

# Chemical Compositions and Antioxidant Activity of Volatile Oils from *Morinda citrifolia* and *Beta vulgaris* Leaves from Nigeria

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## Abstract

*Morinda citrifolia* L. and *Beta Vulgaris* L. leaves are both ethnomedicinal use for the treatment of arthritis, indigestion and skin infections with no reports on their essential oils compositions. The colourless volatile oils with a percentage yield of 0.6 and 0.4 (w/w) for *Morinda citrifolia* L. and *Beta Vulgaris* L. respectively were obtained. Forty-five compounds representing 94.31 % of the total percentage compositions were identified in the leaf essential oil of *M. citrifolia* with the most abundant compound as 14-beta-H-pregna- (33.13%). Forty-eight compounds representing 74.18% of the total oil composition were identified in the leaf oil of *B. Vulgaris* with phytol (24.20%) as the dominant compound. The essential oils showed good free radical scavenging activity when compared to ascorbic acid used as control, with % inhibition varying from  $88.74 \pm 0.010$  to  $96.61 \pm 0.004$  as compared to  $95.68 \pm 0.010$  to  $97.31 \pm 0.003$  of the ascorbic acid at (100 to 6.25 mg/ml) concentrations. The leaves essential oils of *Morinda citrifolia* L. and *Beta Vulgaris* L. contains chemical compounds that might be responsible for their antioxidant activity. This result validates the traditional usage of these plants in the treatment of arthritis, indigestion and skin infections.

**Keywords:** *Morinda citrifolia* L.; *Beta Vulgaris* L.; Antioxidant; free radical scavenging activity volatile oil.

## INTRODUCTION

The *Morinda citrifolia* L. (figure 1), also known as Noni in Nigeria, belongs to the Rubiaceae family (Arunachalam, 2018). It is a plant native to Southeast Asia and Australia that has long been used medicinally. According to reports, *M. citrifolia* can treat a wide range of medical conditions, including arthritis, heartburn, headaches, wounds, and skin infections. Additionally, there are some reports on the antitumor and anticancer activity of this plant and biological activities such as antihypoglycemia have been reported (Algenstaedt *et al.*, 2018).

*Beta Vulgaris* L. (figure 1), popularly known as beetroot, is a member of the Chenopodiaceae family (Mello *et al.*, 2008). It is a biennial herbaceous plant. It is eaten in Nigeria as a vegetable. It has traditionally been believed to treat or prevent conditions like arthritis, colon, prostate, dyspepsia, and skin infections. *Beta Vulgaris* leaf consumption lowers the risk of diabetes mellitus, obesity, and cardiovascular illnesses. It is also recognized for its potential to treat cancer (Kumar *et al.*, 2016). It is known to have a wide range of biological activities, including properties that are antibacterial, antioxidant, anticancer, antiviral, and anti-diabetic (Kavitha *et al.*, 2020). Consequently, in continuation of our search for biologically active compounds from

plants with ethnomedicinal uses (Okpala *et al.*, 2021; Okpala *et al.*, 2022; Onanuga and Oloyede, 2021), we present chemical compositions of the volatile oils and antioxidant activities of leaves of *Beta Vulgaris* L. and *Morinda citrifolia* L. The chemical compositions of the essential oils (EOs) of the leaves of both plants have not been reported in the literature to the best of our knowledge.



Figure 1. The image of the studied plants.

## MATERIAL AND METHODS

### Plant Material

The samples of *Morinda citrifolia* L. leaves were obtained fresh from Akobo Ibadan, Oyo state, South-

west, Nigeria (7°22' 39''N; 3° 54'21''E), on 10th April, 2021 while *Beta Vulgaris* L. leaves were purchased from Bodija market Ibadan, Oyo State. The plants were identified by Dr. S. K. Odewo of Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State.

### Extraction of the Essential Oil

The air dried and pulverized leaves of *M. citrifolia* (200 g) and *B. Vulgaris* (200 g) were weighed and separately subjected to extraction using hydro-distillation method with Clevenger type apparatus for four hours following British Pharmacopoeia specifications with modifications (British Pharmacopoeia, 1980). The samples were added into a 2 L round-bottomed flask containing 1.0 L distilled water and heated to boiling. There was the evaporation of the essential oils together with water vapour and these were collected in a condenser. The upper phase that contained the EOs was separated from the lower one and anhydrous sodium sulphate was used for drying the oils isolated. Oils extracted were preserved in a sealed amber glass vial at 4°C until analyses. The percentage yields (w/w) were determined.

### Gas Chromatograph-Mass Spectrometry (GC-MS) of the Oils

GC-MS analysis of the oils was performed using Gas chromatography 7890 coupled with mass spectrometer 5975 Agilent technology. The chemical components were identified by matching their mass spectra with those recorded in the mass spectra library (W11N17 main). The stationary phase was the column of model Agilent technologies HP-5 MS of length 30 m, the internal diameter of 0.320 mm and thickness of 0.25 µm while the mobile phase was helium gas. The oven temperature was at 80°C held for 2 mins at 12 degrees per minute to the final temperature of 240°C held for 6 mins. The ion source was set at 240°C and electron ionization at 70 eV. The scan ranges were from 50 to 550 amu and the interface temperature between GC and MS was 250°C. A sample of (1.0 µL) of diluted oils in hexane was manually injected into the GC-MS.

### Antioxidant Assay

The antioxidant activity of the essential oils was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging ability method. The concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 µg/mL) of the essential oils were mixed with 100 µM methanol- DPPH solution (2.0 mL) prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol to give a purple solution. The mixture was

shaken vigorously and left to incubate for 30 minutes in the dark at room temperature and the absorbance was then measured at 517 nm and recorded as A<sub>(sample)</sub> using a GS UV-12, UV-VIS Spectrophotometer. In its radical form, DPPH absorbs, but upon reduction by antioxidant species, its absorption reduces. A blank experiment was carried out applying the same procedure without essential oil (DPPH + Methanol) and the absorbance was recorded as A<sub>(control)</sub>. Ascorbic acid was used as a standard antioxidant for comparison. The free radical scavenging activities of each essential oil were then calculated as percentage inhibition according to the following equation:

$$\% I = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

## RESULTS

The physical properties and percentage yields of the volatile oils obtained from *M. citrifolia* and *B. Vulgaris* leaves are presented in Table 1.

Forty five compounds representing 94.31 % of the total percentage compositions were identified in the leaf essential oil of *M. citrifolia* (figure 2). The most abundant compounds are 14-beta-H-pregna- (33.13%), 1-Hexacosane (11.11%), Heneicosane (7.90%) and Tricosane (7.17%). The constituents of the oil were mainly non-terpenoids: hydrocarbon (43.67%), steroids (33.13%), alcohols, esters and fatty acids (9.26%) while the terpenes present are monoterpene (0.40%), diterpene (6.05%) and triterpene (1.28%) Table 2. Forty eight compounds representing 74.18% of the total oil composition were identified in the leaf oil of *B. Vulgaris* (figure 3). The dominant compounds are phytol (24.20 %), 1, 3-dimethylbenzene (14.84%) and neophytadiene (6.13%). The major class of compounds identified are diterpene alcohol (24.20) and aromatic compounds (18.70%) Table 3.

Some similarities of the ethnomedicinal uses of the two plants could be related to the presence of compounds such as phytol, neophytadiene, mesitylene, 3-hexanol, decane, Docosane, tetracosane, eicosane and heneicosane which were identified in the essential oils of both plants. The GC-MS chromatograms of the essential oils are given in Figures 2 and 3. The antioxidant activity of the essential oils of the leaves of *M. citrifolia* and *B. Vulgaris* are presented in Tables 4 and 5. The DPPH scavenging ability of the essential oils was compared with ascorbic acid a known standard antioxidant.

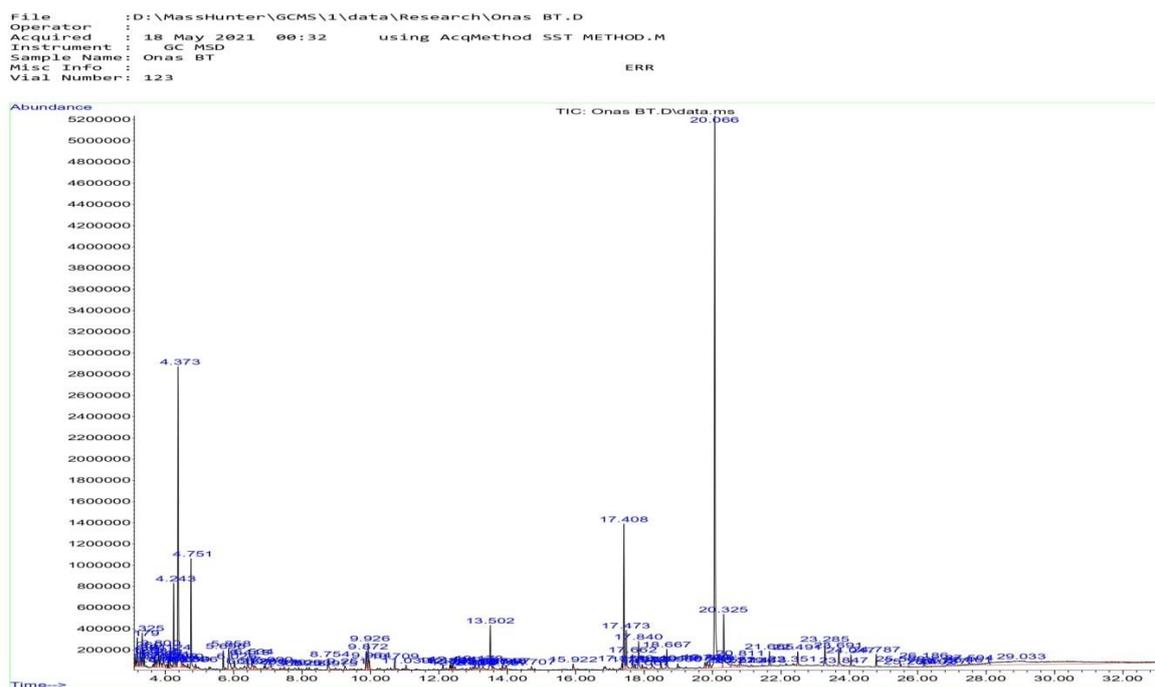


Figure 2. The GC-MS chromatogram of the leaf essential oil of *M. citrifolia*.

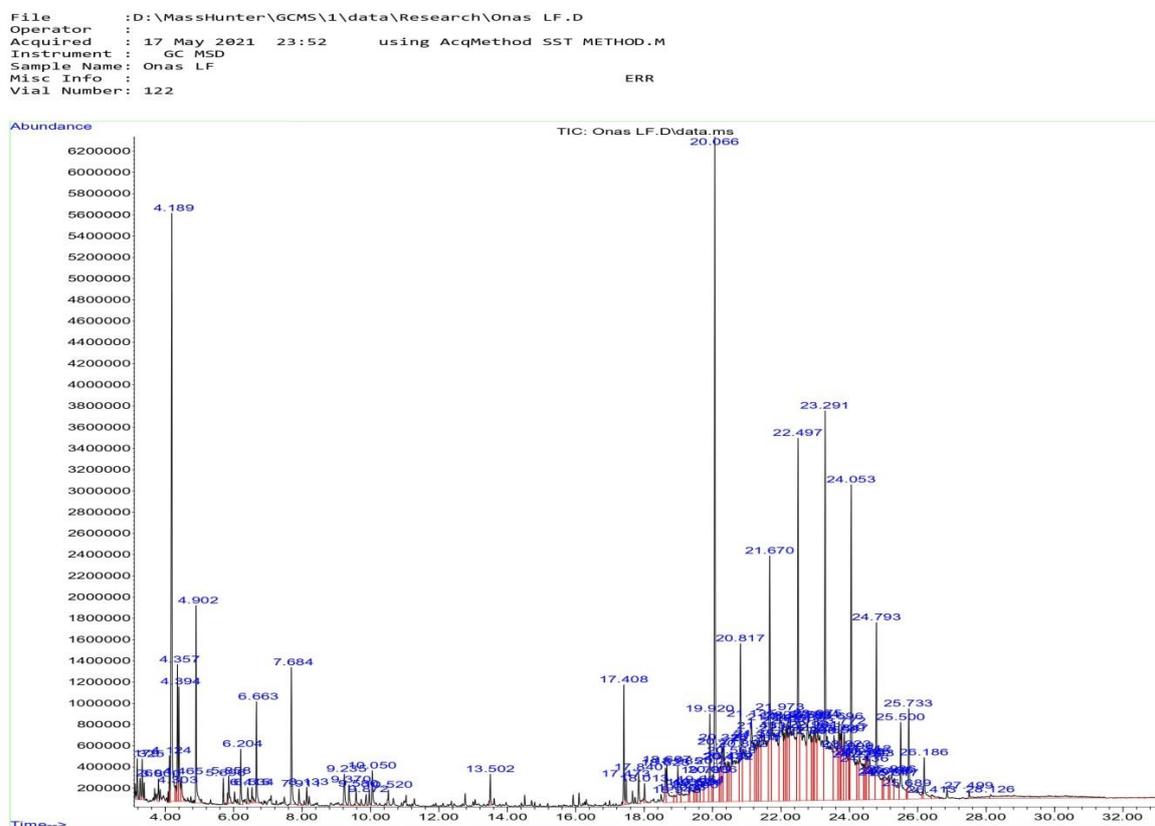


Figure 3. The GC-MS chromatogram of the leaf essential oil of *B. Vulgaris*.

**Table 1.** Physical properties and yields of essential oils from *M. citrifolia* and *B. Vulgaris* leaves.

Plant	Weight of leaf sample (g)	Weight of oil obtained (g)	% (w/w) yield of the oil obtained	Physical Properties
<i>Morinda citrifolia</i> L.	200	1.2	0.6	Colourless, herbaceous
<i>Beta vulgaris</i> L.	200	0.8	0.4	Colourless; leafy and aromatic odour

**Table 2.** Chemical compositions of *M. citrifolia* essential oils.

S/N	RT (min)	Chemical constituents	% composition	Class of compound
1	3.260	3-hexanol	0.14	Alcohol
2	3.325	6-methyl-2-heptanol	0.25	Alcohol
3	3.800	Ethyl-cyclohexane	0.15	Cycloalkane
4	4.124	2-hexenal	0.24	Aldehyde
5	4.189	3-hexen-1-ol (z)	4.66	Alcohol
6	4.357	2-hexen-1-ol (E)	0.77	Alcohol
7	4.394	1-hexanol	0.74	Alcohol
8	4.465	3-methylene-1-vinyl-1-cyclopentene	0.25	Cycloalkane
9	4.902	2-heptanol	1.20	Alcohol
10	5.858	2-hexanol	0.22	Alcohol
11	6.204	1-octen-3-ol	0.35	Alcohol
12	6.415	Mesitylene	0.13	Aromatic
13	6.534	Decane	0.11	Alkane
14	6.663	3-hexen-1-ol, acetate	0.57	Ester
15	7.684	Benzofuran	0.94	Heterocyclic
16	8.133	3-carene	0.12	Monoterpene
17	9.235	Cinnamaldehyde (E)-	0.30	Aldehyde
18	9.872	2-methyl-2-nonen-4-one	0.10	Ketone
19	10.050	Citronellol	0.28	Monoterpene
20	10.520	2-decanal (E) -	0.15	Aldehyde
21	13.502	4-(2,6,6-trimethyl-1-cyclohexen-1-yl 3-buten-2-one	0.19	Ketone
22	17.408	Neophytadiene	0.66	Diterpene
23	17.473	3-octadecene (3E)-	0.18	Alkene
24	17.840	3-eicosene (E)	0.24	Alkene
25	18.013	Nonadecane	0.13	Alkane
26	18.623	<i>n</i> -hexadecanoic acid	0.34	Fatty acid
27	18.985	Eicosane	1.05	Alkane
28	19.093	14-beta-H-pregna	33.13	Steroid
29	19.785	1-nonadecene	0.68	Alkene
30	19.920	Heneicosane	0.85	Alkane
31	20.66	Phytol	5.39	Diterpene
32	20.207	1-heneicosene	0.36	Alkene
33	20.325	1-eicosene	1.20	Alkene
34	20.482	1-tetracosene	0.62	Alkene
35	20.817	Docosane	1.69	Alkane
36	21.670	Tricosane	7.17	Alkane
37	21.750	1-Hexacosene	11.11	Alkene
38	22.491	Tetracosane	3.16	Alkane
39	23.291	Heneicosane	7.90	Alkane
40	24.053	Hexacosane	3.65	Alkene
41	25.317	9-Hexacosene	0.53	Alkene
42	25.500	Octacosane	0.98	Alkane
43	25.733	Supraene	1.28	Triterpene
44	26.186	Nonacosane	0.44	Alkane
45	27.499	3-Ethyl-2,6,10-trimethyl undecane	0.24	Alkane
Total percentage composition			94.31	

**Table 3.** Chemical compositions of *B. Vulgaris* essential oils.

S/N	RT (min)	Chemical constituents	% composition	Class of compounds
1	3.125	3-Hexanone	0.31	Ketone
2	3.152	1-ethyl-3-methyl cyclopentane	0.41	Cycloalkane
3	3.179	2-Hexanone	0.89	Ketone
4	3.260	3-Hexanol	1.22	Alcohol
5	3.250	2,5-dimethyl-1-hepten-4-ol	1.20	Unsaturated alcohol
6	3.384	1, 3-dimethyl-trans- cyclohexane	0.39	Cycloalkane
7	3.730	1,2-dimethylcyclohexane	0.33	Cycloalkane
8	3.800	Ethyl-cyclohexane	0.82	Cycloalkane
9	4.081	1,2,4-trimethyl cyclohexane	0.26	Cycloalkane
10	4.124	2-Hexanal (E)-	0.60	Aldehyde
11	4.243	Ethylbenzene	2.57	Aromatic
12	4.308	Nonane	0.31	Alkane
13	4.470	1,3-dimethylbenzene	14.84	Aromatic
14	4.659	Cis-1-ethyl-3-methyl-cyclohexane	0.13	Cycloalkane
15	4.886	Nonane	0.18	Alkane
16	5.858	2-methyl-3-propyl-trans oxirane	1.30	Cyclo ether
17	6.415	Mesitylene	0.56	Aromatic
18	6.534	Decane	0.61	Alkane
19	6.885	1-ethyl-3-methyl benzene	0.12	Aromatic
20	7.360	1-methyl-3-propylbenzene	0.13	Aromatic
21	7.479	1-ethyl-3,5-dimethyl benzene	0.14	Aromatic
22	7.895	1,2,4,5-tetramethyl benzene	0.13	Aromatic
23	8.203	Nonanal	0.12	Aldehyde
24	8.754	3-methyl-6-(1-methyl ethyl ) cyclohexene	0.68	Cycloalkane
25	9.251	4-acetyl-1-methyl cyclohexene	0.24	Cycloalkane
26	9.872	1-cyclopropyl-2-propanone	0.72	Cycloketone
27	9.964	1-carboxaldehyde, 2,6,6-trimethyl-1 cyclohexene	0.33	Cycloalkane
28	11.039	Tridecane	0.17	Alkane
29	12.363	Tetradecane	0.15	Alkane
30	13.059	6,10-dimethyl, 5,9-undecadien-2-one	0.18	Unsaturated ketone
31	13.173	1-[(E)-3-methylbut-1-enyl]cyclohexene	0.32	Cycloalkane
32	13.281	1-(1,1-dimethylethyl)4-ethyl benzene	0.21	Aromatic
33	13.502	4-(2,6,6- trimethyl-1-cyclohexen-1-yl) 3-buten-2-one	1.83	Cycloketone
34	13.967	Copaene	0.23	Alkene
35	14.707	2-methyl-4-(2,6,6, trimethyl-1-cyclohexen-1-yl) 2 butenal	0.16	Cycloaldehyde
36	15.922	Heptadecane	0.27	Alkane
37	17.354	3,7,11,15 tetramethylhexadec-2-ene	1.78	Alkene
38	17.408	Neophytadiene	6.13	Diterpene
39	17.948	1-Nonadecene	0.18	Alkene
40	19.780	E-15-heptadecanal	0.34	Aldehyde
41	19.839	1-octadecene	0.40	Alkene
42	19.920	Heneicosane	0.29	Alkene
43	20.066	Phytol	24.20	Diterpene
44	20.325	Diallylacetal, palmitaldehyde	3.18	Aldehyde
45	20.811	Docosane	0.87	Alkane
46	21.665	Eicosane	1.98	Alkane
47	22.491	Tetracosane	0.68	Alkane
48	23.285	3-pentacosene (E)	1.09	Alkene
Total percentage composition			74.18	

**Table 4.** Absorbance values at 517 nm of DPPH method of antioxidant assay.

Conc. (mg/ml)	<i>M. citrifolia</i>	<i>B. Vulgaris</i>	Asc. Acid
100	0.251 ± 0.003	0.102 ± 0.004	0.081 ± 0.003
50	0.270 ± 0.002	0.106 ± 0.007	0.097 ± 0.004
25	0.280 ± 0.013	0.116 ± 0.007	0.105 ± 0.009
12.5	0.312 ± 0.005	0.123 ± 0.005	0.127 ± 0.005
6.25	0.339 ± 0.010	0.133 ± 0.005	0.130 ± 0.010

Absorbance values in mean ± standard error; Asc. Acid = Ascorbic Acid at 517nm; ± Standard deviation for measurement, Absorbance of control = 3.010 ± 0.005

**Table 5.** Percentage inhibition calculated from DPPH method of antioxidant assay.

Conc. (mg/ml)	<i>M. citrifolia</i>	<i>B. Vulgaris</i>	Asc. Acid
100	91.66	96.61	97.31
50	91.02	96.48	96.78
25	90.69	96.15	96.51
12.5	89.63	96.91	95.78
6.25	88.74	95.58	95.68

## DISCUSSION

Methyl hexanoate, methyl octanoate, ethyl octanoate and methyl 4 *E*-decanoate have been reported in the volatile oil of *M. citrifolia* fruit, they are found to contain flavonoids as the major phytochemicals (Pino *et al.*, 2010). 14-b-H-Pregna, a steroid considered to be a sex pheromone specific to males, and also a defensive chemical with diabetic retinopathy prevention and treatment effects. The presence of 14-b-H-pregna has been reported in the essential oils from different parts of some plants, including *Scutellaria* plants, *Urginea indica* Kunth, *Allium rotundum*, *Gundelia tournefortii* L (Farhang *et al.*, 2016). Citrus limon (Akhila *et al.*, 2015). Dehpour *et al.* 2012 reported that 14-b-H-pregna was the major compound in the essential oil of lower *Allium rotundum* which displayed antibacterial activity.

Neophytadiene is a good analgesic, antimicrobial, antipyretic, antioxidant and anti-inflammatory compound (Venkata *et al.*, 2012). Phytol, known to exhibit antioxidant and antinociceptive effects is a precursor of synthetic vitamins E and K and is cytotoxic against breast cancer cell lines (MCF7) (Casuga *et al.*, 2016; Sermakkani and Thangapandian, 2012).

The measured absorbance values and calculated percentage inhibition show that the antioxidant activity of the two essential oils and standard (Ascorbic acid) is concentration dependant. From the results, the % inhibition of the essential oils of both plants exhibited good scavenging ability on DPPH radical which were comparable to ascorbic acid at all concentrations (100-6.25 mg/ml) investigated.

## CONCLUSIONS

Analysis of the essential oils of *M. citrifolia* and *B. Vulgaris* leaves showed that they contained different major constituents. The major constituent of *M. citrifolia* leaves essential oil were steroids: 14-beta-H-pregna (33.13%) and 1-Hexacosane (11.11%), while the dominant compounds identified in *B. Vulgaris* were phytol (24.20 %) and 1,3-dimethylbenzene (14.84%). These major constituents of both oils have been reported to possess similar properties such as antibacterial, antioxidant and antinociceptive. Most of the chemical compounds identified from the essential oils of *M. citrifolia* and *B. Vulgaris* were biologically active compounds and the essential oils exhibited good scavenging ability at all concentrations investigated. The plants' parts could be a source of drug development for oxidative diseases.

**Competing Interests:** A. O. ONANUGA and E. O. OKPALA declare that they have no competing interests.

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