti-proliferative activity.[13] The compound Hexadecanoic acid, methyl ester which is fatty acid methyl ester showed various activities like antibacterial, antifungal, antioxidant, decreases blood cholesterol level, anti-inflammatory and also used as nematicide.[14-16] Diterpene compound like phytol possessed antibacterial, anti-fungal, anticonvulsant, anti-arthritis, insulin sensitizing, antidiabetic effect, anti-cancer, antioxidant, neuroprotective, antimicrobial, anti-inflammatory, and antidiuretic activities.[17,18] Triterpene Compound such as Squalene have hypolipidemic, hepatoprotective, cardioprotective, antioxidant and anti-toxicant activity, anticancer, antimicrobial, pesticides, sunscreen, immunostimulatory, antiaging, xenobiotic neutralizer, chemoprotective, atherosclerotic, diuretic, analgesic, hypo-diabetics and antibacterial.[19-21] Cholesterol is a steroid exhibits antifungal activity.[22] Campesterol is also a steroidal compound have anti-inflammatory and cytotoxic activity.[23] Stigmasterol which also belongs to sterol group previously reported anti-osteoarthritic, anti-hypercholesterolemic, anti-inflammatory, antioxidant, cytotoxicity, anti-tumor, hypoglycemia, antimutagenic, and CNS effects.[24] Alkyl group like, Tri-tetracontane has anti-inflammatory activity.[25]

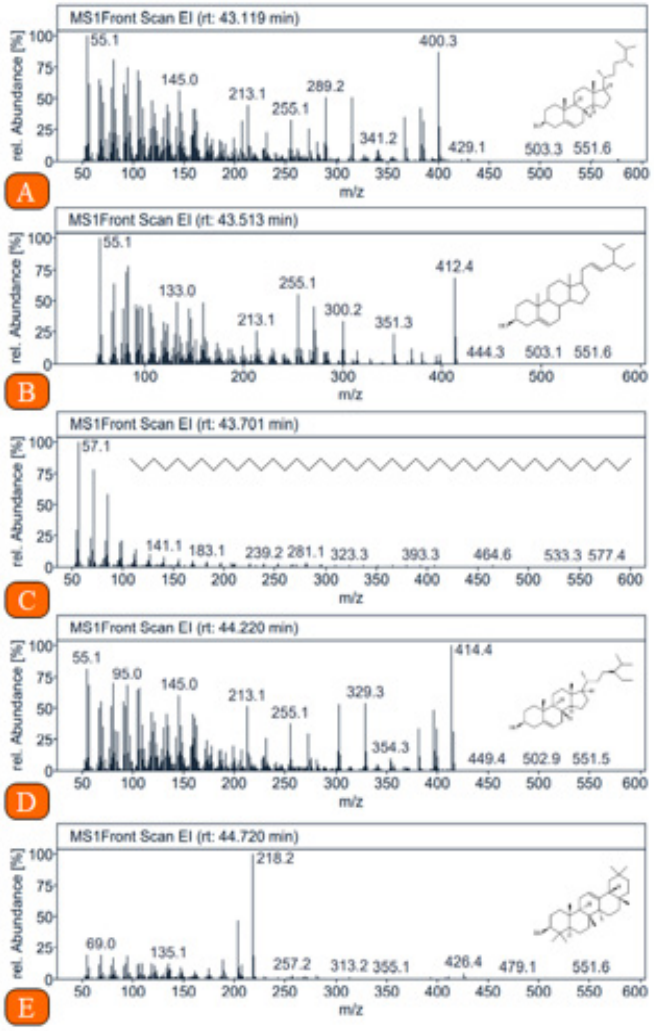
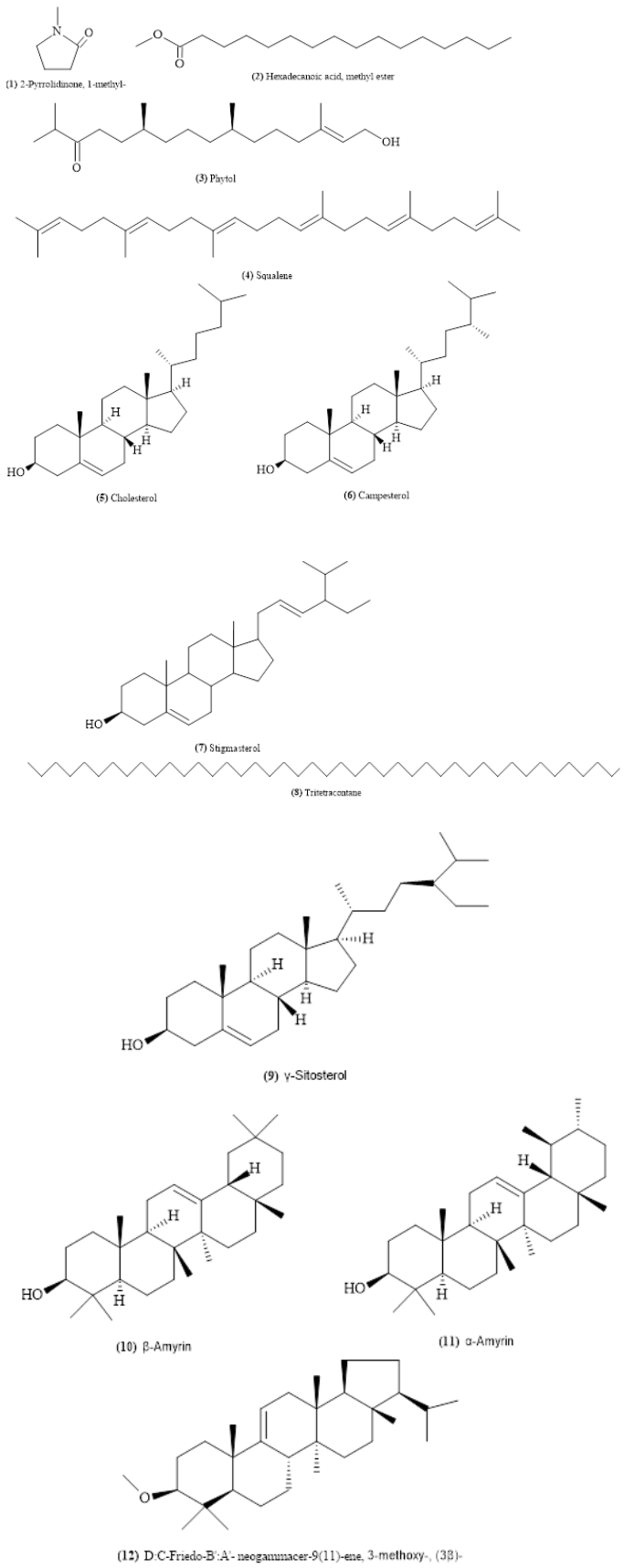
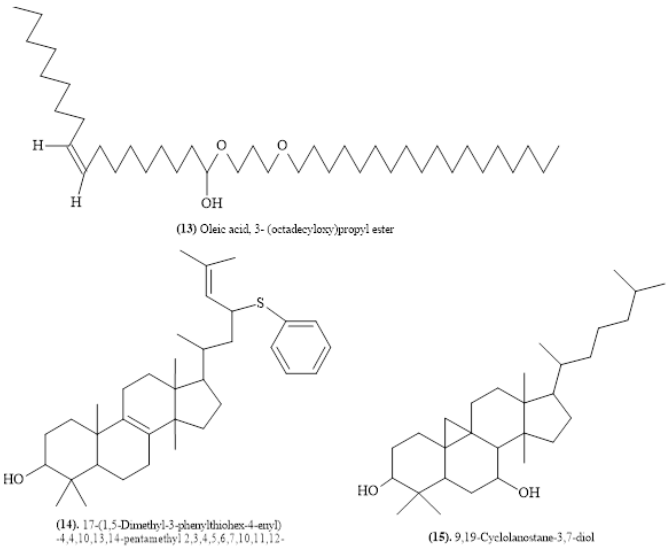


Figure 3: (A)α-Amyrin; (B)D:C-Friedo-B':A'-neogammacer-9(11)-ene, 3-methoxy-, (3β)-; (C)Oleic acid, 3-(octadecyloxy)propyl ester (D) 17-(1,5-Dimethyl-3-phenylthiohex-4-enyl) -4,4,10,13,14-pentamethyl 2,3,4,5,6,7,10,11,12-; (E)9,19-Cyclolanostane-3,7-diol.

Chemical structure of phytocomponents of GC-MS petroleum ether of *U. distachya*



γ-Sitosterol which is C-24 isomer of beta sterol shows a wide range of activities such as anti-hyperglycemic activity, anti-ulcer, anti-microbial, anti-pyretic, and hypolipidemic, hypercholesterolemic, anti-inflammatory, anti-viral (influenza), anti-cancer, anti-bacterial, anti-protozoal (Leishmania), hepatoprotective, antioxidant, anti-cancer, anti-angeogenic and anti-diarrhoeal.[21,26-29] β-Amyrin (ester group) have anti-tumor, anti-inflammatory, anxiolytic, and hepatoprotective effects. It also shows anti-microbial activity against *Streptococcus mutans, Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*.[30,31] α-Amyrin is a Tri-terpenoid compound shows anti-tumor, anti-inflammatory, anxiolytic and hepatoprotective, anti-microbial activities against *S. aureus*.[30,31] Fatty acid like Oleic acid, 3-(octadecyloxy) propyl ester shows anti-fungal effect.[32-34] 9,19-Cyclolanostane-3,7-diolis triterpene compounds have anti-inflammatory effects.[35]



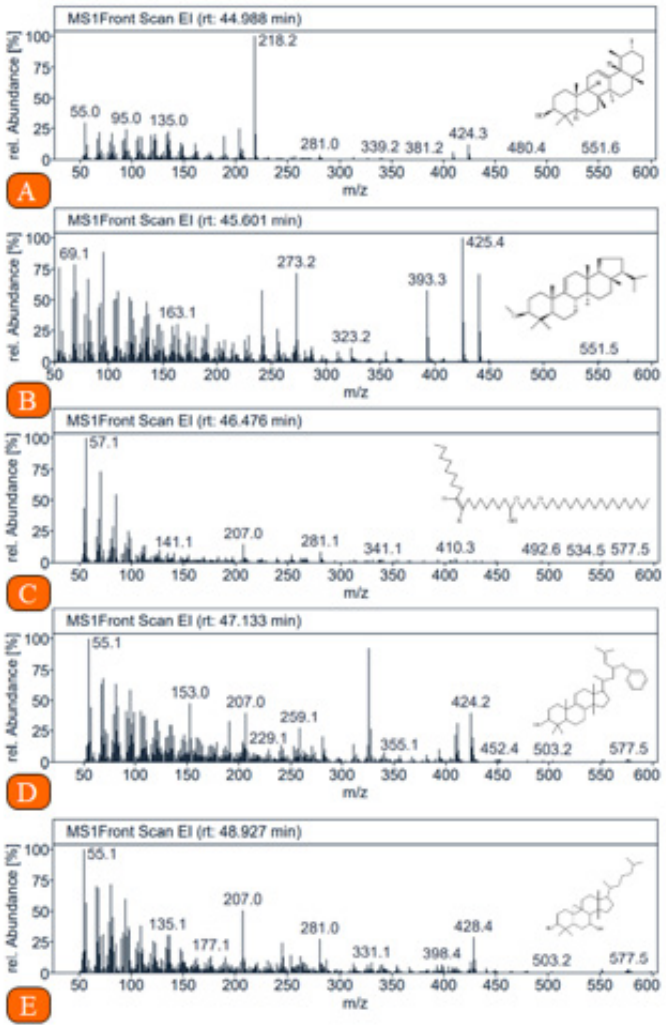


Figure 4: (A)2-Pyrrolidinone, 1-methyl-; (B)Hexadecanoic acid, methyl ester; (C)Phytol; (D) Squalene(E) Cholesterol.

CONCLUSION

The current investigation concluded that the methanolic extracts of *U. distachya* showed good anthelmintic activity against *Eisenia fetida* in concentration dependent manner. The nematicide activity of the compounds 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z) identified in GC-MS analysis might be responsible for anthelmintic activity of the plant. Further study is needed to isolate these compounds and evaluate their pharmacological activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

U. distachya: *Urochloa distachya* (L.); **GC-MS:** Gas Chromatography and Mass spectrometry; **HPLC:** High Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **MUD:** Methanolic extract of *U. distachyaI*; **PUD:** Petroleum ether extract of *U. distachya*.

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