

Chemical study of some species for *Cyperus* L. (Cyperaceae) in Diwaniyah river using Gas Chromatography - Mass spectrometry

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Abstract: The present study reported for the first time from Iraq about some detailed information on chemical composition of the study examined the precise properties of the chemical content of some Cyperaceae family, which included the *Cyperus* L. species. Five species were observed, three of which were chemically analyzed: *C. difformis*, *C. odoratus* and *C. alternifolius*. The samples were collected from the Riverside of the Diwaniyah River and specifically (Sunni area, Daghara area, Diwaniyah district) during the autumn and winter season from 15/10/2016 to 15/3/2017. The chemical content of leaves, which was characterized by its abundance in species under study, the chemical compounds were identified using Gas chromatography-mass spectrometry (GC-MS). The richness of the species under study in secondary metabolites, which ranged from: terpenes, phenols, steroids, Fatty acids, alkanes, alkaloids and esters. and all phytochemicals of the species participated in 7 compounds like : Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Octadecanoic acid, 2,3-dihydroxypropyl ester , Phytol , gamma.-Sitosterol and 1-(+)-Ascorbic acid 2,6-dihexadecanoate. It was characterized by high concentrations of different species that helped to isolate them clearly from each other, also some species were involved in the same compounds like: Squalene and Campesterol. Also distinguished and separated from each other by other compounds such as: Caryophyllene oxide and Phenol, 2,3,5,6-tetramethyl. Which reinforced the taxonomic importance of this study, so this study is of great importance. In the current study, two species were recorded for the first time on the river: *C. odoratus* and *C. alternifolius*.

Keywords: *Cyperus* L, GC-MS Analysis. Chemotaxonomy. Secondary metabolites compounds. Biological effectiveness.

INTERDICTION

Belongs genera *Cyperus* L. to Cyperaceae family which is the third largest family of Monocotyleuons, as it comes after Orchidaceae and Poaceae family. It is composed of

109 genus and 5,500 species [1]. Considered *Cyperus* L is one of the largest species and taxa second after *Carex* L. [2].

The chemical variants shown by Plant Taxa are similar to those in Morphological Characteres, Anatomycal Characters, and other which used for distinguishing between these taxas. These variations are also important from taxonomic point of view, [3])noted that chemical evidence has been used since humans began to label and classify plants according to their economic uses and medicinal properties.[4] has suggested that the addition of chemotaxonomy to transactions obtained as phenotypic and anatomical as well as cellular may provide a solid basis for phytosanitary decisions. He also pointed out that the first successful attempts to link the chemical composition and the physiological body as Barker and Smith (1920) when studying the *Eucalyptus* genus , suggesting that the level of the relationship should reflect the degree of chemical similarity between the species and the secondary metabolites are the most important secondary metabolites Compounds used in plant chemical classification [5].In addition, it is the main source of pharmaceuticals and active substances used in the preparation of pharmaceutical or used as raw materials for the production of a number of chemical compounds that are involved in the manufacture of a number of drugs [6].

Materials and methods of work

1-Extraction

The process of extraction was done according to [7]) method with some modification. Extract 1 gram(g) of dried leaves by 10 mL of methanol (99%) with stirring for 5 minutes, then leaved for 6 hours in a dark place At the temperature of the room and then filtered by a filter connected to a medical syringe (Chinese origin) with an opening capacity of 0.45 micrometers, and then added to it Hexsan (99%) with a volume of (1) ml to concentrate the extract, Drag the floating part that the hexane separated from the water and then estimate the active substances in it

2- Sample analysis in GC-MS device

The active compounds were diagnosed and evaluated by a GC-MS (Shimadzu GC-

MS 2010 plus gas chromatograph mass spectrometry) system manufactured by the GC clarus 500 Perkin Elmer system, which includes the Auto Sampler [AOC-20i + s] for compounds and the bound gas chromatography By mass spectrometry, and according to the following conditions:

General column of the Eliter-1 fused silica capillary column with dimensions (0.25 × 30Mdfµm1 × IDmm). 2. Helium gas (99.999%) was used as a conveyor gas at a continuous flow rate of 1 ml. Min⁻¹. - The size of injected liquid is 2µl and works by split (10:1). The oven temperature is programmed automatically at 40 ° C (the temperature is equal to three minutes) with an increase of 15 ° C. Min⁻¹ up to 180 ° C ,Then 10 ° C. -1 minute to 300 ° C then the temperature stabilizes at 300 ° C for 3 minutes until the end. The mass spectra were taken on a 70 Ev basis with a time interval of 0.5 seconds. The pressure inside the device is 49.5 kpa and the rate of 1 ml .Min⁻¹. The total time to start and end the device working for each sample is 28 minutes. The relative amount of each component was calculated by comparing its average surface area to the total area based on TurboMassVer 5.2.0 for mass spectra and chromatograms supplied with the device and all this information is automatically programmed on the machine.

3- Determination of raw chemical compounds

The components were determined according to GC-MS mass spectrometry and the database of the National Institute of Standards and Technology (NIST) Determination of raw chemical compounds. The output spectrum of the unknown component was compared with a range of known components stored in the NIST library to confirm the name, molecular weight, and structure of the components of the test materials. The test was carried out at the Food Research and Consumer Protection Laboratories of the Food Industries Division- Lab. of GC MS- College of Agriculture- University of Basrah Depending on the importance and the highest percentage of compounds were diagnosed in the current study.

Results

Cyperaceae family species are considered to be a perennial herbaceous plants. Some species have a medical and therapeutic importance in folk medicine the used part of the Plant: roots tubers, which contain many effective substances including the terpenes and terpenes hydroxylation and phenolics and other effective substances(8) (Bhattia et al,1981).

The current study Classified the phytochemical Compounds, the quantity and the quality of Chemical content, was observed, A total of 65 for *Cyperus* species Chemical Compounds were observed for the variety under study, The present study has shown different in type and time of detention phytochemical that have been detected by GC-MS,including leaves for all species under study.

The specie *C.difformis* contain on 18 compounds, table (1),figure (1),where the *C.odoratus* included on 27 compounds,table (2) figure (2),while the last specie *C.alternifolius* included 20 compounds,table (3),figure (3).

The current study showed a variation of phytochemical compounds. They are different according to type and time of detention the Chemical compounds in their distribution varied among species under study for the total terpenes compounds, it has been recorded(13) compound table (4).Two compounds were separated from plants extract for leaves. the highest purity was recorded in compound Phytol, Its concentration was 3.47% and has appearance in 17.355 minutes followed by the compound 3,7,11,15 Tetramethyl-2-hexadecen-1-ol Its appearance was in 14.633 minutes.

They species *C.odoratus* included (12) cultivars with the highest concentration of the compound Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2. Who reached 3.83% and has an appearance in minute 11.565.While Five compounds were separated in specie *C.alternifolius* and the compounds recorded Squalene It has the highest concentration 4.97% in time appearance 23.070 minute.

As well (7) phenolic compound were identification species under study together as .six phenolic compounds were detected,the highest concentration was recoded in specie *C.difformis* for the compound 2-Furancarboxaldehyde, 5-(hydroxymethyl with a concentration 21.13% in the time of appearance was recorded per minute 9.180%.While the presence of phenolic compounds was recorded two compounds in extract leaves specie *C.odoratus* one of them glycosides compounds:alpha.-Tocopherol.-beta.-D-mannoside with a concentration 1.68% and appeared in a minute 25.178. The specie *C.alternifolius* also recorded phenolic compounds in the extracts of leaves, one of which was mentioned above and recorded the highest concentration, which reached 11.05% and the time of appearance per minute 25.198.

In the present study, the presence of phytosterols or cholesterol compounds was observed. In specie *C. difformis* was diagnosed with two compounds, with the highest percentage of 21.32% for gamma.-Sitosterol and its time recording time was 26.526. Species *C.odoratus*. and *C.alternifolius*, three compounds were identified, including gamma.-Sitosterol, which had the highest

concentrations which Its 24.12% In the second species Table 3, followed by Campesterol in the first specie with a concentration of % 5.31 and time of occurrence of 24.857minute. As for the fatty acid compounds that were diagnosed in the current study,

Either for fatty acid compounds, of the two species, *C.difformis* and *C.alternifolius*, the compositions of the compounds between the species mentioned. The study recorded the possession of the first on 5 compounds, while the second has 7 compounds, and recorded the highest concentration between the them to the compound Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester As the proportions reached 23.71% and 9.70% respectively. And 20.841 and 20.840 minute respectively, followed by compound octadecanoic acid (2,3-dihydroxypropyl ester). And this compound was recorded in the last species under study, which represented *C.odoratus* which have highest concentrations in plant extracts among all species under study, with a concentration of 19.18% shown at minute 22.415, and also on seven different compounds, such as 4,8,12,16-Tetramethylheptadecan-4-olide and 1 (2 H) -Naphthalenone, octahydro-4,8a-dimethyl-6 (1-methylthenyl), (4.alpha., 4a. bet.

As well diagnosed and separated Five compounds Alkanes A difference depending on the types and time of detention of phytochemical compounds according to the species under studied was as follows: Specie *C.odoratus* contained four compounds with the highest concentrations of Heptane (2,4-dimethyl), which was 4.37%, the first compound to be recorded.in minute 3.166. While only one compound of *C.alternifolius* was identified which It 2-Methyl-Z-4-tetradecene, with a concentration of 3.06% and an impression time of 20.175. The last specie, *C.difformis*, did not register the current study any compound alkanes in the extracts of the leaves.

In addition to the compounds mentioned, three of the Ester compounds were observed in the species under study .The presence of Dichloroacetic acid, tridec-2-ynyl ester was recorded in extracts of only two species, *C.difformis* and *C.odoratus*.The highest concentration was in the first specie with a concentration of 4.82% and a record time of minute 17.618. A single Esters compound was found in *C.alternifolius* with a concentration of 2.7% in the plant extract: Isophthalic acid, hexadecyl neopentyl ester and its time of occurrence were in minute 26.086.

As for alkaloids compounds which was diagnosed in the current study,two alkaloid were identified one of them: 1, Butanamide, N- (4-methoxyphenyl), and his concentration in *C.alternifolius*, was 1.70% in time of occurrence per minute.23.253. The current study showed the Sucrose compound as the first compound appeared in the analysis and recorded the highest concentration of 6.49% in the leaves extract of the specie *C.alternifolius* and record time of appearance in the 11.100 and limited to exist in this specie only.

The results showed that there are some 14 on compounds among species, within the same gender, which was found in species and did not exist in other species or vice versa. For terpenes compounds, the current study found that Phytol was common in the leaves of all species under study as in figure (4). While the 3,7,11,15-Tetramethyl-2-hexadecen-1-ol compound was common among all species except *C.alternifolius*. Participation observed the specie *C.odoratus* was also observed in two compounds with *C.alternifolius*, as shown in the same mentioned above. Also, Participation observed the phenolic compounds were also observed among species. The presence of alpha-Tocopherol-.beta.-D-mannoside was found in all leaves extracts of the species under study and in different concentrations (Fig. 5).

As for steroids, the compound gamma.-Sitosterol was found to be present in a plant extracts of the species under study and with a different concentration ratio (Fig.9). The presence of Campesterol and Stigmasterol was observed in only two species.

While the common compounds of fatty acids were detected, including four compounds common in all species, namely 1- (+) - Ascorbic acid 2,6-dihexadecanoate and Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester and Octadecanoic acid, 2,3-dihydroxypropyl ester and 9,12-Octadecadienoic acid (Z, Z). The rest of the compounds were reported in Table (4).As can be seen from the same table, the present study reported the presence of one Dichloroacetic acid, tridec-2-ynyl ester, which was found in *C.odoratus* and *C. difformis*.

The presence of some compounds was limited to certain species without other species, For Phenolic compounds, the presence of some compounds was limited to certain species, for example, phenol, 2,3,5,6-tetramethyl, in specie *C.difformis*. The specie *C.alternifolius* was unique to a number of phytoesters, including Ergot-5-en-3-ol, (3.beta). Were Alkanes compounds, for example, the composition of Heptadecane was limited to *C.odoratus*. For Ester compounds, for example, *C.alternifolius* was unique in the presence of a single compound, Isophthalic acid, hexadecyl neopentyl ester .In addition, each of the previous species under study was distinguished by the presence of a number of fatty acid compounds (Table2), if *C.odoratus* was isolated with two compounds, as well as four individual compounds of specie *C.difforims*.

Discussion:

The results of the study showed that the chemical side of the genus *Cyperus* L, using GC-MS technique, was satisfactory. The latter helped to isolate all species by distinguishing them with various chemical compounds as well as clarifying their chemical content, which can be used in all medical aspects, especially in general non-taxonomic research, The results of the analysis of plant extracts of the studied plant parts showed an abundance of phytochemical compounds, which enhances the medical importance of the Cyperaceae family and the species under study [8:9].

[9,10] both them said they contain the family plant on Sesquiterpenes, flavonoids, glycosides and alkaloids. This is identical to the current study, with the same compounds as steroid, esters, vitamins and sugars found in the current study. These compounds have many benefits and medical uses including anti-oxidant, anti-inflammatory, antibacterial, cancer preventive, etc., as shown in Table(6). The present study shows that there are common compounds in all plant extracts of plant parts between species and within the study. The current study indicates that there are variations in the concentrations and time of these compounds and significantly between species. This has enhanced the taxonomic significance of this study and is taken as taxonomic evidence for separating and isolating them. For steroids, it is possible to observe from Figure (6) for gamma.-Sitosterol. Among the species, the lowest percentage was recorded in *C.difformis* leaf extracts, while *C.alternifolius* was characterized by the highest concentration. 26.63%. As for fatty acid compounds, which were diagnosed in the current study between papers, the latter specie was characterized by having the highest concentration of the leaves extracts for the compound 1- (+) - Ascorbic acid 2,6-dihexadecanoate with a concentration of 5.17% figure (7), Which has significantly enhanced the state of isolation from other species.

It was also possible to isolate *C.difformis* in Fig. (8) and (10) with the highest concentration of hexadecanic acid, 2-hydroxy-1 (hydrolymethyl) ethyl ester and 9-12-Octadecadienoic acid (Z, Z) Which has significantly enhanced the state of isolation from other species. The compound hexadecanic acid, 2-hydroxy-1 (hydrolymethyl) ethyl ester is considered of monoacylglyceride derivatives are added to commercial food products in small quantities. This compound acts as an emulsifier to mix fat with water for these products and to protect them from damage [11] while the second compound of unsaturated fatty acids. In addition, the same specie recorded the highest a concentration of leaf extracts for the terpenes compound Phytol figure (4). The highest concentration of the compound Octadecanoic acid, 2,3-dihydroxypropyl ester was of the specie *C.odoratus* and thus could be isolated from the rest of the species figure(9). As for phenolic compounds, the highest concentration of leaf extracts in *C.alternifolius* was found in the form of alpha-Tocopherol-beta.-D-mannoside (5) with 11.05%. This ratio is high when compared with other species under study and thus can be separated and distinct from the rest of his genera. Table (5) showed that the studied species, except *C.difformis*, were observed with two compounds, Campesterol and Stigmasterol (Fig.).

These compounds are naturally occurring phytosterols and are similar in their chemical composition to cholesterol compounds It is a soluble powder in alcohols. This compound is used medically as it is mixed with the anti-prostate Medication and in the reduction of hypercholesterolemia[12].

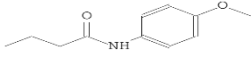

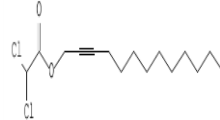
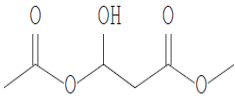
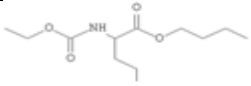
Also, from the same table, we observe that the species under study [13] are a common compound, indicating that they belong to one family. The similarity of the plants in the chemical content is a sign of a relationship between them. As for the rest of the compounds contained in the tables and non-common species are considered as insulating compounds and useful in the diagnosis and isolation of each specie. The present study agreed with [12:13]) in the presence of terpenes and phenols in the leaves.

[14] pointed out to the presence of 29 compounds, mostly monoterpenes and Sesquiterpenes, while the current study recorded in leaves extract only two terpenes compounds in addition to 16 compounds effective diversity between phenols and steroids along with some esters and as shown in Table (1).

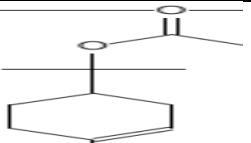
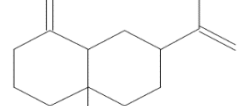
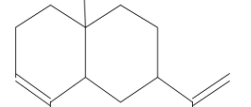
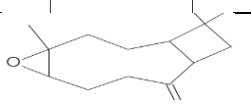

The difference in the ratio of active substances, their presence or their disappearance in plants may be due to several reasons: Genetic factors and plant species play an important role in the concentration of compounds, including phenolic compounds [15:16], concluded that alkanes (a type of hydrocarbons) increase in the autumn and winter due to low temperature and therefore lack of evaporation, which leads to accumulation in the plant. The obtained compounds depend on several factors, such as the type of extract used. [17] indicated that the type of extract and extraction method has an effect on the type of compounds shown and analyzed by GC-MS. For the type of column used in the gas chromatograph - mass spectrometer the effect on the type of compounds resulting from solvent type analysis and based on the polar quality of the extract is chosen column separation which is often associated with the user. For example, hexane can be used to obtain non-polar compounds such as fatty acids and volatile oils such as terpenes, esters and ketones, and then analyze them using a non-polar column, Polar compounds such as alkanes and phenols can be obtained using methanol as solvent and using a polar separation column. If we want to get both, we use a general column, which consists of non-polar and polar parts of 95% and 0.5%, respectively.

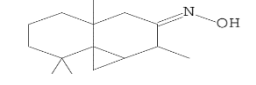
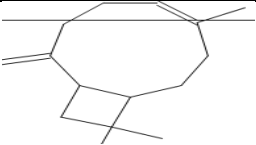



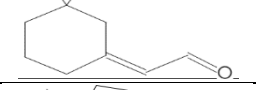
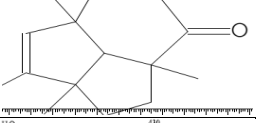
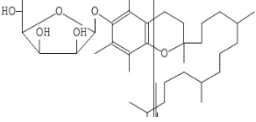
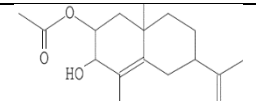
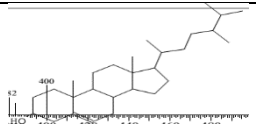
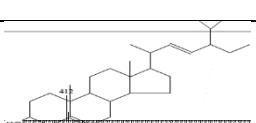
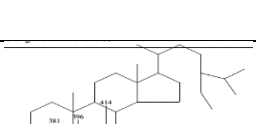
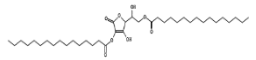
Table(1) Analysis GC-MS of extract of Leaves of *C.difformis*


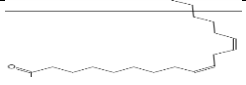
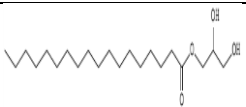
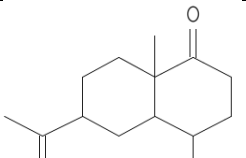
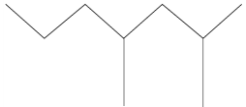
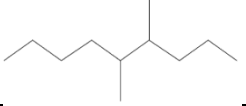


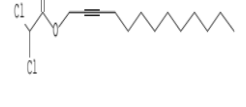
Nature of compound	NO	Compound Name	Structure	Molecular Formula	Peak Area%	Retention time (min)	N.Peak
Terpenes compound	1	3,7,11,15 Tetramethyl-2-hexadecen-1-ol-		C ₂₀ H ₄₀ O	2.36	14.633	8
	2	Phytol		C ₂₀ H ₄₀ O	3.47	17.335	10
Phenolic compounds	3	2-Furancarboxaldehyde, 5-(hydroxymethyl)		C ₆ H ₆ O ₃	21.13	9.180	2
	4	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester		C ₁₄ H ₃₂ O ₃	0.74	14.082	7
	5	alpha.-Tocopherol-.beta.-D-mannoside		C ₃₅ H ₆₀ O ₇	0.92	25.179	17
	6	Phenol, 2,3,5,6-tetramethyl-)-		C ₁₀ H ₁₄ O	0.87	9.896	4
Stroides compound	7	Cholestan-3-one, 4,4-dimethyl-, (5.alpha.)-		C ₂₉ H ₅₀ O	3.39	20.683	13
	8	.gamma.-Sitosterol		C ₂₉ H ₅₀ O	1.81	26.513	18
Fatty acid	9	1-(+)-Ascorbic acid 2,6-dihexadecanoate		C ₃₈ H ₆₈ O ₈	3.37	15.911	9
	10	Butyl 9,12,15-octadecatrienoate		C ₂₂ H ₃₈ O ₂	5.38	22.256	15
	11	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester		C ₁₉ H ₃₈ O ₉	23.71	20.841	14
	12	Octadecanoic acid, 2,3-dihydroxypropyl ester		C ₂₁ H ₄₂ O ₄	16.37	22.424	16
	13	9,12-Octadecadienoic acid (Z,Z)-		C ₁₈ H ₃₂ O ₂	1.78	17.557	11

Alkaloids compounds	14	Butanamide, N-(4-methoxyphenyl)-		C ₁₁ H ₁₅ NO ₂	0.83	13.158	6
	15	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene		C ₈ H ₁₅ N ₃ O	1.71	13.115	5
Ester compounds	16	Dichloroacetic acid, tridec-2-ynyl ester		C ₁₅ H ₂₄ Cl ₂ O ₂	4.82	17.618	12
	17	3-Acetoxy-3-hydroxypropionic acid, methyl ester		C ₆ H ₁₀ O ₅	0.95	9.335	3
	18	l-Norvaline, N-ethoxycarbonyl-, butyl ester		C ₁₂ H ₂₃ NO ₄	6.40	8.232	1

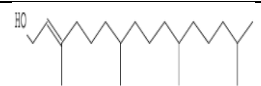
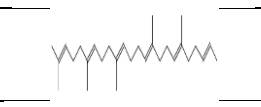
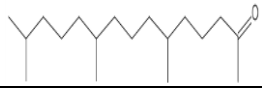
Table(2) Analysis GC-MS of an extract of Leaves of *C.odoratus*.

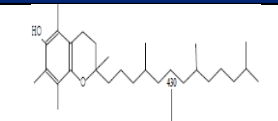
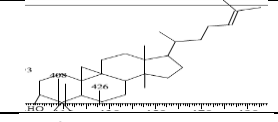
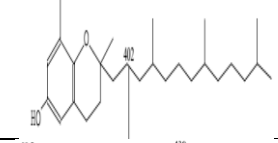
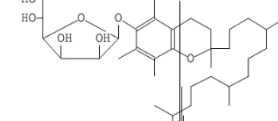
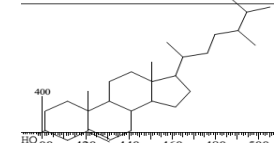
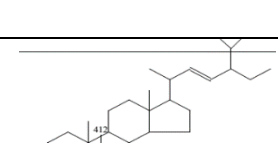
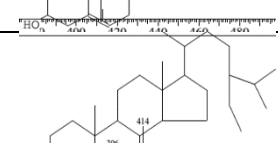
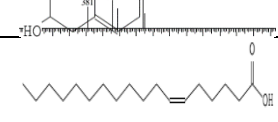
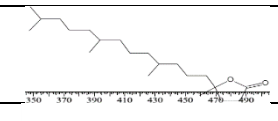

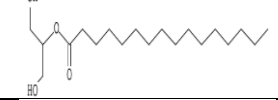
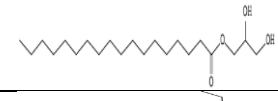
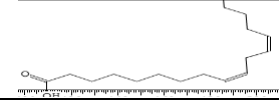
Nature of compounds	NO	Compound Name	Structure	Molecular Formula	Peak Area%	Retention time (min)	N.Prak
Terpenes compound	1	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate		C ₁₂ H ₂₀ O ₂	0.58	10.202	3
	2	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha		C ₁₅ H ₂₂	2.38	11.507	6
	3	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2		C ₁₅ H ₂₄	3.83	11.565	7
	4	Caryophyllene oxide		C ₁₅ H ₂₄ O	0.73	12.287	8
Terpenes	5	Squalene		C ₃₀ H ₄₈	1.04	23.053	25

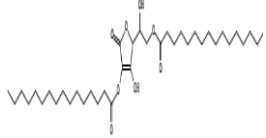

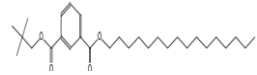
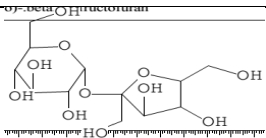
	6	Cyclopropa[d]naphthalen-3-one, octahydro-2,4a,8,8-tetramethyl-, oxime		C ₁₅ H ₂₅ NO	0.58	12.748	10
	7	Caryophyllene		C ₁₅ H ₂₄	0.69	13.613	13
	8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		C ₂₀ H ₄₀ O	1.24	14.630	15
Terpenes compound	9	2-Pentadecanone, 6,10,14-trimethyl-		C ₁₈ H ₃₆ O	0.88	14.684	16
	10	Phytol		C ₂₀ H ₄₀ O	1.52	17.333	18
	11	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-		C ₁₀ H ₁₆ O	0.94	13.140	12
	12	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-en-8-one		C ₁₅ H ₂₂ O	0.62	11.315	5
Phenolic compounds	13	.alpha.-Tocopherol-.beta.-D-mannoside		C ₃₅ H ₆₀ O ₇	1.68	25.178	27
	14	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphth		C ₁₇ H ₂₆ O ₃	2.27	13.812	14
Stroides compounds	15	Campesterol		C ₂₈ H ₄₈ O	3.55	25.852	28
	16	Stigmasterol		C ₂₉ H ₄₈ O	4.75	26.040	29
	17	.gamma.-Sitosterol		C ₂₉ H ₅₀ O	13.51	26.516	30
Fatty acid	18	1-(+)-Ascorbic acid 2,6-dihexadecanoate		C ₃₉ H ₆₈ O ₈	4.36	15.900	17

	19	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester		C19H38O4	15.92	20.826	22
	20	9,12-Octadecadienoic acid (Z,Z)-		C18H34O2	1.04	17.554	19
	21	Octadecanoic acid, 2,3-dihydroxypropyl ester		C21H42O4	18.25	22.415	24
	22	1(2H)-Naphthalenone, octahydro-4,8a-dimethyl-6-(1-methylethenyl)-, (4.alpha.,4a.beta)		C15H24O	1.23	13.058	11
Alkanes compound	23	Heptane, 2,4-dimethyl-		C9H20	4.11	3.167	1
	24	Nonane, 4,5-dimethyl-		C11H24	0.64	8.752	2
	25	Tetradecane		C14H30	0.66	10.661	4
	26	Heptadecane		C17H36	0.53	12.333	9
Ester compound	27	Dichloroacetic acid, tridec-2-ynyl ester		C15H24Cl2O2	3.11	17.610	20

Table(3) Analysis GC-MS of extract of Laeves of *C.alternifolius*

Nature of compound	NO	Compound Name	Structure	Molecular Formula	Peak Area%	Retention time (min)	N.Prak
Terpenes compound	1	Phytol		C20H40O	1.32	17.351	5
	2	Squalene		C30H50	4.97	23.070	11
	3	2-Pentadecanone, 6,10,14-trimethyl-		C18H36O	1.84	14.701	3

	4	Vitamin E.		C20H50O	1.89	24.688	14
	5	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-		C30H50O	1.44	27.232	20
Phenoles compounds	6	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2		C27H46O2	2.42	23.411	13
	7	alpha.-Tocopherol-.beta.-D-mannoside		C35H60O7	11.05	25.198	16
Stroides compounds	8	Campesterol		C28H48O	2.62	25.876	17
	9	Stigmasterol		C29H48O	4.52	26.064	18
	10	.gamma.-Sitosterol		C29H50O	26.63	26.540	19
Fatty acid	11	6-Octadecenoic acid, (Z)-		C18H45O2	1.63	17.625	6
	12	4,8,12,16-Tetramethylheptadecan-4-olide		C21H40O2	4.16	19.484	7
	13	1-Dodecanol		C12H26O	3.53	13.170	2
	14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester		C9H38O4	7.34	20.839	9
	14	Octadecanoic acid, 2,3-dihydroxypropyl ester		C21H42O4	6.15	22.427	10
	16	9,12-Octadecadienoic acid (Z,Z)-		C18H32O2	0.53	23.253	12

	17	1-(+)-Ascorbic acid 2,6-dihexadecanoate		C38H68O8	5.17	15.915	4
Alkanes	18	2-Methyl-Z-4-tetradecene		C15H30	3.06	20.175	8
Ester	19	Isophthalic acid, hexadecyl neopentyl ester		C29H48O4	2.7	25.086	15
Carbohydrate	20	Sucrose		C12H22O11	6.49	11.100	1

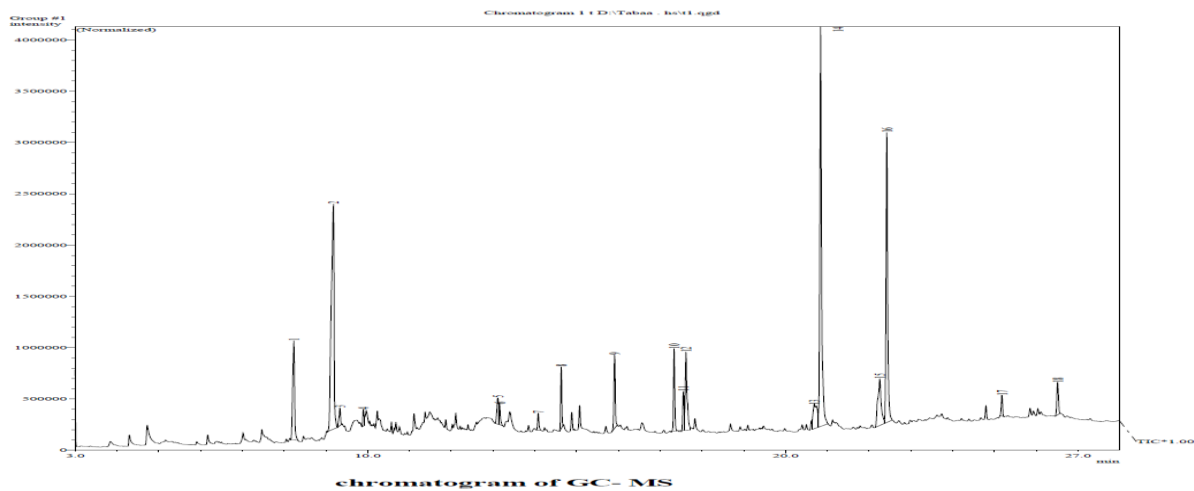
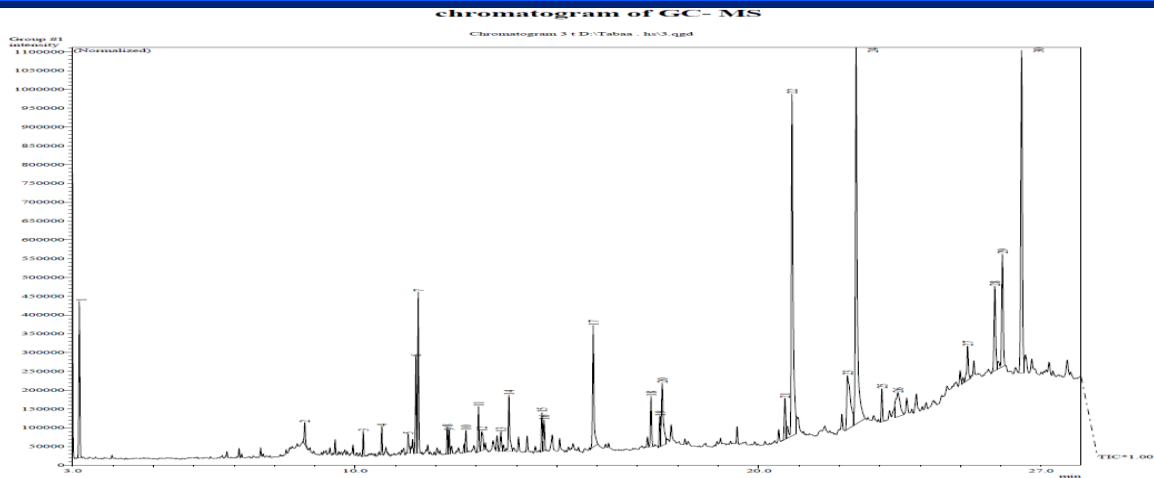


Figure (1) GC-MS chromatogram of extract of *C.difformis*



Figure(2) GC-MS chromatogram of extract of *C.odoratus*

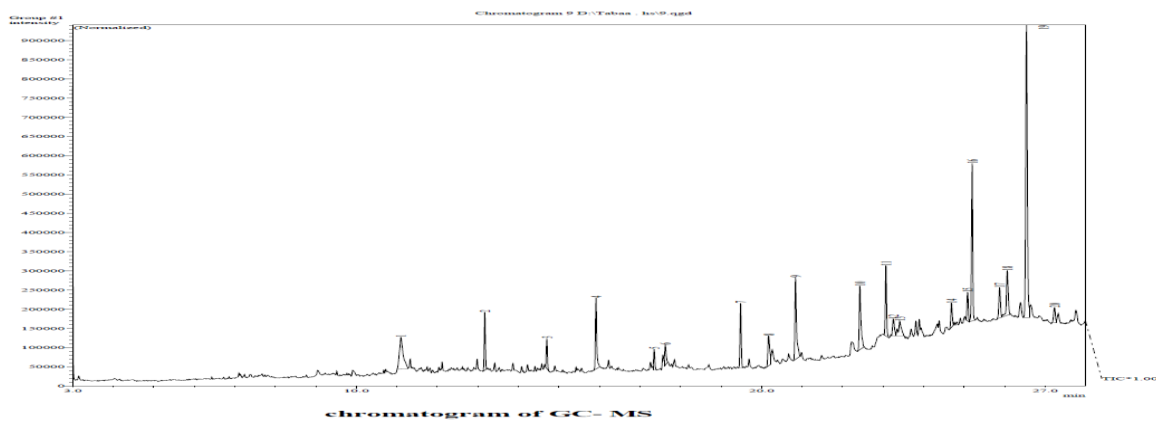


Figure (3) GC-MS chromatogram of extract of *C.alternifolius*

Table (4) Common compound Secondary chemicals of the species under study

NO	Name of compound	Specie Name		
		<i>C.alternifolu</i>	<i>C.odoratus</i>	<i>C.difformis</i>
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-	+	+
2	l-(+)-Ascorbic acid 2,6-dihexadecanoate	+	+	+
3	Phytol	+	+	+
4	9,12-Octadecadienoic acid (Z,Z)-	+	+	+
5	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	+	+	+
6	Octadecanoic acid, 2,3-dihydroxypropyl ester	+	+	+
7	alpha.-Tocopherol-.beta.-D-mannoside	+	+	+
8	gamma.-Sitosterol	+	+	+

9	Campesterol	+	+	-
10	Stigmasterol	+	+	-
11	2-Pentadecanone, 6,10,14-trimethyl-	+	+	-
12	Squalene	+	+	-
13	Dichloroacetic acid, tridec-2-ynyl ester	-	+	+

Table(5) The Compounds of the species under study) (+)The presence of the compound (-) the absence of the compound

NO	Name of compound	Specie Name		
		<i>C.alternifolius</i>	<i>C.odoratus</i>	<i>C.difformis</i>
1.	3,7,11,15 Tetramethyl-2-hexadecen-1-ol-	-	+	+
2.	Phytol	+	+	+
3.	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	-	+	-
4.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha	-	+	-
5.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2	-	+	-
6.	Caryophyllene oxide	-	+	-
7.	Squalene	+	+	-
8.	Cyclopropa[d]naphthalen-3-one, octahydro-2,4a,8,8-tetramethyl-, oxime	-	+	-
9.	Caryophyllene	-	+	-
10.	2-Pentadecanone, 6,10,14-trimethyl-	+	+	-
11.	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-	-	+	-
12.	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-en-8-one	-	+	-
13.	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	+	-	-
14.	Vitamin E	+	-	-
15.	2-Furancarboxaldehyde, 5-(hydroxymethyl	-	-	+
16.	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester	-	+	+
17.	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphth	-	+	-
18.	alpha.-Tocopherol-.beta.-D-mannoside	+	+	+
19.	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2	+	-	-
20.	Phenol, 2,3,5,6-tetramethyl)-	-	-	+
21.	.gamma.-Sitosterol	+	+	+
22.	Campesterol	+	+	-
23.	Stigmasterol -	+	+	-
24.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	+	+	+
25.	Butyl 9,12,15-octadecatrienoate	-	-	+
26.	Octadecadienoic acid (Z,Z)-methylcyclohexylidene	-	-	+

27.	9,12-Octadecatrienoic acid, (Z,Z)-	+	+	+
28.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	+	+	+
29.	Octadecanoic acid, 2,3-dihydroxypropyl ester	+	+	+
30.	6-Octadecanoic acid,(Z)-	+	-	-
31.	1(2H)-Naphthalenone, octahydro-4,8a-dimethyl-6-(1-methylethenyl)-, (4.alpha.,4a.beta)	-	+	-
32.	4,8,12,16-Tetramethylheptadecan-4-olide	-	+	-
33.	Nonane, 4,5-dimethyl-	-	+	-
34.	Heptane, 2,4-dimethyl-	-	+	-
35.	Tetradecane	-	+	-
36.	Heptadecane	-	+	-
37.	2-Methyl-Z-4-tetradecene	+	-	-
38.	Dichloroacetic acid, tridec-2-ynyl ester	-	+	+
39.	3-Acetoxy-3-hydroxypropionic acid, methyl ester	-	-	+
40.	Isophthalic acid, hexadecyl neopentyl ester	+	-	-
41.	l-Norvaline, N-ethoxycarbonyl-, butyl ester	-	-	+
42.	Sucrose	+	-	-
43.	Butanamide, N-(4-methoxyphenyl)-	-	-	+

Table (6) Biological effectiveness of some compounds of the species under study

NO	Name of compound	Nature of compound	Biological effectiveness
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol,	Terpene alcohol	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory, Fungicide) 2015,Jamil(
2	Phytol	Diterpene	Anti-inflammatory, Anticancer Diuretic- Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide Insectifuge, , Antieczemic Kavitha et al(2015)/, Antimicrobial, Antiasthmatic Rukshana <i>et al.</i> ,(2017)
3	9,12-Octadecadienoic acid (Z,Z)-	Lipidic acid	Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge- Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic Kavitha <i>et al.</i> ,(2015)
4	Squalene	triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide Kavitha <i>et al.</i> ,(2015)
5	Vitamin E	Vitamin Compound	Antiageing, Analgesic Antidiabetic Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer,

			Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary ,Kavitha et a(2015)
6	Heptadecanoic acid	Margaric acid	Antioxidant (Ponnamma and Manjunath(2012)
7	Campesterol	Steroids	Antioxidant, Hypocholesterolemic (Ponnamma and Manjunath(2012)
8	Stigmasterol	Steroids	Antihepatotoxic, Antiviral, Antioxidant, Cancerpreventive, Hypocholesterolemic Ponnamma and Manjunath(2012)
9	4H-pyran-4-one,2,3,-dihydro-3,5-dihydroxy-6methyl	Flavanoid	Anti-tussive, antibacterial activities and antioxidant,AL-altameme <i>et al.</i> ,(2015)
10	l-(+)-Ascorbic acid 2,6 dihexadecanoate	Fatty acid	Antioxidant, Sosa <i>et al.</i> ,(2016)
11	Caryophyllen oxide	Sesquiterpene	Antibacterial, Anti-inflammatory, Analgesic, Antioxidan Elezabeth and Arumugam(2014)
12	Phenol, 2,6-dimethoxy	phenol	Ant ifungal Antihelminthic, Elezabeth and Arumugam(2014)

Percentage of chemical compounds in the leaves of the species under study

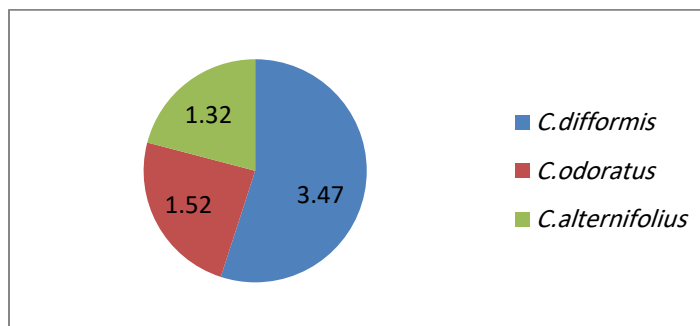


Figure (4) Percentage of Phytol compound concentrations

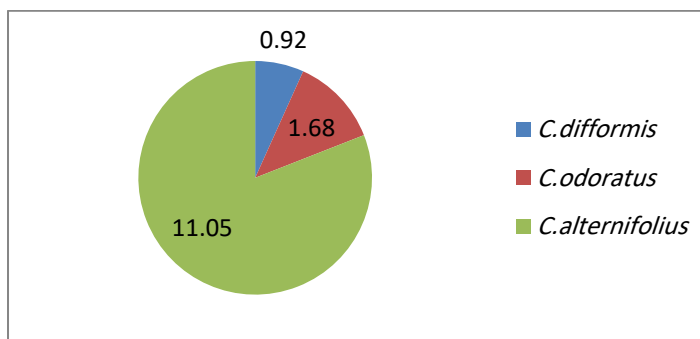


Figure (5) Percentage of alpha.-Tocopherol-.beta.-D-mannoside compound concentrations

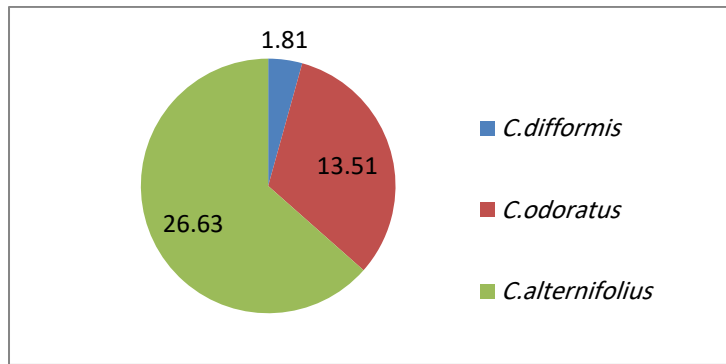


Figure (6) Percentage of gamma.-Sitosterol compound concentrations

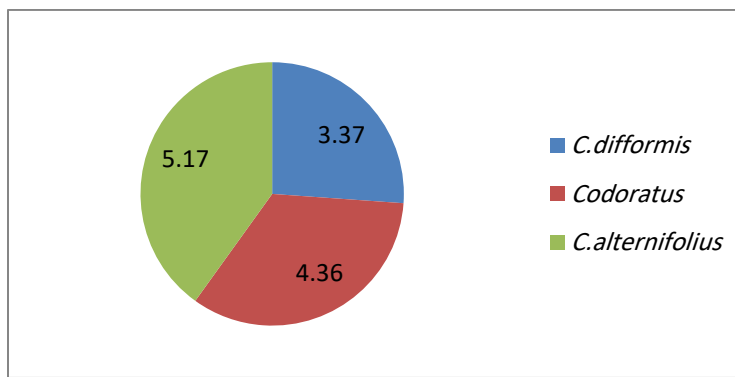


Figure (7) Percentage of 1-(+)-Ascorbic acid 2,6-dihexadecanoate compound concentrations

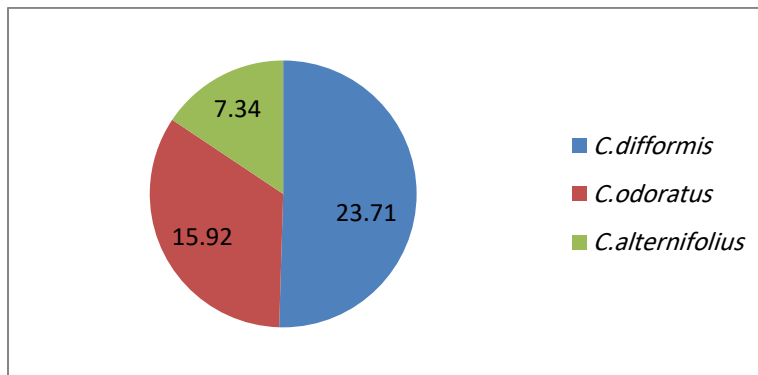


Figure (8) Percentage of Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester compound concentrations

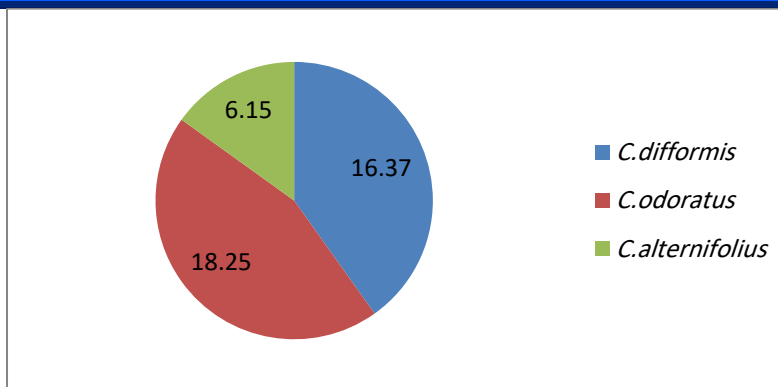
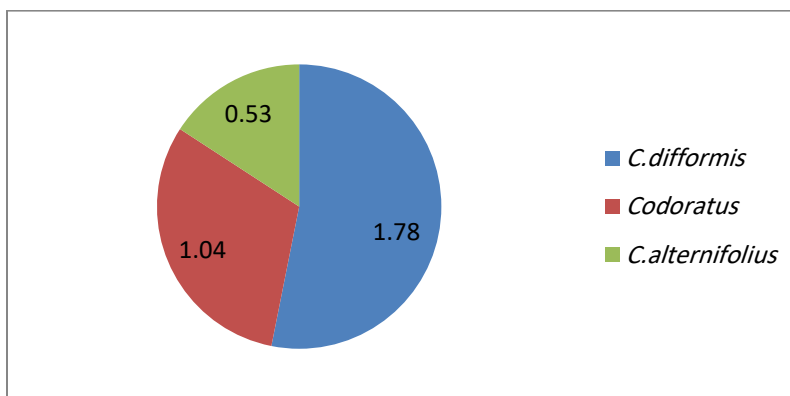


Figure (9) Percentage of Octadecanoic acid, 2,3-dihydroxypropyl ester compound concentrations



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