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Phytochemical Characterization and GC-MS Analysis of Methanolic Extracts of *Cipadessabaccifera* and *Orthosiphonaristatus*

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ABSTRACT

The aim of the current study was to characterize the phytochemicals present in the methanolic extract *Cipadessabaccifera*(Roth) Miq. and *Orthosiphonaristatus*(Blume) Miq through chemical tests and GC-MS analysis. The methanolic extract of leaves of *C. baccifera* and *O. aristatus* were screened for the presence of terpenoids, tannins, flavonoids, alkaloids, Phenolic compounds, saponins, steroids, and glycosides. The bioactive compounds were identified by GC-MS method. The phytochemical screening revealed the presence of flavonoids, alkaloids, terpenoids, glycosides and phenolic compounds in both the extracts. GC-MS analysis revealed the presence of phytol Shyobunone (RT: 40.06; %Peak area: 26.67), 9,12,15-Octadecatrienoic acid, methyl ester (RT: 34.76; %Peak area: 9.25), and 2-Furancarboxaldehyde, 5-(hydroxymethyl) (RT: 11.82; %Peak area: 7.50) as major compounds in the methanolic extract of *C. baccifera*. Phytol (RT: 33.67; %Peak area: 34.47), n-Hexadecanoic acid (RT: 30.65; %Peak area: 23.24) and 9,12,15-Octadecatrienoic acid (RT: 35.02; %Peak area: 7.51. The results revealed the presence of therapeutically active active compounds.

Keywords: *Cipadessabaccifera*, *Orthosiphonaristatus*, phytochemical, and GC-MS.

Introduction

The phytochemicals present in the plants are responsible for preventing diseases and promoting the health. Identification and isolation of chemical components are the crucial steps to establish the underlying mechanism of action[1] and to investigate the lead compound for further new drug development. The secondary metabolites of plants like terpenoids, flavonoids , steroids, alkaloids and tannins are known to have pharmacological activities such as antidiabetic, cytotoxic, antioxidant, anti-inflammatory, antimicrobial and antihypertensive activities.

*C. baccifera*is a shrub belongs to family Meliaceae, usually 1-4 m tall and leaves are 830 cmlong[2]. It is one of the most popular traditional medicines in India for the treatment of rheumatoid arthritis, dysentery and pruritus[3]. The paste of root, leaf and bark of this plant is applied topically to cure psoriasis[4]. The bark has a bitter taste and its decoction has been utilized to treat dysentery, skin itches and malaria fevers by the tribal community[5]. Phytochemical characterization of ethanolic extract of cipadessabaccifera leaves showed the presence of glycosides, tannins, alkaloids, flavonoids, terpenoids, and saponins[6].

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O. aristatus is a perennial herb belongs to family Lamiaceae, growing from 25-200 cm tall.It has been utilised in the folk or traditional medicine of Asian cultures and is frequently used for the treatment of renal inflammation, kidney stones and dysuria[7]. Over the years, enormous number of secondary metabolites from O.aristatus have been identified; the majority of these phytochemicals are of the terpenes family. Other chemical constituents isolated from *O. aristatus*include, but are not limited to flavonoids, caffeic acid derivatives, methylripariochromene A, and ursolicacid[8]. Based on a reported quantitative analysis using aqueous methanolic extract, sinensetin is the major flavonoid constituent of the plant[9,10].

The present study investigated the phytochemicals present in the methanolic extract of *O.aristatus* and *C. baccifera* through phytochemical screening and GC-MS analysis.

Materials and Methods

Plant Material collection and preparation of extract:

The leaves of *C. baccifera* were collected from Puliarai village, Tirunelveli district, Tamilnadu and the leaves are *O. aristatus* leaves were collected from Kulasekaram, Kanyakumari district, Tamilnadu. Powdered leaf sample

of *C. baccifera*.(100 g each) was taken with 1000 ml of methanol and kept for shaking at room temperature for 5 h. Then, the extract was separated using filtration and the volume of the extract was measured. Then the solvent was removed by distillation and the extract was lyophilized. The extract was re-suspended in ethanol at 10 mg/ml ratio and used for further analysis. The same procedure had been followed for the preparation of methanolic extract of *O. aristatus.*

The residues of the extracts obtained were tested for the presence of various chemical constituents by conducting following chemical tests.

Test for Sterols and Steroids:

Libermann-Burchard Test: To the chloroform solution, a few drops of acetic anhydride and 1ml of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. Reactions observed and recorded.

Salkowski Test: To the chloroform solution, few drops of concentrated sulphuric acid added, shaken and allowed the reaction to take place. Reactions observed and recorded.

Test for tannins:

A small quantity of the extract was added with water and to the aqueous extract, few drops of ferric chloride solution was added. Reactions observed and recorded

Test for flavanoids:

Shinoda test: A little amount of the extract was treated with alcohol and filtered. To the alcohol solution, few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for 5 minutes. Reactions observed and recorded.

To alcoholic extract 10% potassium hydroxide solution and ammonia were added. To the alcoholic extract, a few drops of lead acetate solution was added. Reactions observed and recorded.

Test for alkaloids:

Small amount of extract was mixed with 1gm of calcium hydroxide and 5ml of water and was made into a smooth paste and set aside for five minutes. It was then heated to dryness, in a porcelain dish on a water bath. 20ml of chloroform was added, mixed well and then refluxed for half an hour on a water bath. Then it was filtered and evaporated. To this 5ml of diluted sulphuric acid was added, followed by 2ml of each of the following reagents.

MAYER'S Test (Potassium mercuric iodide): Extract was treated with mayer's reagent. Reactions observed and recorded.

WAGNER'S Test (Iodine and Potassium iodide solution): Extract was treated with Wagner's reagent. Reactions observed and recorded.

DRAGENDORFF'S Test: (Potassium Bismuth Iodide Solution): Extract was treated with drogendroff's reagent. Reactions observed and recorded.

*HAGER'S Test: (Saturated Picric Acid)***:** Extract was treated with Hager's reagent. Reactions observed and recorded

Test for Terpenoids

The extract was treated with cholorform solution and filtered. A little chloroform solution was warmed gently with tin and thionyl chloride. Reactions observed and recorded.

GC-MS analysis:

The methanolic extracts were analyzed using Gas Chromatographic system coupled with Mass Spectrometry (GC-MS, Make: Perkin Elmer, Model: Clarus-500) operated with Turbomass software (Version 5.2.0). Silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness, Elite-5 MS non-polar fused) was used. Oven temperature was programmed with an increase of 6°C/min to 150°C with 2 min holding time and then 4°C/min upto280°C with 5 min holding time. The injector temperature was 280°C and carrier gas was helium with the flow rate of 1 ml/min. Sample (1.0 µl) was injected with split ratio of 1:10. Ionization energy 70 ev was used in the electron ionization mode; ion source temperature was set at 160-200°C, mass was scanned in the range of 40-600 amu. The resulted mass spectrum was compared with inbuilt NIST library database and fragments of various compounds present in the extracts were identified.

Results and Discussion

The results of phytochemical screening by chemical methods are captured in Table 1. Phytochemical screening revealed the presence of flavonoids, alkaloids, terpenoids, tannins and phenolic compounds in both the extracts, which could be the reason for pharmacological activities of these extracts.

The GC-MS analysis of methanolic extract of cipadessabaccifera revealed the presence thirty eight compounds. The major components were shyobunone(RT: 40.06; %Peak area: 26.67), 9,12,15-Octadecatrienoic acid, methyl ester (RT: 34.76; %Peak area: 9.25), 2-Furancarboxaldehyde, 5-(hydroxymethyl) (RT: 11.82; %Peak area: 7.50),2H-1-Benzopyran-2-one (RT: 16.40; %peak area: 6.37) and n-Hexadecanoic acid (RT: 30.47; %Peak area: 4.73) (Table 2 and Figure 1). Shyobunone is a sesquiterpenoid. Sesquiterpenes usually have a wide range of pharmacological activities, monocyclic sesquiterpenoids has allergic and anti-inflammatory properties. Many sesquiterpenoid lactones with chemical and structural properties for selective activity towards tumor and cancer stem cells[11]. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (RT: 9.66; %Peak area: 3.02), a potent anti-inflammatory and antioxidant compound[12]. Coumarin (2H-1-Benzopyran-2-one) has medicinal value.

Coumarins and other benzopyrones are known to stimulate macrophages to degrade extracellular albumin, allowing faster resorption of edematousfluids[13, 14]. Presence of these therapeutically active compounds in *C.baccifera* is an indication for its efficacy against various ailments.

The GC-MS analysis of methanolic extract of *O. aristatus* revealed the presence of various major volatile phytochemicals such as Phytol (RT: 33.67; %Peak area: 34.47), n-Hexadecanoic acid (RT: 30.65; %Peak area: 23.24), 9,12,15-Octadecatrienoic acid (RT: 35.02; %Peak area: 7.51), and 2, 6,10,14,18,22-Tetracosahexaene (RT: 48.62; %Peak area: 6.34). Squalene (2,6,10,14,18,22-Tetracosahexaene), an isoprenoid compound structurally similar to beta-carotene, is an intermediate metabolite in the synthesis of cholesterol, The primary therapeutic use of squalene currently is as an adjunctive therapy in a variety of cancers.Presence of these volatile phytochemicals in the methanolic extract could be responsible for the antioxidant and anticancer activities exhibited by *O. aristatus*(Table 3 and Figure 2).

Conclusion

The study revealed the presence of therapeutically active compounds in the mathanolic extract of C. baccifera and O. aristatus leaves. Further screening is required to confirm the pharmacological activities of these plants.

Table 1

Phytochemical screening of methanolic extract of *C. baccifera*and *O. aristatus*(leaves)

Phytochemical Constituents	*C.baccifera*	*O. aristatus*
Terpenoids	+	+
Tannins	+	+
Flavanoids	+	+
Alkaloids	+	+
Phenolic compounds	+	+
Steroids	-	+

Table 2:

Chemical constituents of *Cipadessabaccifera*

S.No.	Peak Name	Retention time	Peak area	% Peak area
1.	Name: 3-Amino-2-oxazolidinone Formula: $C_3H_6N_2O_2$ MW: 102	2.83	14029049	1.5504
2.	Name: 1H-Imidazole, 1,5-dimethyl- Formula: $C_5H_8N_2$ MW: 96	3.39	398094	0.0440
3.	Name: 2-Furanmethanol Formula: $C_5H_6O_2$ MW: 98	3.70	2602944	0.2877
4.	Name: 1,2-Cyclopentanedione Formula: $C_5H_6O_2$ MW: 98	4.88	13780054	1.5229
5.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	5.70	3876337	0.4284
6.	Name: 2,4-Imidazolidinedione, 1-methyl- Formula: $C_4H_6N_2O_2$ MW: 114	7.45	8497442	0.9391
7.	Name: 2,5-Dimethyl-4-hydroxy-3(2H)-furanone Formula: $C_6H_8O_3$ MW: 128	7.71	3913078	0.4325
8.	Name: 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl- Formula: $C_6H_{10}N_2O$ MW: 126	8.35	26715970	2.9525
9.	Name: N-Acetyl-3,4-dehydroproline Formula: $C_7H_9NO_3$ MW: 155	8.62	3519211	0.3889

10.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	9.66	27358216	3.0235
11.	Name: Benzofuran, 2,3-dihydro- Formula: C_8H_8O MW: 120 Name: Benzaldehyde, 4-methyl- Formula: C_8H_8O MW: 120	10.16	38388328	4.2425
12.	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: $C_6H_6O_3$ MW: 126	11.82	67849688	7.4984
13.	Name: Pentanoic acid, 3-hydroxy-4-methyl-, methyl ester Formula: $C_7H_{14}O_3$ MW: 146	12.16	8396379	0.9279
14.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	13.45	3636005	0.4018
15.	Name: 3-Methyl-4-methylamino-1,2,4-triazole-5-thiol Formula: $C_4H_8N_4S$ MW: 144	13.69	2369438	0.2619
16.	Name: Phenol, 2,6-dimethoxy- Formula: $C_8H_{10}O_3$ MW: 154	14.27	5439170	0.6011
17.	Name: Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]- Formula: $C_{15}H_{24}$ MW: 204	15.61	2948544	0.3259
18.	Name: 2H-1-Benzopyran-2-one Formula: $C_9H_6O_2$ MW: 146	16.40	57677988	6.3743
19.	Name: Sucrose Formula: $C_{12}H_{22}O_{11}$ MW: 342	17.63	29113206	3.2175
20.	Name: D-Allose Formula: $C_6H_{12}O_6$ MW: 180	18.61	12134230	1.3410
21.	Name: 4'-Ethoxy-2'-hydroxyoctadecanophenone Formula: $C_{26}H_{44}O_3$ MW: 404	19.83	3489173	0.3856
22.	Name: Caryophyllene oxide Formula: $C_{15}H_{24}O$ MW: 220	20.15	4075990	0.4505
23.	Name: 10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0] undecan-5á-ol Formula: $C_{15}H_{24}O$ MW: 220	21.75	558708	0.0617
24.	Name: à-L-Galactopyranoside, methyl 6-deoxy- Formula: $C_7H_{14}O_5$ MW: 178	22.31	1872969	0.2070
25.	Name: Dodecanoic acid Formula: $C_{12}H_{24}O_2$ MW: 200	25.23	3367572	0.3722

26.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	26.69	16366131	1.8087
27.	Name: 2-Butanone, 4-(2,2,6-trimethylcyclohexyl)- Formula: $C_{13}H_{24}O$ MW: 196	28.40	3191888	0.3528
28.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	30.47	42760936	4.7257
29.	Name: Hexadecanoic acid, ethyl ester Formula: $C_{18}H_{36}O_2$ MW: 284	30.64	5729230	0.6332
30.	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	33.57	54806128	6.0569
31.	Name: 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- Formula: $C_{19}H_{32}O_2$ MW: 292	34.76	83695168	9.2496
32.	Name: 2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde Formula: $C_{15}H_{22}O_2$ MW: 234 Name: D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3á)- Formula: $C_{30}H_{50}O$ MW: 426	35.32	52220244	5.7711
33.	Name: 6áBicyclo[4.3.0]nonane, 5á-iodomethyl-1á-isopropenyl-4à,5à-dimethyl-, Formula: $C_{15}H_{25}I$ MW: 332	37.26	2665824	0.2946
34.	Name: 1-Naphthalenepropanol, à-ethenyldecahydro-3-hydroxy-à,5,5,8a-tetramethyl-2-methylene- Formula: $C_{20}H_{34}O_2$ MW: 306 Name:.Isotenulin Formula: $C_{17}H_{22}O_5$ MW: 306	38.30	8086586	0.8937
35.	Name: Shyobunone Formula: $C_{15}H_{24}O$ MW: 220 Name: 5à-Androst-15-en-17á-ol, 17-methyl- Formula: $C_{20}H_{32}O$ MW: 288	40.06	241339472	26.6717
36.	Name: 2-Buten-1-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)- Formula: $C_{13}H_{20}O$ MW: 192	40.67	6152575	0.6800
37.	Name: Stigmastane-3,6-dione, (5à)- Formula: $C_{29}H_{48}O_2$ MW: 428	42.88	11993303	1.3254
38.	1.All-trans-Squalene Formula: $C_{30}H_{50}$ MW: 410	48.30	29836018	3.2973

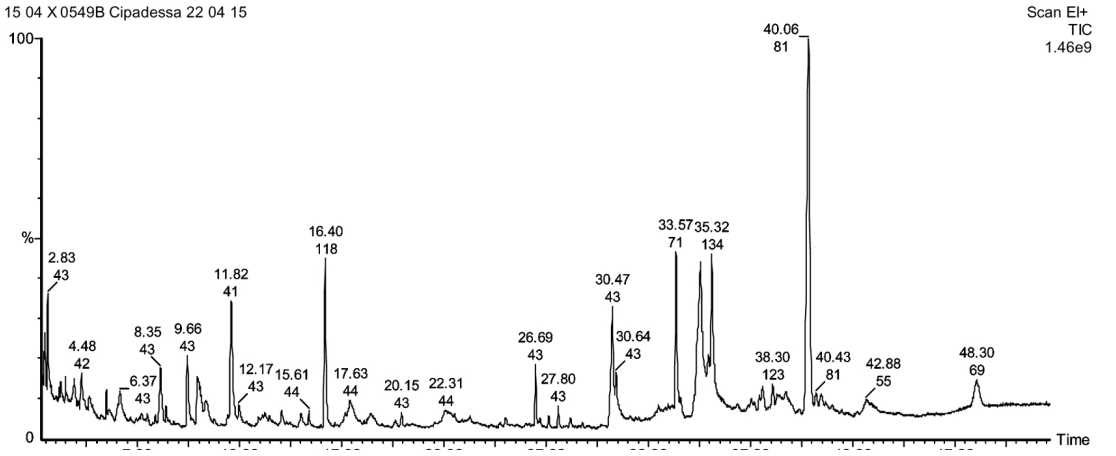


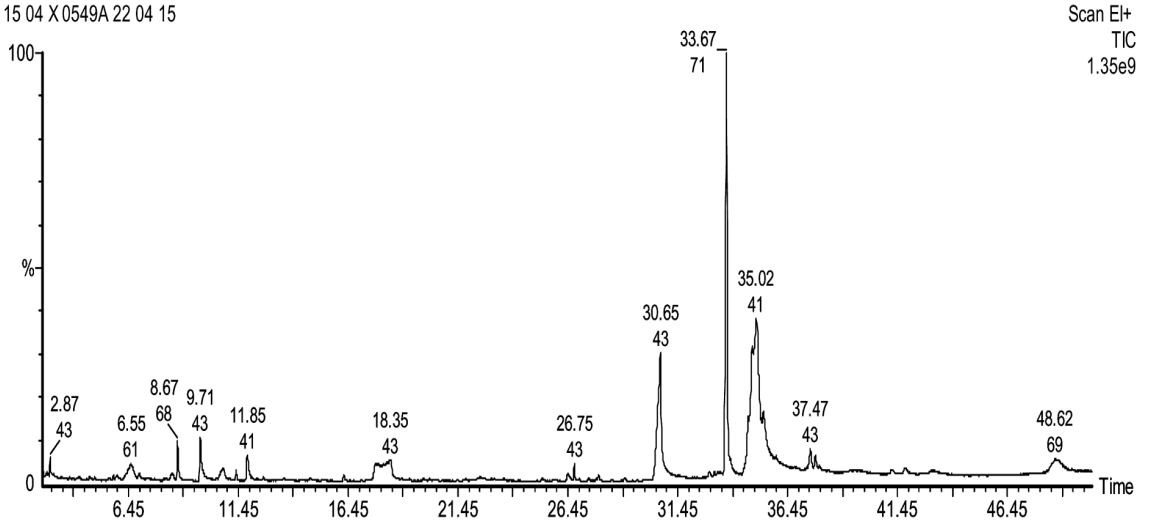
Table 3:

Chemical constituents of Orthosiphonaristatus

S.No.	Peak Name	Retention time	Peak area	% Peak area
1.	Name: 2-Furanmethanol Formula: $C_5H_6O_2$ MW: 98	3.76	316622	0.0883
2.	Name: 2-Cyclopentene-1,4-dione Formula: $C_5H_4O_2$ MW: 96	4.17	1012801	0.2823
3.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	4.91	511544	0.1426
4.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	5.76	529275	0.1475
5.	Name: Propylamine, N,N,2,2-tetramethyl-, N-oxide Formula: $C_7H_{17}NO$ MW: 131	6.55	13711603	3.8222
6.	Name: 2H-Pyran-2-one, tetrahydro-5,6-dimethyl-, trans- Formula: $C_7H_{12}O_2$ MW: 128	6.92	1430000	0.3986
7.	Name: 1H-Pyrrole, 2,5-dihydro- Formula: C_4H_7N MW: 69	8.67	8019723	2.2356
8.	Name: N-Acetylpyrrolidone Formula: $C_6H_9NO_2$ MW: 127	9.71	15509450	4.3234
9.	Name: 4-Amino-1,5-pentandioic acid Formula: $C_7H_{13}NO_4$ MW: 175	10.74	7270334	2.0267
10.	Name: L-Proline, 1-acetyl- Formula: $C_7H_{11}NO_3$ MW: 157	11.34	1730944	0.4825

11.	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: $C_6H_6O_3$ MW: 126	11.84	10633802	2.9643
12.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	13.52	636228	0.1774
13.	Name: N-(3-Methylbutyl)acetamide Formula: $C_7H_{15}NO$ MW: 129	14.72	1045901	0.2916
14.	Name: Dianhydromannitol Formula: $C_6H_{10}O_4$ MW: 146	16.24	1763314	0.4915
15.	Name: Benzaldehyde, 2-hydroxy-4-methyl- Formula: $C_8H_8O_2$ MW: 136	17.68	6128615	1.7084
16.	Name: 4-Acetamidobutyric acid Formula: $C_6H_{11}NO_3$ MW: 145	18.35	7416892	2.0675
17.	Name: 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- Formula: $C_{15}H_{26}O$ MW: 222 CAS	19.25	461721	0.1287
18.	Name: (-)-Spathulenol Formula: $C_{15}H_{24}O$ MW: 220	20.13	620288	0.1729
19.	Name: trans-Z-à-Bisabolene epoxide Formula: $C_{15}H_{24}O$ MW: 220	21.80	684908	0.1909
20.	Name: 3-Dodecen-1-ol Formula: $C_{12}H_{24}O$ MW: 184	22.96	756897	0.2110
21.	Name: 2,11-Dodecadiene, 4-acetoxy- Formula: $C_{14}H_{24}O_2$ MW: 224	23.09	718194	0.2002
22.	Name: Octadecanal Formula: $C_{18}H_{36}O$ MW: 268	23.54	668922	0.1865
23.	Name: Tetradecanoic acid Formula: $C_{14}H_{28}O_2$ MW: 228	25.28	989706	0.2759
24.	Name: Cyclohexene, 1-acetyl-2-(1-hydroxyethyl)- Formula: $C_{10}H_{16}O_2$ MW: 168	26.44	3259275	0.9086
25.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	26.75	3209848	0.8948
26.	Name: 2-Undecanone, 6,10-dimethyl- Formula: $C_{13}H_{26}O$ MW: 198	26.98	632441	0.1763
27.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	27.38	602097	0.1678

28.	Name: 2-Nonanone, 9-hydroxy- Formula: $C_9H_{18}O_2$ MW: 158	28.45	529776	0.1477
29.	Name: Tridecanoic acid, methyl ester Formula: $C_{14}H_{28}O_2$ MW: 228	29.01	931337	0.2596
30.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	30.65	83378752	23.2426
31.	Name: 2-Nonadecanone Formula: $C_{19}H_{38}O$ MW: 282	33.38	691945	0.1929
32.	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	33.67	123642880	34.4666
33.	Name: 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Formula: $C_{18}H_{30}O_2$ MW: 278	35.02	26942416	7.5105
34.	Name: Octadecanoic acid Formula: $C_{18}H_{36}O_2$ MW: 284	35.36	6110604	1.7034
35.	Name: 2-Nonadecanone Formula: $C_{19}H_{38}O$ MW: 282	37.89	999089	0.2785
36.	Name: 2-Propenoic acid, 2-methyl-, 2-(dimethylamino)ethyl ester Formula: $C_8H_{15}NO_2$ MW: 157	41.19	2492671	0.6949
37.	Name: 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- Formula: $C_{30}H_{50}$ MW: 410 All-trans-Squalene	48.62	22741492	6.3394



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