



## ANTIMALARIAL EFFICACY OF *Vitellaria paradoxa* GAERTN (FAMILY: SAPOTACEAE) LEAVES AND STEM BARK



Wandayi Emmanuel Amlabu<sup>1\*</sup> and Ishaya Haruna Nock<sup>2</sup>

<sup>1</sup>Department of Zoology, Ahmadu Bello University, Zaria, Nigeria

<sup>2</sup>West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, Legon, Accra, Ghana

\*Corresponding author: [wandayiamlabu@gmail.com](mailto:wandayiamlabu@gmail.com)

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**Abstract:** The antiplasmodial activity of *Vitellaria paradoxa* (Sapotaceae) leaves and stem bark 70% aqueous methanol crude extracts were evaluated and their phytochemical components elucidated. The study was carried out using the SYBRGreen 1 assay in an *ex vivo* culture of parasitized red blood cells along with a Giemsa stained microscopy study of same to ascertain the validity of the results. The leaves extract gave an IC<sub>50</sub> of 39 µg/ml against the 3D7 strain of *Plasmodium falciparum* while the stem bark showed no antiplasmodial effect with an IC<sub>50</sub> of 66 µg/ml. The photomicrographs of the treated cells show some parasite growth arrest in the case of treatment with the leaves extract but showed the contrary in terms of the stem bark extract. The presence of Alkaloids, Tannins, Terpenoids and Flavonoids were detected using chemical tests in the leaves and stem bark extracts but with the Flavonoids not found in the stem bark extract. Hence, the leaves of *Vitellaria paradoxa* has proven to have some antimalarial active compounds which if isolated would be valuable in the development of new antimalarials.

**Keywords:** *Vitellaria paradoxa*, *Plasmodium falciparum*, SYBRGreen 1 assay, microscopy, sapotaceae

### Introduction

Malaria is the most important parasitic disease in the world, taking into account its global distribution, the number and severity of infections, and the appalling public burden. Efforts to control malaria are being conducted on many fronts which spans from vector control, improved diagnosis and treatment, which includes the development of new drugs and drug combinations against the resistant parasite strains (Richie and Parekh, 2009). Malaria is a preventable and curable disease but most deaths occur among children living in Africa in where a child dies every minute from malaria (WHO, 2010). Of the approximately 3.4 billion people worldwide who are exposed annually, 1.2 billion are at high risk; the World Health Organization (WHO) states that there were 198 million cases of symptomatic malaria in 2013 (WHO, 2014). Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigeria's population while the remaining 3% of the population live in the malaria free highlands (United States Embassy in Nigeria, 2011). Therefore, concerns by all stakeholders to control and possibly eradicate this disease burden cannot be overemphasized.

The malarial infection begins when *Plasmodium* sporozoites are injected directly into the bloodstream from the salivary glands of a mosquito during a blood meal (mainly between dusk and dawn). The sporozoites enter liver cells where they begin a phase of multiplication called 'exoerythrocytic schizogony', during which thousands of uninucleate merozoites are formed. These released liver merozoites enter red blood cells in which they undergo a second phase of multiplication or erythrocytic schizogony. This is the only stage causing the complex and varying spectrum of symptoms characterizing the disease in humans. There are mainly four human adapted *Plasmodium* parasites with a fifth one which has just recently found humans as its new definitive hosts, these are namely; *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* (Largely a simian adapted species) (Garnham, 1966; Krotoski, 1985; Gueriard *et al.*, 2010).

In this study we sort to explore the possible new antimalarial lead compounds from a plant source which has been reported by folkloric claim to cure some ailments with symptoms associated to malaria. *Vitellaria paradoxa* (formerly *Butyrospermum parkii*) commonly known as the Shea butter tree which belongs to the Family of flowering plants called Sapotaceae and the Order: Ericales; has a pan-tropical

distribution (Aleza *et al.*, 2018) and with a gross economic impact on the persons living wherever it's found in the tropics. It is reported to find its usage as a rich source of cooking oil, edible fruits, cosmetics for skin and hair care, its wood serves for charcoal making, furniture construction, its latex serves as glue and its decoctions of leaves and barks are traditionally used in Burkina Faso to treat malaria, fever, pain, inflammation, and gastrointestinal disorders. The antibacterial and antifungal properties of *Vitellaria paradoxa* have also been demonstrated for different parts of this plant (Abbiw, 1990; Lovett and Haq, 2000a; Cidell and Alberts, 2006; Ogunwande *et al.*, 2001; Jansen *et al.*, 2010). However, we explored its antiplasmodial efficacy with the aim of eventually having new lead compounds that could serve for future antimalarial interventions.

### Materials and Methods

#### Plant materials

The leaves and stem bark of *Vitellaria paradoxa* Gaertner (Sapotaceae) were collected from the wild in Zaria (11.0667° N, 7.7000° E), northern Nigeria West Africa and identified and documented with the voucher number 9540 at the Herbarium, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. The plant materials were coded using Numbers and Alphabets that indicate the parts of the plant in used in this study.

#### Plant extracts preparation

The plant materials were air dried at room temperature for 2 weeks, after which they were ground to powder. Five grams (5 g) of the powdered dried leaves and stem bark were macerated individually in 300 ml of 70% (v/v) aqueous methanol and kept in a shaker (at 130 rpm, 28°C) for 17 hours. Filtrates were poured into a Rotary evaporator for condensation and dryness (Rotavapor Buchi R210/V, Vacuum controller V-850) at a vacuum pressure of 109 mbar, temperature of 50°C and at a speed of 4 rpm. After which the crude extracts obtained were kept safe in a refrigerator till usage. Extracts yield of 22.4 and 4.2% were obtained for the leaves and stem bark respectively.

#### Phytochemical screening of the plant extracts using chemical tests

##### Test for alkaloids using Dragendoff's reagent

Plant extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were treated with Dragendoff's Reagent (solution of Potassium Bismuth

Iodide). The formation of red precipitate would indicate the presence of alkaloids (Evans, 1996).

**Test for terpenoids**

The extracts were treated with chloroform then concentrated Sulphuric acid was added, the formation of reddish brown colouration indicates the presence of terpenoids (Evans, 1996).

**Test for phenolic compounds using 1% ferric chloride (which includes anthraquinones, flavonoid and tannins)**

The extracts were treated with 3-4 drops of ferric chloride solution. The formation of bluish black colour indicates the presence of phenols (Evans, 1996).

**Test for flavonoids**

NaOH solution was added to the water filtrate of the plant extract to give a yellow mixture. Two-three drops of dilute HCl were then added. The formation of a colourless solution from the yellow coloured mixture would indicate the presence of flavonoids (Evans, 1996).

**Cultivation of Plasmodium falciparum 3D7 in vitro and the antiplasmodial activity assay**

The leaves and stem bark crude extracts of *Vitellaria paradoxa* were subjected to an *in vitro* antiplasmodial screening using the chloroquine sensitive *Plasmodium falciparum* 3D7 (*Pf* 3D7). The parasites were maintained in media composed of RPMI 1640 containing 0.2% sodium bicarbonate, 0.5% albumax I, 45 µg/mL hypoxanthine, 50 µg/mL gentamicin and incubated under a gas mixture of 5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% N<sub>2</sub> in a CO<sub>2</sub> incubator at 37°C.

SYBR green I-based fluorescence assay was used as described by Smilkstein *et al.* (2004). The fluorescence counts were plotted against the drug concentrations and the 50% inhibitory concentration (IC<sub>50</sub>) was determined by analysis of dose-response curves (Kaushik *et al.*, 2013; Amlabu *et al.*, 2018).

**Dilutions of control drugs and test plant materials**

A parent stock of the test samples was formulated by dissolving the test extracts at 25 mg/ml in DMSO. Ten microliter (10 µl) of these parent stock solutions were aliquoted with mixing into different wells of the microtiter plate each of which contained 90 µl of distilled water. The resulting working dilutions were now 2.5 mg/ml in 10 % DMSO. The working dilutions (50 µl) were then two fold diluted two fold using 8 channel multipipette in triplicate wells containing 50 µl of 10 % DMSO to obtain a series of concentrations all in 10 % DMSO. This plate containing working dilutions in 10 % DMSO was designated as master plate. Four microliter (4 µl) of each concentration in triplicate from the master plate were then transferred to three wells of the daughter plate triplet wells containing 4 µl chloroquine (1mM) and triplet wells with 4 µl cRPMI served as positive (100 % growth inhibition) and negative (full growth) controls. Finally, a multipipette was used to dispense 96 µl of ring stage synchronized culture of malaria parasite to give 1% parasitemia and 2% hematocrit. In the process, the samples undergo 25 fold dilution of solute to a final DMSO concentration of 0.4% (v/v) in cRPMI (this concentration of DMSO is non-toxic to the growth of malaria parasite). The cultures were then incubated at 37°C under standard gas composition (5% O<sub>2</sub>, 5% CO<sub>2</sub>, 90% N<sub>2</sub>) (Amlabu *et al.*, 2018).

**Morphological effect of the plant extracts on Plasmodium falciparum**

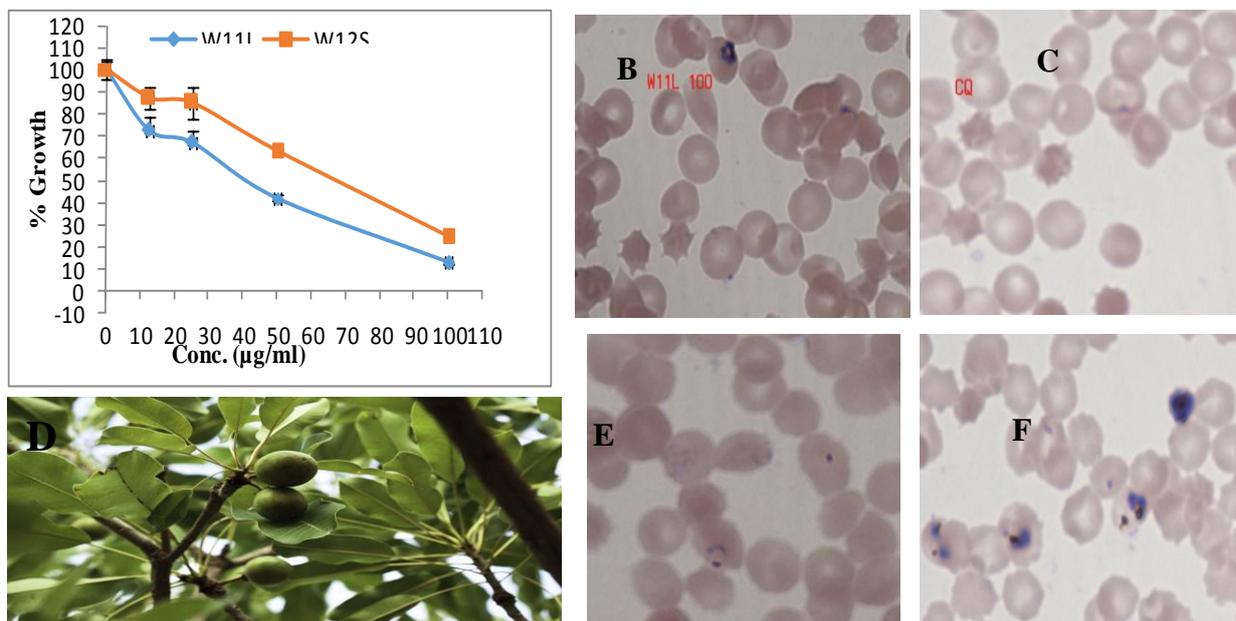
A study of the effect of the plant extracts on the morphology of the parasites was conducted using microscopy after incubation of parasites with the test materials (Table 2). The

test materials were incubated with synchronized ring stage parasites for 48 h. Thin smears of the incubated cells on glass slides were made and stained with Giemsa stain. The slides were viewed under a microscope (Nikon Eclipse 50i) to observe the nature of the parasite cells after treatment. The reference drugs and the untreated parasites served as positive and negative controls, respectively.

**Results and Discussion**

*Vitellaria paradoxa* leaves and stem bark extracts were tested for their antimalarial activity. The results obtained showed that the leaves extract shows some slight activity against the parasites *in vitro* while the stem bark extract showed no activity (Fig. 1; Table 1). The findings here run contrary to that reported by Jansen *et al.* (2010), where slight antiplasmodial activity was found in the stem bark in their report and no antiplasmodial activity was shown by the leaves extract. The disagreement seen in the two findings could be attributed to some opinions which range from the differences in the class of secondary metabolites present in the plant samples screened in the two different studies, the extraction methods used and possibly differences in the geographical locations where the plant materials were obtained. These reasons have been proven biologically to affect the availability of certain secondary metabolites found in a plants organism, which were known to be originally produced by plants to help them in fighting diseases in them or any challenges they encounter as a result of invasion by foreign bodies or any changes from their external environment that might affect their normal body functions or metabolism. And which further translate into becoming metabolites that have been used medicinally by man and other organisms to fight certain ailments in them. The same secondary metabolites were found in both the leaves and stem bark extracts in this study, but with the exception of flavonoids; which were absent in the stem bark extract. Flavonoids have been implicated as having medicinal properties against diseases of humans, like being anti-cancer, anti-inflammatory, anti-oxidant, combats cardiovascular diseases etc (Williams *et al.*, 2004; Friedman, 2007; Cazarolli, 2008; Ravishankar *et al.*, 2013; Siasos *et al.*, 2013; van Dam *et al.*, 2013; Cappello *et al.*, 2015), therefore, its presence might have buttressed the antiplasmodial activity shown by the leaves extract. However, the other secondary metabolites found in the extracts of this plant have been reported to have medicinal properties and their synergistic effects when administered traditionally could result in the therapeutic effect. It shows against various ailments as reported traditionally. In this study we report the antiplasmodial efficacy *in vitro* of the *Vitellaria paradoxa* leaves extract and recommend a further compound isolation efforts in order to obtain the bioactive compound for possible further development into new antimalarial therapeutic.

The GC-MS fingerprint of the leaves crude extract has elucidated the rich array of compounds that have known and unknown bioactivities (Fig. 2; Table 3). This study has revealed the profile of compounds found in *Vitellaria paradoxa* leaf which provides the chemotaxonomic blueprint of this particular plant from its micro locality of Zaria. This information would serve as a baseline data for future comparisons of compound composition for check listing, possible speciation and species preservation.



**Figures 1:** (A) Dose dependent growth inhibition of *Plasmodium falciparum* (3D7) against the crude extracts of *Vitellaria paradoxa* leaves and stem bark (B) The morphology of *Plasmodium* after being treated with 100 µg/ml of the crude leaf extract (W11L) showing slight parasite growth inhibition with an  $IC_{50}$  of 39 µg/ml (C) Parasites treated with Chloroquine which serves as the positive control, showing complete parasite growth inhibition or clearance (Plate D) Picture of *Vitellaria paradoxa* Plant (E) The morphology of *Plasmodium* after being treated with 100 µg/ml of the crude stem bark extract (W12S) showing no parasite growth inhibition with an  $IC_{50}$  of 66 µg/ml (F) Parasite without treatment serving as negative control

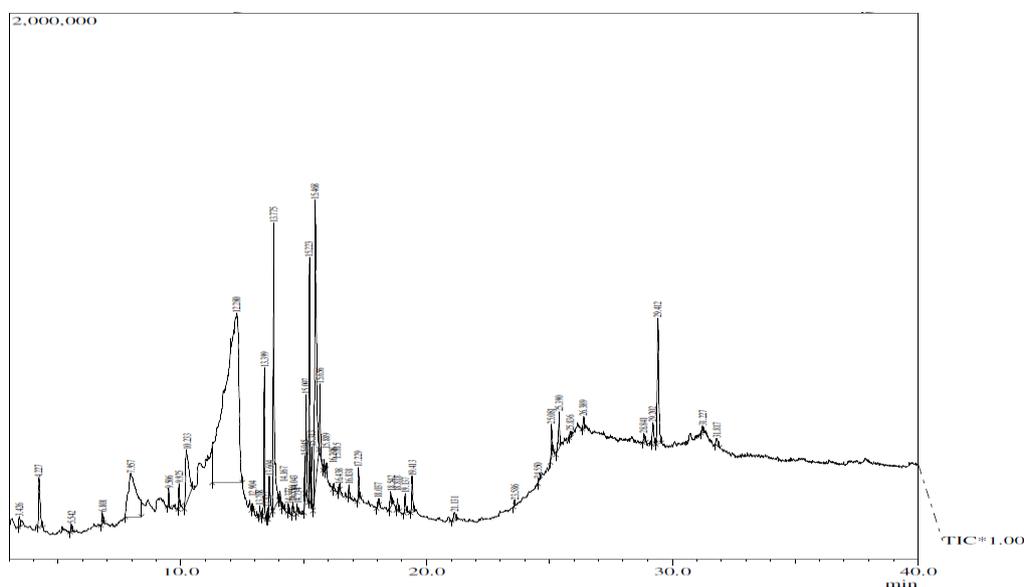
**Table 1: Antiplasmodial activity of 70% aqueous methanolic crude extracts of *Vitellaria paradoxa* whole plant**

Plant name	Plant parts used	Starting/Recovery weight (g)	% Yield	<i>P. falciparum</i> 3D7 $IC_{50}$ (µg/ml)
<i>Vitellaria paradoxa</i>	Leaves (W11L)	5/1.12	22.4	39
	Stem Bark (W12S)	5/0.21	4.2	66

**Table 2: Secondary Metabolites profile of *Vitellaria paradoxa* using chemical test methods**

Plant name	Plant parts used	Alkaloid	Terpenoids	Flavonoids	Tannins
<i>Vitellaria paradoxa</i>	Leaves (W11L)	+	+	+	+
	Stem Bark(W12S)	+	+	-	+

+ = Present, - = Absent



**Fig. 2: Gas Chromatography –Mass Spectrometry (GC-MS) Chromatograph of 70% aqueous methanol crude extract of *Vitellaria paradoxa* leaf**

**Table 3: Phyto-components identified in *Vitellaria paradoxa* (W11L) 70% aqueous methanol crude leaf extract by GC-MS Fingerprinting**

Peak No.	RT	Name of Compound	Peak Area %	MW	MF
1	3.426	2-Hexanone, 3-methyl-4-methylene-	0.28	126	C <sub>8</sub> H <sub>14</sub> O
2	4.227	4h-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1.57	144	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>
3	5.542	2,6-Octadien-1-ol, 3,7-dimethyl-, (e)-	0.18	154	C <sub>10</sub> H <sub>18</sub> O
4	6.801	6-Octen-1-ol, 3,7-dimethyl-, acetate	0.17	198	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>
5	7.957	1,2,4-Benzenetriol	7.25	126	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
6	9.506	Rose phenone	0.25	266	C <sub>10</sub> H <sub>6</sub> C <sub>13</sub> O <sub>2</sub>
7	9.925	1,2-Benzenedicarboxylic acid, diethyl ester	0.39	222	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>
8	10.233	1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid	3.45	192	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>
9	12.280	Inositol, 1-deoxy-	47.53	164	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>
10	12.904	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.08	278	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>
11	13.208	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	0.18	210	C <sub>14</sub> H <sub>26</sub> O
12	13.399	Hexadecanoic acid, methyl ester	2.13	270	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
13	13.527	Cyclohexane, 1e,5e-diisopropenyl-2a,3a-dimethyl-	0.14	192	C <sub>14</sub> H <sub>24</sub>
14	13.604	9-Hexadecenoic acid	1.08	254	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>
15	13.775	n-Hexadecanoic acid	6.18	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
16	14.043	1-Hexadecanol	0.18	242	C <sub>16</sub> H <sub>34</sub> O
17	14.167	Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pentylcyclopropyl)methyl	0.07	374	C <sub>25</sub> H <sub>42</sub> O <sub>2</sub>
18	14.377	Octadecanoic acid, methyl ester	0.10	298	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>
19	14.556	9-Octadecenoic acid (z)-	0.21	282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
21	15.045	Methyl octadeca-9,12-dienoate	0.53	294	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
22	15.097	9-Octadecenoic acid (z)-, methyl ester	1.79	296	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
23	15.223	Phytol	3.50	296	C <sub>20</sub> H <sub>40</sub> O
24	15.313	Octadecanoic acid, methyl ester	0.69	298	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>
25	15.468	Oleic acid	9.95	282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
26	15.656	Octadecanoic acid	1.17	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
27	15.815	6.beta.bicyclo[4.3.0]nonane, 5.beta.-Iodomethyl-1.beta.-isopropenyl-4.alpha.,5.	0.13	332	C <sub>15</sub> H <sub>25</sub> I
28	15.889	1-docosanol	0.09	326	C <sub>22</sub> H <sub>46</sub> O
29	16.208	11,14-eicosadienoic acid, methyl ester	0.14	322	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>
30	16.438	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	0.09	330	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>
31	16.838	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	0.21	568	C <sub>35</sub> H <sub>68</sub> O <sub>5</sub>
32	17.229	Cyclohexane, eicosyl-	0.54	364	C <sub>26</sub> H <sub>52</sub>
33	18.037	1-(1-heptadecynyl)cyclopentanol	0.15	320	C <sub>22</sub> H <sub>40</sub> O
34	18.542	e,e-2,13-Octadecadien-1-ol	0.35	266	C <sub>18</sub> H <sub>34</sub> O
35	18.818	Methyl dihydromalvalate	0.25	296	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
36	19.137	Octadecanal	0.43	268	C <sub>18</sub> H <sub>36</sub> O
37	19.413	Cyclohexane, eicosyl-	0.85	364	C <sub>26</sub> H <sub>52</sub>
38	21.131	13-Tetradecenal	0.32	210	C <sub>14</sub> H <sub>26</sub> O
39	23.586	(6e,10e,14e,18e)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-	0.09	426	C <sub>30</sub> H <sub>50</sub> O
40	24.550	Azacycloheptan-2-one, 3-[(3-phenylpropionyl)amino]- n-(2-oxo-3-azepanyl)-3-phenylpropanamide	0.07	260	C <sub>15</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>
41	25.081	9(11)-Dehydroergosteryl benzoate	0.60	498	C <sub>35</sub> H <sub>46</sub> O <sub>2</sub>
42	25.390	Neergosterol, acetate	0.93	422	C <sub>29</sub> H <sub>42</sub> O <sub>2</sub>
43	25.836	Pregn-5-ene-3,8,11,12,14,20-hexol, (3.beta.,11.alpha.,12.beta.,14.beta.)-	0.10	382	C <sub>21</sub> H <sub>34</sub> O <sub>6</sub>
44	26.389	Vitamin E	0.24	430	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>
45	28.841	Methyl commate a	0.19	500	C <sub>32</sub> H <sub>52</sub> O <sub>4</sub>
46	29.202	6s-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene	0.66	204	C <sub>15</sub> H <sub>24</sub>
47	29.412	Lupeol	3.81	426	C <sub>30</sub> H <sub>50</sub> O
48	31.227	Cholesta-17(20),24-diene-3,6-diol, (3.beta.,5.alpha.,6.alpha.)-	0.20	400	C <sub>27</sub> H <sub>44</sub> O <sub>2</sub>
49	31.817	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-	0.28	470	C <sub>31</sub> H <sub>50</sub> O <sub>3</sub>

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