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Phytochemical analysis of *Commelina diffusa* Burm. F. through GC-MS method

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Abstract
The plant *Commelina diffusa* Burm. f. (*C. diffusa*) is the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Hence we have selected shoots of *C. diffusa* for premilinary phytochemical study and GC-MS analysis. For the premilinary phytochemial analysis dried powdered shoots of *C. diffusa* was extracted with methanol, aqueous and petroleum ether. Prepared extracts were used qualitative phytochemical tests and the methanol extracts were used for GC-MS. In the preliminary phytochemical test of the methanolic extract of shoots were showed higher amount of steroids, alkaloids, phenolic compounds, tannins, saponins, amino acid and phytosteroids. And the Gas chromatography - mass spectrometry results revealed presence of 21 phytoconstituents in methanol extract.

Keywords: *C. diffusa*, GC-MS, phytochemical, secondary metabolites

1. Introduction
Commelina diffusa belongs to family commelinaceae. It is also known as climbing dayflower or spreading dayflower. The weeds are commonly found in many crop fields during June to September 1998. In India, almost 95% of the prescriptions have been reported to be plant based in the traditional systems of unani, siddha, ayurveda and homeopathy [1, 2]. This herb is also distributed throughout Bangladesh and other South Asian countries. In Chinese traditional medicine it is used to reduce swelling and inflammation. In different countries of Asia, Africa and America it is commonly used in urinary tract infections, to remove cough with sticky phlegm and in diarrhea, hemorrhoids, enteritis, eye irritation, conjunctivitis and other eye problems like ophthalmia [3-5].
Phytochemicals are present in plant have healing properties. *C. diffusa* contain minerals, vitamins, alkaloids, saponins, phenols, tannins, phytosterols, triterpens, terpenoids as secondary metabolites. Plants produce diverse array of low molecular weight products [6-8]. These phytochemicals are responsible for pharma industry and at higher concentration toxic to animals [9].
As the aerial parts of the plants have many pharmacological activity, hence the present investigation to evaluate that phytoconstituent of aerial parts of the plant with the help of preliminary phytochemical study and GC-MS, which will help for further pharmacological evaluation.

2. Materials and Methods
2.1 Plant Material: The plant sample, *C. diffusa* was collected from field side of the thanthonimalai and the college campus of Government Arts College, Karur.

2.1.1 Preparation of plant extract: Fresh plants of *C. diffusa* were collected, washed and air dried at room temperature. The dried material was homogenized to obtain a coarse powder and stored in air-tight bottles. About 20 gm of the powdered material was subjected 3 different extracts like methanol, aqueous and petroleum ether and for the further study filtrates were used. The methanol extract was employed in GC-MS analysis [10-13].

2.32Preliminary phytochemical analysis
The phytochemical analysis of the plant was carried out by the standard methods for the preliminary phytochemcial analysis the plant extracts were screened for the presence of bioactive compounds such as alkaloids, flavonoids, carbohydrates, phytosterols, proteins, phenolics, tannins, saponins and terpenoids [14-16].

2.3 GC-MS analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol extracts of *C. diffusa* were performed using a GC-MS (Model; Thermo GC – Trace Ultra) equipped with a VF 5ms fused silica capillary column of 30m length, diameter 0.25µm film thickness. The column over temperature was programmed from 70ºC to 260ºC for 6ºC/min. Ionization of the sample components was performed in electron impact mode (EI, 70 Ev). Helium (99.9995% purity) was the carrier gas fixed with a flow rate of 1.0ml min-1. The mass range from 40-1000 m/z was scanned at a rate of 3.0 scans/s. 1.0µL of the methanol extract of *C. diffusa* was injected to the GC-MS manually for analysis. Total running time of GC-MS is 40min. The relative percentage of the each extract constituents was expressed as percentage with pack area normalization [17-19].

2.4 Identification of components

The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library [20, 21].

3. Results

Phytochemicals play an important role in plant defense mechanism and the stress between interspecies protections, these phytochemicals are used as a traditional medicine. In the current study, phytochemical screening of the *C. diffusa* extracts revealed the presence of various phytochemical such as alkaloids, phenolic compounds, tannins, steroids, flavonoids, proteins and phyosteriods (Table 1). Among the three extracts, methanol extracts showed the presence of these compounds with varying degree of alkaloids, phenolic compounds, tannins, saponins and proteins. Whereas the other two extract namely chloroform and acetone showed highest degree of tannins, flavonoids, alkaloids, phenolic compounds respectively [22-25].

Table 1: Preliminary phytochemical compounds in *C. diffusa* from different extracts

Phytochemical studies	Methanol	Aqueous	Petroleum ether
Steroids	+	+	+
Tannins	+	-	-
Flavonoids	+	+	+
Amino acid	+	+	-
Proteins	-	+	-
Phytoseroids	+	+	-
Glycosides	+	+	-
Anthraquinones	-	-	-
Phenols	+	-	+
Alkaloids	+	+	-

The results pertaining to GC-MS analysis led to the identification of number of compounds from the methanolic extract of *C. diffusa*. These compounds were identified through mass spectrometry attached with GC. The sample of methanolic extract was run for 45 minutes. The spectrum profile of GC-MS confirmed the presence of 21 phytochemical components from *C. diffusa.*

The phytoconstituents identified were mainly sterols, terpenoids and alkanes. The major components detected were identified by GC-MS analyzed (Fig. 1). The bioactive components with their retention time (RT), molecular formula and area (%) in the methanol extract of *C. diffusa* Burm. f. are presented in (Table 2). The results revealed that 1-Phenyl-2-butanone (1.62%), Phenol, 4-ethenyl-, acetate (17.90%), 2-Methoxy-4-vinylphenol (0.26%), Piperidin-1-yl-acetic acid, hydrazide (0.33%), 2,6-Difluorobenzoic acid, 3,5-difluorophenyl ester (0.71%), 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)- (1.12%), Cyclopentaneundecanoic acid (2.60%), 1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2 en (0.77%), Cyclopentaneundecanoic acid (0.09%), Di-n-octylphthalate (1.37%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.31%), Cyclopropanedodecanoic acid, 2-octyl-, methyl ester (1.40%), n-Hexadecanoic acid (1.93%), 9,12-Octadecadienoic acid, methyl ester (10.67%), Phytol (0.91%), Methyl stearate (1.00%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (9.59%), Octadecanoic acid (41.49%), Hexadecanoic acid, 15-methyl-, methyl ester (0.02%), Carbonic acid, 2-dimethylaminoethyl propyl ester (0.15%), 9-(2',2'-Dimethylpropanoilhydrazono)- 3,6-dichloro-2,7-bis-[2-(d (0.61%), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z, (1.00%). The other components were present in minute quantities.

The most prevailing major components were Phenol, 4-ethenyl-, acetate (17.90%), n-Hexadecanoic acid (1.93%), 9,12-Octadecadienoic acid, methyl ester (10.67%), Phytol (0.91%), Methyl stearate (1.00%), 9,12,15-Octadecatrienoic acid,(Z,Z,Z)- (9.59%), Octadecanoic acid (41.49%) etc., other major and minor compounds were also present.

Several phytoconstituents analyses have been carried out in many parts of the world by GC-MS technique. Similar to the present analysis, eleven compounds were detected from *Commelina benghalensis* through GC-MS analysis [26] and also eleven compounds from ethanolic leaf extract of *C. benghalensis* [27] by GC-MS method. Among the 21 different phytochemicals, 10 components are having biological activity are reported (Table 3).

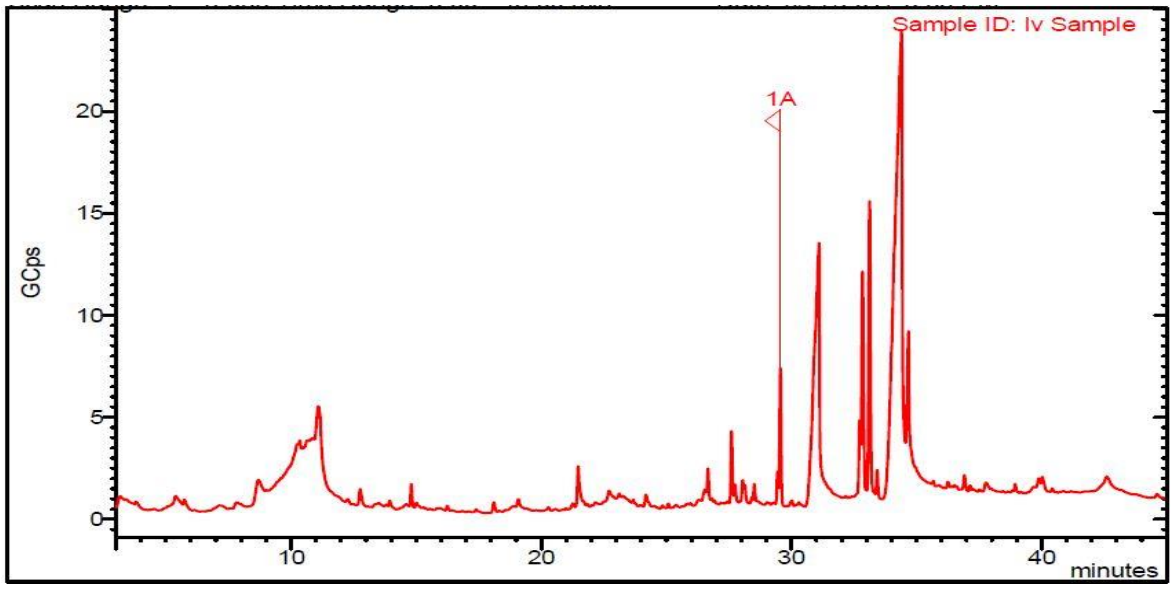

Fig 1: GC-MS Chromatogram of methanolic leaf extract of *C. diffusa*

Table 2: Phytocomponents identified in the methanolic shoot extracts of *C. diffusa* by GC-MS

S. No	Retention Time	Name of the compounds	Molecular Formula	Molecular Weight	Peak Area %
1	5.548	1-Phenyl-2-butanone	$C_{10}H_{12}O$	148	1.162
2	11.106	Phenol, 4-ethenyl-, acetate	$C_{10}H_{10}O_2$	162	17.904
3	12.789	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.268
4	14.552	Piperidin-1-yl-acetic acid, hydrazide	$C_7H_{15}N_3O$	157	0.338
5	18.172	2,6-Difluorobenzoic acid, 3,5-difluorophenyl ester	$C_{13}H_6F_4O_2$	270	0.711
6	19.149	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	$C_9H_{12}O_2$	152	1.120
7	21.595	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	2.608
8	24.183	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2-en	$C_{14}H_{20}O_3$	236	0.776
9	26.732	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	0.096
10	28.083	Di-n-octylphthalate	$C_{24}H_{38}O$	390	1.374
11	28.521	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.319
12	29.716	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	$C_{24}H_{46}O_2$	366	1.401
13	31.066	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.932
14	32.725	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	10.679
15	33.107	Phytol	$C_{20}H_{40}O$	296	0.912
16	33.444	Methyl stearate	$C_{19}H_{38}O_2$	298	1.000
16	34.401	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	9.593
17	34.660	Octadecanoic acid	$C_{18}H_{36}O_2$	284	41.491
18	36.908	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	0.023
19	38.963	Carbonic acid, 2-dimethylaminoethyl propyl ester	$C_8H_{17}NO_3$	175	0.156
20	40.084	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(d	$C_{30}H_{42}CI_2N_4O_3$	576	0.616
21	42.627	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{36}O_4$	352	1.000

Table 3: Biological activities of the compounds identified in the plants of *C. diffusa*

S. No	Retention Time	Name of the Compound	Nature of Compound	Biological Activities
1	12.789	2-Methoxy-4-vinylphenol	Phenolic compound	Antioxidant, Antimicrobial, Anti-inflammatory
2	28.521	3, 7, 11, 15-Tetramethyl-2 hexadecen-1-ol	Terpene alcohol	Antimicrobial Anti-inflammatory
3	29.716	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	Fatty acid ester compound	No activity reported
4	31.066	n-hexadecanoic acid	Palmitic acid	Antioxidant, Nematicide, Antiandrogenic.
5	32.725	9,12-octadecadienoic acid, methyl ester	Linoleic acid	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic,
6	33.107	Phytol	Diterpene	Anti-inflammatory, Antioxidant, Diuretic
7	33.444	Methyl stearate	Fatty acid methyl esters	Anti-diarrheal, cytotoxic and antiproliferative activity
8	34.401	9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Polyenoic fatty acid	Anti-inflammatory, Hypocholesterolemic, Cancer
9	34.660	Octadecanoic acid	Fatty acid	Hypocholesterolemi, Cosmetic, Flavour, Lubricant
10	42.627	9,12,15-octadecatrienoic acid (Z,Z,Z)-2,3 dihydroxypropyl ester	--	Analgesic, antipyretic, anticonvulsant, antiseptic

4. Conclusion

In the present study, twenty one compounds have been identified from methanol extract of the shoots of *C. diffusa* by GC-MS analysis. Hence the phytochemical studies are helpful in finding chemical constituents in the plant material that may help to their quantitative assessment and also in locating the source of pharmacologically active chemical compound.

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