JCBPS; Section B; August 2018 – October 2018, Vol. 8, No. 4; 811-819, E- ISSN: 2249 –1929
[DOI: 10.24214/jcbps.B.8.4.81119.]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences
Available online atwww.jcbsc.org
Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Volatile Organic Constituents of Two Fractions of Leaves of *Ficus vogelii* and their Potential Health Implication

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Received: 15 July 2018**; Revised:** 26 July 2018; **Accepted:** 03 August 2018

Abstract: The leaf of *Ficus vogelii* is commonly used as a green-leafy vegetable in Northern Cross River State of Nigeria. Its ethanol extract is used by adults for well-being, while, its aqueous extract is used for weaning children and for treatment of pediatric anemia. In this study, the methanol and n-hexane fractions of the leaves were analyzed for volatile organic composition using GC-MS, in order to determine the class of constituents that may be responsible for the amelioration of anemia, and sustenance of well-being in adults. GC-MS analysis of n-hexane and methanol fractions revealed the presence of several organic constituents including, twenty one (21) volatile compounds in n-hexane fraction and, thirty five (35) compounds in methanol fraction. The dominant compounds in the n-hexane fraction included, Hexadecanoic acid (3.14%), n-Nonadecanoic acid (17.81%), Phytol (38.45%), Oleic acid (21.20%) and E-2-Octadecadecen-1-ol (4.77%); while the dominant compounds in methanol fraction included, Glycerin (8.44%), Dimethyl sulphoxide (7.44%), 2(R), 3(S)-1,2,3,4-Butane tetrol (6.47%), 17α-OH-17 β-Cyano-Preg-4-en-3-one (3.10%), Ethyl-β-d-glucopyranoside (7.25%), Bicyclo[3.1.0]hexan-3-ol (10.11%); n-Hexadecanoic acid (15.42%) and Oleic Acid (21.40%). It was concluded that the presence of Palmitaldehyde diisopentyl acetate (2.52%) in the n-hexane fraction may contribute significantly to the pleasant flavor of the extract fraction and its nutritional acceptability. It was also concluded that the high content of oleic acid and phytol in the plant may be responsible for the cardiovascular benefits the plant confers on the populations consuming it, as both compounds are known to lower blood cholesterol lipids in adult humans.

Key Words. *Ficus vogelii*, methanol fraction, n-hexane fraction, volatile organic compounds, GC-MS

INTRODUCTION

Ficus vogelii is a small tree of about 10-20m tall, which grows in most tropical climates[1]. It belongs to the family of Moraceae. The tree is widely distributed in Africa. It is found in all of West African countries including Nigeria, Ghana, Senegal and Mali; and grows in the Congo on to some East African countries including, Uganda, Cameroun and Tanzania[2].

Ficus vogelii is commonly called West African Rubber Tree [1]. The leaves of *Ficus vogelii* are alternate and spirally arranged. They are bluish-green with pale green veins. Its fruits are sessile and yellow when ripe with a fuzzy surface[3]. *Ficus vogelii* produces fruits which resemble inverted flower[4].

The leaves of *Ficus vogelii* and *Ficus asperifolia* look alike externally, except for slight differences in their venation. They co-occur in most tropical and sub-tropical regions of the world. *Ficus vogelii* is called kujung by the Obudu people of Northern Cross River State. Its leaves are used for the treatment of diarrhea, dysentery and anemia in traditional medicine. The latex of members of the *Ficus genus* has been reported to give protection from physical assault by pests[5]. *Ficus vogelii* leaf is used by the Obudu and Bekwarra people of Cross River State as a green leafy vegetable, and as a medicinal herb in ameliorating anemia and diabetic conditions, as well as other endocrine complications.

The leaves are prepared as vegetables in soups and used traditionally in the treatment of anemia, and for well-being in adults. This is because adults believe that eating various dishes prepared with the leaves guarantees them good health and well-being. This study discovered that traditionally, there are claims that the bark and root are used in treating urinary tract infection, asthma, diabetes and malaria. This claim was supported by literature in which the bark was reported to have been used in treating urinary infection, cardiovascular diseases, and kidney diseases and cough[6].

It was on account of these claims, that we started research work on the chemical and photochemical constituents of this plant in order to relate the medicinal activities of the plant to its natural and bioactive constituents.

MATERIALS AND METHODS

Collection of Plant Material: Fresh mature *Ficus vogelii* leaves were harvested from a farm in Obudu Local Government Area of Cross River State of Nigeria. The plant was then authenticated by a botanist in the Botany Department of the University of Calabar, Nigeria. Rat chow feed was purchased from Grand Cereals Limited in Aba, Abia State of Nigeria.

Sample preparation for GC-MS Analysis for Volatile Organic compounds: One kg of the leaves was washed, cut into small pieces and air-dried at room temperature (27±1.50ºC) for seven days. The leaves were then blended using a manual blender into coarse powder and stored in air tight plastic containers until use. 500g of the coarse powder was macerated with 2000ml of 80% ethanol in a ratio of 1:4 for two days. This allowed the sample to have sufficient contact with the solvent to increase its extraction efficiency of bioactive volatile compounds. The extract was then filtered using a cheese cloth and concentrated *in vacuo* using a rotary evaporator to remove all alcohol and afford 55g of the crude extract which was stored under refrigeration.

Fractionation of Extract by Column Chromatography: Glass column chromatography was packedwith silica gel (Silane 343, pore size 0.15µm) for reverse phase fractionation of the crude extract. The packed column was washed with 30% methanol and stabilized by washing with distil water. 5g of the crude extract was applied to the column and allowed to stand for 5 minutes. The column was then eluted with n-hexane, followed by 30% methanol to afford n-hexane and 30% methanol fractions respectively. All fractions obtained were concentrated *in vacuo* to afford alcohol-free extracts which were later diluted 1:20 in their respective solvents for GC-MS analysis.

GC-MS Analysis of Methanol Fraction: Diluted samples (1/20 in n-hexane and 1/20 in methanol) were injected manually through the injector port. An Agilent 6890 GC coupled with a 5973i mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was used. The GC was equipped with a HP-5MS capillary column (30m × 250µm i.d. × 0.25µm, Agilent Technologies). Helium was the carrier gas with a constant flow of 1mL/min to the column. The initial oven temperature was at 40°C, holding for 2 min, then raised to 150°C at 5 °C/min; and finally raised to 280°C at 15°C/min, holding for 2 min. The injection port was maintained at splitless mode. The mass detector was operated at 150°C in electron impact (EI) mode at 70 eV. The ion source temperature was at 230°C and the transfer line temperature was maintained at 250°C. The chromatograms were recorded by monitoring the total ion currents in the 15–450 mass range. MS was detected with 2 min solvent delay. Analysis of the sample at each condition was repeated twice to ensure consistency. C6-C24 *n*-alkanes were ran under the same chromatographic conditions in order to calculate the retention indices (RI) of detected compounds. Identification of the oil and volatile constituents was based on retention indices relative to n-alkanes (C8-C24), and computer matching with the WILLEY 275.L library, and those contained in the NIST08 database; and confirmed by comparison of the retention times (RIs), as well as by comparison of their mass spectral fragmentation patterns with those reported in literature.

Compounds were identified by comparing their mass spectra with those contained in the NIST08 database, and confirmed by comparison of the retention times of the separated constituents with those of the authentic samples and by comparison of retention indexes (RIs) of the separated constituents with the RIs reported in the literature

RESULT

Twenty one (21) compounds were identified in the n-hexane fraction (**Figure1, Table 1),** while thirty five (35) compounds were identified in the methanol fraction (**Figure 2, Table 2**). The dominant compounds in the n-hexane fraction included, Hexadecanoic acid (3.14%), n-Nonadecanoic acid (17.81%), Phytol (38.05%), Oleic acid (21.20%) and E-2-Octadecadecen-1-ol (4.77%); while the dominant compounds in the methanol fraction included, Glycerin (8.44%), Dimethyl sulphoxide (7.44%), 2(R), 3(S)-1,2,3,4-Butane tetrol (6.47%), 17α-OH-17β-Cyano-Preg-4-en-3-one(3.10%), Ethyl-β-d-glucopyranoside (7.25%), Bicyclo[3.1.0]hexan-3-ol (10.11%); n-Hexadecanoic acid (15.42%) and Oleic Acid (21.40%). Several organic acids partitioned into the n-hexane solvent fraction and these included C9–C24 compounds. The C9 compound was shown to be n-Nonanoic Acid (Mol. Wt. $C_9H_{18}O_2$) and per cent abundance of 1.19%. The C24 organic acid was identified to be oleic acid (Mol. wt. $C_{18}H_{34}O_2$), and 21.20% abundance in the plant. Several sugars were extracted by the methanol fraction and these included, D-Erythro-2-deoxy-pentose sugar(2.11%), 1-Deoxy-d-Arabitol (0.56%), Ethyl-α-d-glucopyranoside (7.25%), Methyl-α-d-galactopyranoside (1.01%),Methyl-β-d-galactopyranoside (1.20%), Ethyl-β-d-Riboside (0.32%), and 1,6-Anhydro-β-D-glucopyranoside (0.12%).

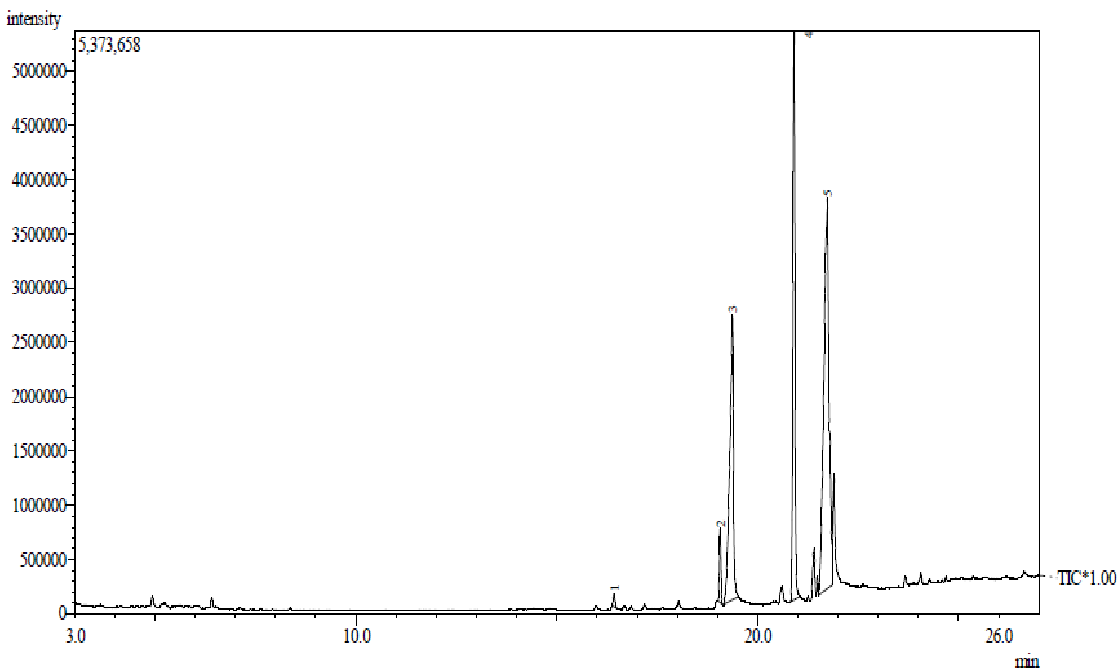

Figure 1: GC-MS Chromatogram of n-Hexane fraction of *Ficus vogelii*

Table 1: Volatile Organic Constituents of n-Hexane fraction of *Ficus vogelii* using GC-MS Analysis

Peak No	Ret Time (Min)	Name of Compound	Mol. Form	Mol. wt.	% Abundance
1	16.47	n-Tetradecen-1-ol acetate	$C_{16}H_{10}O_2$	254	0.32
2.	16.82	1-Nonyne	C_9H_{16}	124	0.22
3	16.93	1,2-Epoxytetradecane	$C_{14}H_{28}O$	212	0.17
4	17.44	2-Decan-1-ol	$C_{10}H_{20}O$	156	0.19
5	17.81	1-Dodecyne	$C_{12}H_{22}$	166	0.06
6	19.07	Hexadecanoic Acid	$C_{18}H_{36}O_2$	284	3.14
7	19.08	Pentadecanoic Acid	$C_{20}H_{40}O_2$	312	0.45
8	19.11	Docosanoic Acid	$C_{24}H_{48}O_2$	368	0.32

9	19.14	Octadecanoic Acid	$C_{20}H_{40}O_2$	312	0.59
10	19.25	Tridecanoic Acid	$C_{15}H_{30}O_2$	242	0.40
11	19.37	n-Nonadecanoic Acid	$C_{19}H_{38}O_2$	298	17.81
12	19.45	n-Decanoic Acid	$C_{10}H_{20}O_2$	172	2.22
13	19.71	n-Nonanoic Acid	$C_9H_{18}O_2$	158	1.19
14	20.91	Hexadecan-1-ol (Phytol)	$C_{20}H_{40}O$	296	38.05
15	20.95	Palmitaldehyde diisopentyl acetate	$C_{26}H_{54}O_2$	398	2.52
16	20.98	2, 6-dimethyl-1,7-Octadien-3-ol	$C_{10}H_{18}O$	154	2.15
17	21.73	Oleic Acid	$C_{18}H_{34}O_2$	282	21.20
18	21.77	E-2-Octadecadecen-1-ol	$C_{18}H_{36}O$	268	4.77
19	21.84	(9E)-9-Octadecenal	$C_{18}H_{34}O$	266	2.19
20	21.91	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	226	0.55
21	21.97	E-9-Tetradecenoic Acid	$C_{14}H_{26}O$	226	0.21
				Total	**100.00**

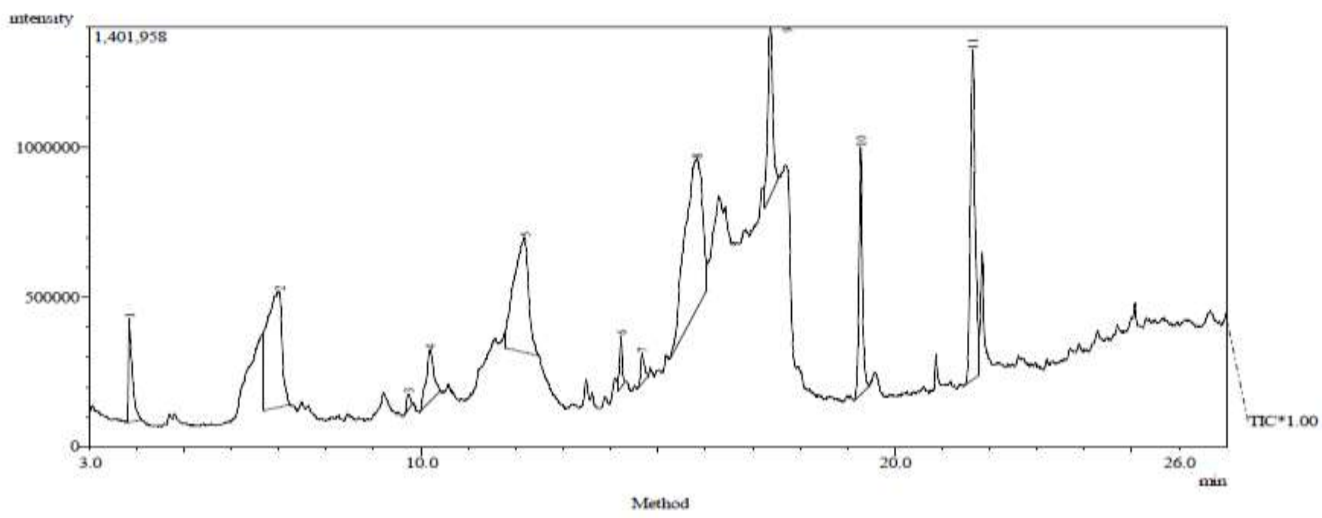

Figure 2: GC-MS Chromatogram of Methanol fraction of *Ficus vogeli*

Table 4: Organic Constituents of Methanol fraction of *Ficus vogelii* using GC-MS

Peak No	Ret Time (Min)	Name of Compound	Mol. Form	Mol. wt.	% Abundance
1	3.84	Dimethyl Sulphoxide	C_2H_6OS	78	7.44
2.	7.01	1,2,3-Propanetriol (Glycerin)	$C_3H_8O_3$	92	8.44
3	9.73	Phenoxyethylene	C_8H_8O	120	0.85
4	9.94	6-Methylene-bicyclo[3.2.0]	C_8H_8O	120	0.25
5	10.19	Hept-3-en-2-one D-Erythro-2-deoxy-pentose sugar	$C_5H_{10}O_4$	134	2.11
6	10.72	1-Deoxy-d-Arabitol	$C_5H_{12}O_4$	136	0.56
7	10.91	1,2,3-Butanetriol	$C_4H_{10}O_3$	106	0.29
8	11.01	2-Isopropoxyethylamine	$C_5H_{13}NO$	103	0.80
9	12.18	2(R),3(S)-1,2,3,4-Butane tetrol	$C_4H_{10}O_4$	122	6.49
10	12.66	3,4-Furandiol	$C_4H_8O_3$	104	1.27
11	14.22	3,4-Dimethyl -2-prop-2-enyl-2,5- Dihydrothio phene 1,1-dioxide	$C_9H_{14}O_2S$	186	0.55
12	14.26	17α-OH-17β-Cyano-Preg-4-en-3-one	$C_{20}H_{27}NO_2$	313	3.10
13	14.32	Z,Z,Z-1,4,6,9 – Nonadecatetraene	$C_{19}H_{32}$	260	0.21
14	14.44	3-Bromo-7-methyl-1-adamantane Carboxylic acid	$C_{12}H_{17}BrO_2$	272	0.11
15	14.59	5, 8, 10-Undecatrien-3-ol	$C_{11}H_{18}O$	166	0.31
16	14.67	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene,1,1 Dioxide	$C_{14}H_{16}O_2S_2$	280	1.77
17	14.71	3-Octyn-2-ol	$C_8H_{14}O$	126	0.21
18	14.79	5-Isopropenyl-1,2-dimethyl-cyclo- Hex-2-enol	$C_{11}H_{18}O$	166	0.20
19	14.84	Acetic acid, 7-oxo-bicyclo[3.2.1] Hept-2-yl ester	$C_9H_{12}O_3$	168	0.03
20	14.97	Trans-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$	0.07	0.07
21	15.81	Ethyl-α-d-glucopyranoside	$C_8H_{16}O_6$	7.25	7.25
22	15.94	Methyl-β-d-galactopyranoside	$C_7H_{14}O_6$	1.20	1.20
23	15.97	Methyl-α-d-galactopyranoside	$C_7H_{14}O_6$	1.01	1.01
24	15.99	Ethyl-β-d-Riboside	$C_7H_{14}O_5$	0.32	0.32
25	16.03	1,6-Anhydro-β-D-glucopyranoside	$C_6H_{10}O_5$	162	0.12
26	17.36	Bicyclo[3.2.0]-hexan-3-ol	$C_{10}H_{18}O$	154	10.11
27	17.45	(E)-9-Tetradecen-1-ol, acetate	$C_{16}H_{30}O_2$	254	1.02
28	17.62	3,5-Octadienoic acid	$C_9H_{14}O_3$	170	0.11
29	17.88	α-Limonene diepoxide	$C_{10}H_{16}O_2$	168	1.01
30	19.28	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.42
31	19.44	n-Nonadecanoic acid	$C_{19}H_{38}O_2$	298	1.20
32	19.67	n-Decanoic acid	$C_{10}H_{20}O_2$	172	0.22
33	19.89	n-Capric acid	$C_{13}H_{26}O_2$	214	1.10
34	21.64	Oleic acid	$C_{18}H_{34}O_2$	282	21.40

35	21.98	E-11-Tetradececenoic acid	$C_{14}H_{26}O_2$	226	2.55
				Total	**100.00**

DISCUSSION

GC-MS analysis of n-hexane and methanol fractions revealed the presence of fifty six (56) bioactive organic constituents including, twenty one (21) volatile compounds in the n-hexane fraction and thirty five (35) compounds in the methanol fraction. The dominant compounds in the n-hexane fraction included, Hexadecanoic acid (3.14%), n-Nonadecanoic acid (17.81%), Phytol (38.45%), Oleic acid (21.20%) and E-2-Octadecadecen-1-ol (4.77%). On the other hand, the dominant compounds in the methanol fraction included, Glycerin (8.44%), Dimethyl sulphoxide (7.44%), 2®, 3(S)-1,2,3,4-Butane tetrol (6.47%), 17α-OH-17 β-Cyano-Preg-4-en-3-one (3.10%), Ethyl-β-d-glucopyranoside (7.25%), Bicyclo[3.1.0]hexan-3-ol (10.11%); n-Hexadecanoic acid (15.42%) and Oleic Acid (21.40%). Others include, D-Erythro-pentose sugar; tetrahydro-3,4-furandiol; 5,8,10-Undecatrien-3-ol; 3-Octyn-2-ol; and levo-glucosan.

The presence of Palmitaldehyde diisopentyl acetate (2.5%) in the n-hexane fraction, suggests the biogenetic origin of most of the volatile organic compounds detected in the plant. Also, Palmitaldehyde diisopentyl acetate may be the major contributing aldehydic ester to the pleasant flavor of the extracts of this plant and its nutritional acceptability by the consumers. Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils, and is classified as a monounsaturated Omega-9 fatty acid. Oleic acid has been reported to reduce high blood pressure, increase fat burning to help with weight loss, protect cells from free radical damage, and may prevent type 2 diabetes[7]. It is also said to prevent ulcerative colitis and generates brain myelin[8]. As an Omega-9 fatty acid, it contributes to reduction of HDL-cholesterol and confers protection to the heart[9].

n-Nonadecanoic acid, is a 19-carbon long chain saturated fatty acid with the chemical formula $CH_3(CH_2)_{17}COOH$. It is found widely distributed in animal fats and vegetable oils. It is also used by insects as pheromones[10].17α-OH-17 β-Cyano-Preg-4-en-3-one is a chemical intermediate in the biosynthesis of many other endogenous steroids, including androgens, estrogens, glucocorticoids, and mineralocorticoids, as well as neuro-steroids[11,12]. It is the precursor to 17α-OH-progesterone (17α-OHP) which is an agonist of the progesterone receptor (PR) similarly to progesterone[13,14]. 17α-OHP increases in the third trimester of pregnancy primarily due to fetal adrenal production[15].

Phytol is the product of chlorophyll metabolism in plants. It is chemically called an acrylic diterpene alcohol which is used in the manufacture of Vitamin E and K [16]. Both of these vitamins are known to play very important functions in the human body. The use of phytol in the human body is indispensable. It is essential in activating enzymes that have a positive effect in the production of insulin. It was also reported to be effective in decreasing blood cholesterol levels[16]. Hexadecanoic acid (Palmitic acid) is a saturated fatty acid and the main acid in Red Palm Oil (RPO). It is commonly found in both animals and plants. Many medical authorities, such as the World Health Organization, say dietary intake of saturated fats such as palm oil (palmitic acid) increases the risk of cardiovascular diseases[17]. However, in moderation, palmitic acid might not be entirely bad for you, as it does display mild antioxidant and anti-atherosclerotic properties, at least in animal studies. In general, diets higher in unsaturated fats are considered healthier[17].

Several sugars were extracted by the methanol fraction and these included, D-Erythro-2-deoxy-pentose sugar(2.11%), 1-Deoxy-d-Arabitol (0.56%), Ethyl-α-d-glucopyranoside (7.25%), Methyl-α-d-galactopyranoside (1.01%), Methyl-β-d-galactopyranoside (1.20%), Ethyl-β-d-Riboside (0.32%), and 1,6-Anhydro-β-D-glucopyranoside (0.12%). These sugars are glucose precursors and its isomers, which may provide energy to children recovering from anemic conditions and its complications such as PEM. The sugars may contribute to the overall well-being reported by adults consuming the extracts. D-Erythro-2-deoxy-pentose sugar is a Deoxy-ribose sugar. The term "2-deoxyribose" may refer to either of two enantiomers: the biologically important D-2-deoxyribose and to the rarely encountered mirror image L-2-deoxyribose[18]. D-2-deoxyribose is a precursor to the nucleic acid DNA. 2-deoxyribose is an aldopentose, which is a monosaccharide with five carbon atoms and having an aldehyde functional group [18].

CONCLUSION

The study revealed the volatile organic constituents in the leaves of *Ficus vogelii*. It was concluded that the high content of oleic acid and phytol in the plant may be responsible for the cardiovascular benefits the plant confers on the populations consuming it.

ACKNOWLEDGEMENT

We thank FEDOROSA CATOLINI for the financial support given to the first author to carry out this study.

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Online publication Date: 03 .08.2018