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Phytochemical screening and GC-MS analysis of *Cyperus dubius*, Rottb. (*Cyperaceae*)

S Srinivasan and V Priya

Abstract
In the present study the investigation on phytochemical screening was carried along with GC-MS analysis to identify the phyto-constituents present in extracts of *Cyperus dubius*, Rottb. Phytochemical analysis in different extracts (methanol, hexane) revealed the presence of secondary metabolites like alkaloids, flavonoids, phenols, terpenoids, glycosides, Phytoserols, quinone. Whereas, the GC-MS analysis showed 30 peaks indicates the presence of 30 compounds and among these Stigmast-5-en-3-ol, (3.beta.)-[31.39%] was found to be major compound followed by Stigmasterol (9.40%), Guanosine (6.44%), Ergost-5-en-3-ol, (3.beta.)-(6.39%), Olivetol, dimethyl ether (5.75%), 1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid (4.39%), 2-methyl-2-[2-(2,6,6-trimethyl-3-methylene-cyclohex-1-enyl)-vinyl]-[1,3]dioxolane (4.14%), 9-octadecenoic acid (Z)-(3.96%), Methyl commate C (3.91%), 2,5-dimethoxybenzylamine (3.73%), 4-formyl-2-methoxyphenyl acetate (3.47%) and Gamma.-tocopherol (2.99%) which possess pharmacologically and industrially potential phyto compounds like antioxidants, phytosteroids and number of unsaturated fatty acids.

Keywords: *Cyperus dubius* Rottb, whole plant, preliminary phytochemical screening,

Introduction
The family *Cyperaceae* or Sedges is the third largest family among monocotyledons which have cosmopolitan in distribution and more concentration in tropics (Mishra and Chaunan 2013) [21]. They greatly resemble with grasses and rushes but they are characterized by stems with triangular cross sections and three ranked spirally arranged leaves (Ball *et al*., 2002) [7]. The grasses are well known for their food and fodder nature and number of research work has been carried on them. But therapeutic importance of the grasses especially in the *Cyperaceae* is least studied (Babu and Savithramma 2014) [4].

Cyperus dubius, Rottb. is a quite common plant. It is erect herb and tufted perennials with short rhizome; culms are slender, 15 to 40 cm long, densely tufted, longitudinally sulcate, triquetrous, base swollen into a bulb shape and clothed with many brown rudimentary membranous leaf sheaths. Leaves many, basal, linear, gradually acuminate at apex, shorter than to subequaling culm, sheath brownish membranous, flaccid and flat. Involucral bracts 3–5, leaf like, longer than inflorescence. Inflorescence capitate, sub-globose, with 1-3 spikes. Spikes dense with many spikelets ovoid to narrowly ovoid, rachilla broadly winged. Glumes pale to greenish on both surfaces but middle green, densly arranged. Stamens 3, anthers linear-oblong, connective prominent beyond anthers, Style of medium length, stigma 3. Fruits are nutlet dark grayish brown, obovoid to ellipsoid, 3-sided, densely punctate. It is a perennial herb growing in seasonally flooded regions and in pockets of soil in rocks. Mostly they do not confined to wetlands and is sometimes found as a weed in fields and near the sea on sandy beaches (Cook 1996); it is also seen in open shady places (Bhargavan, 1989) [8]. It is a common and widely distributed species. Medicinal values of this plant species are still not reported. It is used as cattle fodder (Kitto and Alexander, 2003) [16].

The literature research and study reveals that still no work have been conducted on this plant species. With this knowledge the present study was aimed to determine preliminary phytochemical screening and phytochemical profiling of the methanolic extract of *Cyperus dubius* Rottb using GC-MS.

(A) Habit **(B) Inflorescence**

(C) Sheathing base **(D) Fibrous root**

Fig 1: Morphology of *Cyperus dubius* Rottb.

Materials and Methods

Collection of the Plant material

The entire plant material *Cyperus dubius* Rottb. both underground and upper parts of the plant was collected during flowering stage (without any diseases) from chinnaru, kodanthur region, Udumalpet, Thirupur district, Tami Nadu. In the month of December 2018, the collected plant material was identified and authenticated by Botanical Survey of India, Southern regional center, Coimbatore.

Preparation of plant powder and extract

The entire plant of *Cyperus dubius* was properly washed and shade dried for 1 month. After complete drying, the plant sample was ground into fine powder mechanically and stored in the airtight container. 50 grams of air-dried plant material was subjected to extraction by soxhlet apparatus with 2 different polar solvents (Hexane & Methanol). Each time before extracting with the next solvent, the powdered material was dried. Obtained 2 different solvents plant extracts were stored in 2 separate sterile conical flasks for further analysis.

Phytochemical screening

Both methanolic and hexane extracts of plant sample was subjected into qualitative phytochemical analysis to identify different secondary metabolites like alkaloids, flavonoids, terpenoids, phenols, tannins, glycosides, Phytosterols, saponins, and quinone by the standard method described by Harborne, 1973.

GC-MS analysis

Gas chromatography-Mass spectrometry analysis of the extracts was performed using a GC-MS (Model; QP 2010 ultra-series, Shimadzu, Tokyo, Japan) equipped with thermal desorption system TD 20. Injection Mode: Split, Flow Control Mode: Linear Velocity, Pressure: 81.9 kPa, Linear Velocity: 40.5 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 50.0. For GC-MS detection [GC-2010], Helium gas (99.99%) was used as a carrier gas at a constant flow rate- total flow: 64.7 mL/min. and column flow: 1.21 mL/min. injector and mass transfer line temperature were set at 200 and 240°C respectively. The oven temperature was programmed Column Oven Temp.: 80.0 °C and Injection Temp.: 260.00 °C. Total running time of GC-MS is 46.28 minutes. The relative% amount of each component was calculated by comparing its average peak area to the total area, software adopted to handle mass spectra and chromatograms was a Turbo mass. The relative percentage of each extract constituents was expressed as percentage with peak area.

Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST), USA and WILEY- 8 library. Library possesses more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular structure and weight of the compounds of the test samples were ascertained.

Results and Discussion

Qualitative phytochemical screening of 2 different extracts of *Cyperus dubius* results are shown in Table-1. The results showed the presence of alkaloids, flavonoids, phenols, terpenoids, glycosides, Phytoserols, quinone and the absence of saponins and tannins in both extracts.

Table 1: Preliminary phytochemical screening of *Cyperus dubius*

S.no.	Secondary metabolites	Hexane extract	Methanolic extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Phenols	+	+
4	Tannins	-	-
5	Glycosides	+	+
6	Terpenoids	+	+
7	Steroids	+	+
8	Saponin	-	-
9	Quinone	+	+

Presence- (+); Absence (-)

GC-MS results in methanolic extract of *Cyperus dubius* (entire plant) was figured out in the Table-2 with their compound name, molecular formula, molecular weight, retention time and peak value (area percentage) and structure of the compound, medicinal properties are figured out separately in the Table-3 and Table-4. The results revealed that presence of 30 phyto compounds and among these Stigmast-5-en-3-ol, (3.beta.)-[31.39%] was found to be major compound followed by Stigmasterol (9.40%), Guanosine (6.44%), Ergost-5-en-3-ol, (3.beta.)-(6.39%), Olivetol, dimethyl ether (5.75%), 1,3,4,5-tetrahydroxy-yclohexanecarboxylic acid (4.39%), 2-methyl-2-[2-(2,6,6-trimethyl-3-methylene-cyclohex-1-enyl)-vinyl]-[1,3]dioxolane (4.14%), 9-octadecenoic acid (Z)-(3.96%), Methyl commate C (3.91%), 2,5-dimethoxybenzylamine (3.73%), 4-formyl-2-methoxyphenyl acetate (3.47%), Gamma.-tocopherol (2.99%), Cis-vaccenic acid (2.19%), Phenanthrene,7-ethenyl-1,2,3,4,4A,4B,5,6,7,9,10,10A-dodecahydro-1,1,4A,tetramethyl-[4AS-(4A.alpha.,4B.beta.,7.beta.,10A.beta)] (2.15%), Squalene (1.40%) and 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(1.16%).

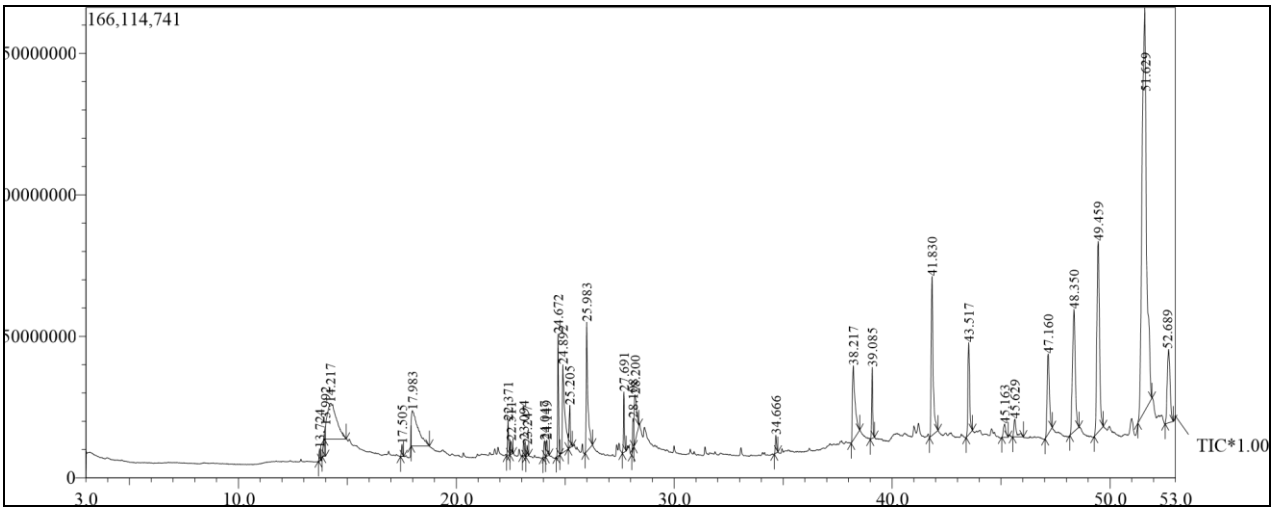
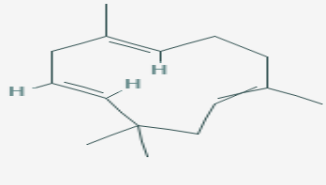


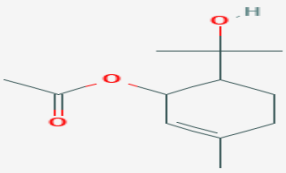
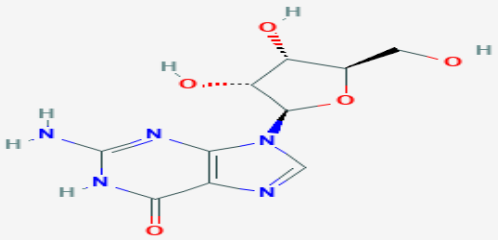
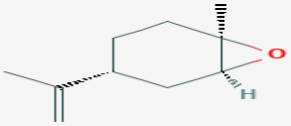
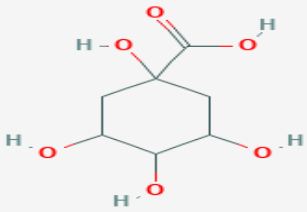
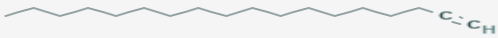
Fig 1: GC-MS chromatogram of the methanolic extract of *Cyperus dubius*

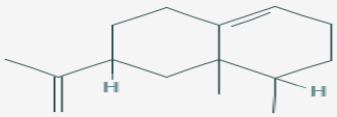
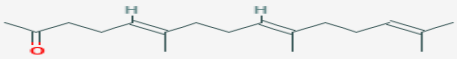
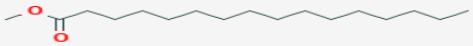
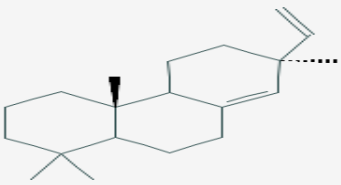
Table 2: Phyto compounds detected in the methanolic extract of *Cyperus dubius*

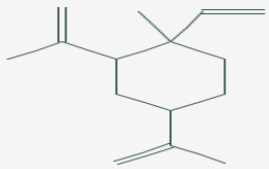
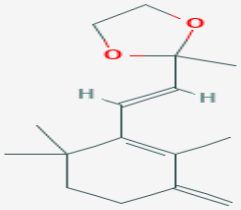
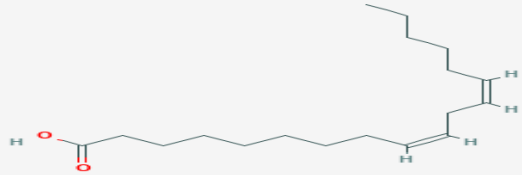
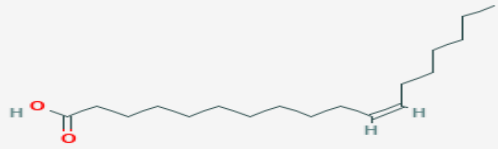
S.no	Compound name	Molecular formula	Molecular weight	Rt	Peak area
1	1,4,8-cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)-	$C_{15}H_{24}$	204.357	13.724	0.21
2	6-(1-hydroxy-1-methylethyl)-3-methyl-2-cyclohexen-1-yl acetate	$C_{12}H_{20}O_3$	212.289	13.992	0.93
3	Guanosine	$C_{10}H_{13}N_5O_5$	283.244	14.217	6.44
4	(+)-limonene oxide	$C_{10}H_{16}O$	152.237	17.505	0.24
5	1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192.167	17.983	4.39
6	1-Octadecyne	$C_{18}H_{34}$	250.47	22.371	0.68
7	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268.485	22.511	0.29
8	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.al	$C_{15}H_{24}$	204.357	23.094	0.27
9	9-eicosyne	$C_{20}H_{38}$	296.539	23.247	0.21
10	5,9,13-pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	$C_{18}H_{30}O$	262.437	24.047	0.40
11	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.457	24.149	0.42
12	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-, [4as-(4a.alpha.,4b.beta.,7.beta.,10a.beta.)]	$C_{20}H_{32}$	272.47	24.672	2.15
13	9-octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282.468	24.892	3.96
14	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1s-(1.alpha.,2.beta.,4.beta.)]-	$C_{15}H_{24}$	204.357	25.205	0.99
15	2-methyl-2-[2-(2,6,6-trimethyl-3-methylene-cyclohex-1-enyl)-vinyl]-[1,3]dioxolane	$C_{16}H_{24}O_2$	248.366	25.983	4.14
16	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296.539	27.691	1.16
17	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.452	28.108	0.65
18	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282.468	28.200	2.19
19	Pentadecanal-	$C_{15}H_{30}O$	226.404	34.666	0.39
20	2,5-dimethoxybenzylamine	$C_9H_{13}NO_2$	167.208	38.217	3.73
21	Squalene	$C_{30}H_{50}$	410.73	39.085	1.40
22	Olivetol, dimethyl ether	$C_{13}H_{20}O_2$	208.296	41.830	5.75
23	Gamma.-tocopherol	$C_{28}H_{48}O_2$	416.69	43.517	2.99
24	9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	$C_{32}H_{52}O_2$	468.766	45.163	0.61
25	2h-1-benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, acetate, [2R-[2R*(4R*,8R*)]]-	$C_{31}H_{52}O_3$	472.754	45.629	0.85
26	4-formyl-2-methoxyphenyl acetate	$C_{10}H_{10}O_4$	194.186	47.160	3.47
27	Ergost-5-en-3-ol, (3.beta.)-	$C_{28}H_{48}O$	400.691	48.350	6.39
28	Stigmasterol	$C_{29}H_{48}O$	412.702	49.459	9.40
29	Stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414.718	51.629	31.39
30	Methyl commate C	$C_{31}H_{50}O_4$	486	52.689	3.91

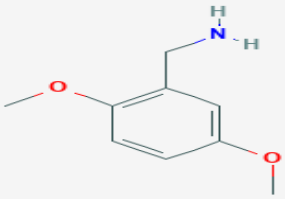
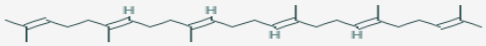
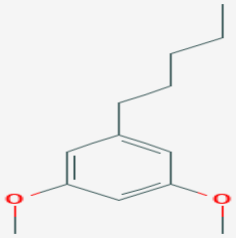
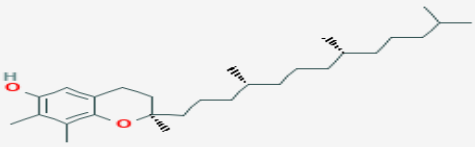
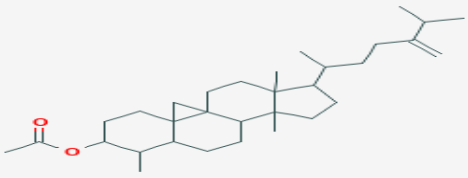
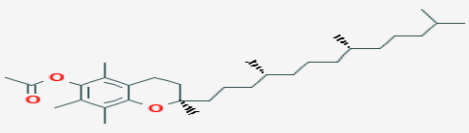
Table 3: Structure of the phytocompunds detected by GC-MS analysis in methanolic extract of *Cyperus dubius*

S.no	Compound name	Structure
1	1,4,8-cycloundecatriene, 2,6,6,9-tetramethyl-, (e,e,e)-	

2	6-(1-hydroxy-1-methylethyl)-3-methyl-2-cyclohexen-1-yl acetate	
3	Guanosine	
4	(+)-limonene oxide	
5	1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	
6	1-octadecyne	
7	Hexahydrofarnesyl acetone	

8	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1r-(1.alpha.,7.beta.,8a.al	
9	9-eicosyne	
10	5,9,13-pentadecatrien-2-one, 6,10,14-trimethyl-, (e,e)-	
11	Hexadecanoic acid, methyl ester	
12	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-, [4as-(4a.alpha.,4b.beta.,7.beta.,10a.beta.)]	
13	9-octadecenoic acid (z)-	

14	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1s-(1.alpha.,2.beta.,4.beta.)]-	
15	2-methyl-2-[2-(2,6,6-trimethyl-3-methylene-cyclohex-1-enyl)-vinyl]-[1,3]dioxolane	
16	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-	
17	9,12-octadecadienoic acid (z,z)-	
18	Cis-vaccenic acid	
19	Pentadecanal-	

20	2,5-dimethoxybenzylamine	
21	Squalene	
22	Olivetol, dimethyl ether	
23	Gamma.-tocopherol	
24	9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	
25	2h-1-benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, acetate, [2r-[2r*(4r*,8r*)]]-	

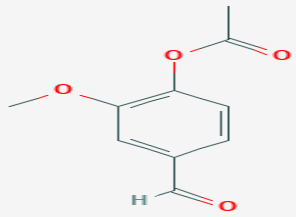
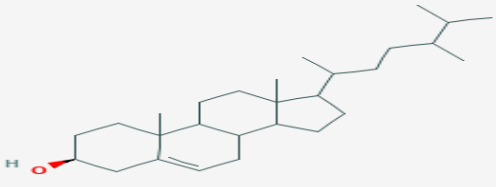
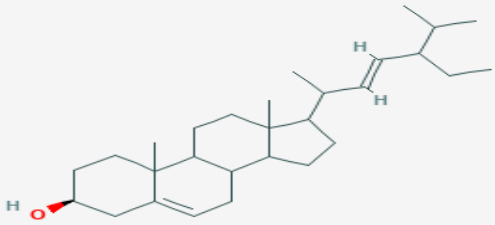
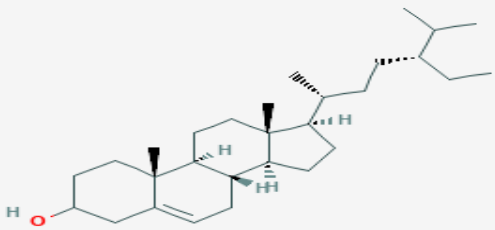
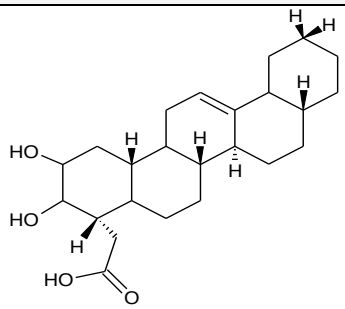
26	4-formyl-2-methoxyphenyl acetate	
27	Ergost-5-en-3-ol, (3.beta.)-	
28	Stigmasterol	
29	Stigmast-5-en-3-ol, (3.beta.)-	
30	Methyl commate c	

Table 4: Medicinal properties of the phyto compounds isolated in the GC-MS analysis in methanolic extract of *Cyperus dubius*

S.no	Compound name	Medicinal properties
1	1,4,8-cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)-	• Anti-inflammatory (Passos *et al*., 2007, Fernandes *et al*., 2007) [11, 25, 11, 25]
2	Guanosine	• Endogenous modulator of glutamatergic excitotoxicity and promote neuro protection (Reus *et al*., 2016)
3	1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	• Antimicrobial and anti-inflammatory properties (Mujeeb *et al*., 2014) [22]
4	9-eicosyne	• Antimicrobial properties (Aadesariya *et al*., 2019) [1]
5	Hexadecanoic acid, methyl ester	• Antioxidant, antimicrobial, anti-fibrinolytic, haemolytic, anti-alopecic, lubricant, nematicide, alpha reductase inhibitor, flavouring agent (Ponnamma and Manjunath 2013)

		• Hypocholesterolemic (Kumbum and sivarao 2012) [19] • Used in perfumes and cosmetics (Rao and Naika *et al*., 2017)
6	9-octadecenoic acid (Z)-	• Antiviral property (Wafaa *et al*., 2007) [32]
7	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1s-(1.alpha.,2.beta.,4.beta.)]-	• Antimicrobial properties (Arun kumar and Paridhavi 2013) [3]
8	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	• Antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immunostimulatory, anti-diabetic, used in cosmetics, shampoos, household cleaners (Kumbum and sivarao 2012) [19] • Nonmutagenic, common food additive, antischistosomal properties (de Moraes *et al*., 2014) [9] • Used in vaccine formulations (Krishnamoorthy subramaniam 2014) [18]
9	9,12-octadecadienoic acid (Z,Z)-	• Antioxidant (Ha and Pariza 1990) [24] • Antiarthritic and anti-inflammatory properties (Jones 2012) • Used in commercial preparation of oleates and lotions and as pharmaceutical solvent (Dirckx 1997) [10]
10	Cis-vaccenic acid	• Used as chemotaxonomic marker (Kleiman and Payne-Wahl 1984)
11	Pentadecanal-	• Antiviral property (Wafaa *et al*., 2007) [32]
12	Squalene	• Antibacterial, antioxidant, pesticide, antitumour, cancer preventive, immunostimulant, chemopreventive, lipogenase inhibitor activity (Sermakkani and Thangapandian, 2012) [31] • Anti-inflammatory, anti-atherosclerotic, anti-neoplastic, adjuvant activities, neutralize different xenobiotics (Kumbum and Sivarao 2012) [19] • Used to make chemicals such as drugs and rubber chemicals. It is used in cosmetics and moisturizers (Thomson and Montvale 2008) [27]
13	Olivetol, dimethyl ether	• Used as aromatics, intermediates, pharmaceuticals, fine chemicals and intermediate in various syntheses of tetrahydrocannabinol (Bailey and Toft, 1973, Poldy *et al*., 2009) [6]
14	Gamma.-tocopherol	• Antioxidant, anti-inflammatory, anticancer, hypocholesterolemic, cardioprotective (Ponnamma and Manjunath 2012) [28]
15	9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)- (Cycloeucalenol acetate)	• Antioxidant activity (Kandasamy *et al*., 2014) [15]
16	2h-1-benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, acetate, [2R-[2R*(4R*,8R*)]]- (Vitamin-E acetate)	• Stable form of vitamin-E, antioxidant, used in cosmetic formulations for skin care benefits (Kalaivanan *et al*., 2015) [14] • Wound healing properties (Pannin *et al*., 2004)
17	4-formyl-2-methoxyphenyl acetate (Vanillin acetate)	• Used as flavouring agent • Used in the preparation of 2-nitrohomovanillic acid, 2-nitrovanildin acetate (Macdonald, 1948) [20], 2-nitro-3,4-dimethoxybenzaldehyde (Weinstock *et al*., 1967) [33]
18	Ergost-5-en-3-ol, (3.beta.)-	• Antimicrobial, anti-inflammatory (Mujeeb *et al*., 2014) [22] • Antioxidant and hypo cholesteremic properties (Ponnamma and Manjunath 2012) [28]
19	Stigmasterol	• Anticancerous phytosterol (Bradford and Awad, 2007) • Antioxidant and antibacterial properties (Mujeeb *et al*., 2014) [22]
20	Stigmast-5-en-3-ol, (3.beta.)-	• Antimicrobial, antioxidant,anti-asthmatic and diuretic (Mujeeb *et al*., 2014) [22] • Anti-inflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and oestrogenic effects. Beta-sitosterol is mainly known and used for its cholesterol lowering property (Patra *et al.*, 2010) [26]
21	Methyl commate C	• cytotoxic, antibacterial, antimicrobial, antiviral, insecticide, nematicide anticoagulant, hemolytic, antiparasitic wound healing and antitumor activities (Bahrami and franco, 2016) [5].

Conclusion

The present study helps in acquiring knowledge about phytochemical compounds in the plant species *Cyperus dubius*, Rottb. The screening reveals that the plant possess pharmacologically active phytoconstituents. The secondary metabolites like alkaloids, flavonoids, phenols, terpenoids, sterols, glycosides are biologically active compounds. GC-MS analysis of whole plant methanolic extract results in Identification 30 different phyto compounds. Which possess pharmacologically and industrially potential phyto compounds like antioxidants, phytosteroids and number of unsaturated fatty acids. There is no literature available in this plant *Cyperus dubius*, Rottb. The phyto compounds in the plant which has been medicinally utilized was not yet reported. Hence further more elaborate pharmacological and physiochemical studies are needed.

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