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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OILS FROM WHITE AND GREEN LEAVES OF NIGERIA-GROWN *Ficus microcarpa*

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ABSTRACT

The chemical composition and antioxidant potentials of the essential oils obtained from white and green leaves of *Ficus microcarpa* were investigated. The essential oils were derived using hydro-distillation method and Gas Chromatography - Mass Spectrometry analysis was used to detect the different constituents in the essential oils. The 1,1-diphenyl-2-picryl radical antioxidant assay was performed to investigate the free radical scavenging capacity of the essential oils. Twenty phytochemicals were common to both essential oils while some notable differences were observed between them. Phytol (40.90%) and copaene (15.85%) were the most abundant compounds in the white leaves while megastigmatrienone (14.98%), 1,3-cyclohexadiene (9.20%) and 1-hexanol (8.80%) were the most abundant in the green leaves. The essential oil from the white leaves with a higher concentration of phytol exhibited the greater antioxidant potential. The plant may, therefore, be employed as a viable phytochemical bio-resource in medical applications.

Keywords: *Ficus microcarpa*, phytol, cubene, copaene, megastigmatrienone, antioxidant

INTRODUCTION

Ficus microcarpa Linn is a common ornamental tree grown in many humid regions of the world and also a notorious invader in Hawaii, Florida, Bermuda, and Central / South America[1]. It is one of the largest genera in the family Moraceae. The species name "*microcarpa"* stems from its small-sized fruit. It is native throughout the tropics with a few species extending towards the semi-warm temperate regions[2]. The classification of members of this genus is based on their differences in habit and their florescence morphologies[3, 4]. Some of the species of the plant produce green and yellow leaves while others produce reddish-green, purplish, blackish and whitish leaves at maturation. The conspicuous difference in the color of the leaves produced by the same plant is usually an attraction which makes it useful as an ornamental plant[4].

Several efforts have been targeted at unveiling the bioactive chemical constitution of various parts of the plant. Eight compounds (triterpenoids and fatty acids) were identified and isolated from the aerial roots of *ficus microcarpa*[5]. Steroids, triterpenoids, phenols, tannins and flavonoids have been identified as principal phytoconstituent of the leaf [6]. A comparative investigation of the chemical composition of essential oils from different parts of the plant was examined[7]. Compounds which include glycosides, coumarins, triterpenes and fatty acids have been isolated and characterized from the aerial root of the plant [8, 9, 5]. The extracts of the leaves are reported to possess hypoglycaemic, antifungal and cytotoxic effects[1,10,11], while the methanolic extracts of the bark, fruits, and leaves were known to possess high antibacterial properties towards gram-positive and gram-negative bacteria[12,13].

However, to the best of our knowledge, the comparison of the chemical constituents of the green and the white leaves of *Ficus microcarpa* has not been reported. Hence this work was designed to determine the

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chemical composition and antioxidant potential of the essential oils from the green and white leaves of Nigeria-grown *Ficus microcarpa*.

EXPERIMENTAL

Plant Materials

The White and Green leaves of *Ficus microcarpa* were obtained from Redeemer's University, Mowe, Ogun State, Nigeria in October 2013. A botanist at the herbarium domiciled in Botany Department, University of Lagos taxonomically identified the plant and a voucher specimen number (LUH 6064) was assigned. The white and green leaves were obtained fresh, sorted for uniformity from other parts and kept for use.

Reagent and Solvents

All reagents were of analytical grade and some solvents were re-distilled before use.

Extraction of Essential Oil from *Ficus microcarpa* Leaves

Wet plant materials (300g) of both leaves were subjected to hydro-distillation differently for five hours each, using the Clevenger's distillation apparatus. The volatile oils were stored in air-tight glass vials at 4^{o}C after their collection over anhydrous sodium sulphate in preparation for analysis.

GC/GC-MS Analysis

The GC-MS (Gas Chromatograph - Mass Spectrometry) analysis was carried out using Gas Chromatograph (Agilent technology 7890A). The fused silica column, the injection and the operating condition of the GC are as previously described by Adeosun *et al*.[14, 15].

Comparing the MS spectra data with NIST (2008) library, the relative percentages of the constituents were directly calculated using the GC-MS peak areas without any correction.

DPPH Assay

The antioxidant potential of the essential oils was determined by using DPPH (1,1-diphenyl1-2-picrylhydrazyl) assay following standard procedure[14, 16]. DPPH solution (0.1mM) was prepared in methanol and incubated at room temperature overnight in the dark before use. Different concentrations of the essential oils (1, 0.5, 0.2, 0.1, 0.05 mg/mL) were also prepared in methanol. 1 mL of methanol and 2 mL DPPH solution were added to each sample and each mixture was incubated in the dark for 30 minutes. The absorbance was afterward measured at 517 nm. Ascorbic acid (AA) was used as the standard antioxidant while a blank solution was prepared using only DPPH in methanol. The percentage antioxidant capacity (%AC) was calculated using the following formula:

$$\%\,AC = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} X\ 100$$

RESULTS AND DISCUSSION

Chemical Constitution of the Essential Oils

The hydro-distillation of the wet leaf samples yielded the essential oils from white leaves (FW) and green leaves (FG) respectively. The constitution of these essential oils was then examined using GC-MS. The chemical composition of FG and FW are shown in Table-1. Both FW and FG indicated the presence of twenty (20) phytochemicals each with marked differences between the compositions of the two samples. They both have twelve (12) common compounds which includes1,3-cyclohexadiene, copaene, tridecane, *alpha*-cubene, 1H-cyclopenta[1,3]cyclopropa[1, 2]benzene, *beta*-caryophyllene, 1H-Cycloprop[e]azulene,3-hexen-1-ol, 2-pentadecanone, phytol, 1,6-cyclodecadiene and megastigmatrienone at varying proportions while eight (8) compounds present in FW were not detectable in FG and vice versa. Phytol (40.90%) andcopaene (15.85%) were the most abundance compounds in FW while megastigmatrienone (14.98%), 1,3-cyclohexadiene (9.20%) and 1-hexanol (8.80%) were the most abundance in FG. Other compounds were detected in a relatively low amount.

However, cyclic hydrocarbons (Figures 1, 2) were the most prominent class of compounds in the samples as it accounted for approximately 47% and 63% of the essential oil in FW and FG respectively. Aliphatic

hydrocarbons and terpenes were detected in significant amount compared to aromatic hydrocarbons, cyclic oxygenated compounds and fatty acids/esters obtained in relatively low yield in both essential oils.

Table-1: The GC-MS Analysis of Essential Oil From White and Green Leaves of *Ficus microcarpa*.

Peak	Compounds	RT	% Area in FW	% Area in FG
1	1-Hexanol	07.40	-	8.80
2	Undecane	09.80	-	6.83
3	Methyl salicylate	13.30	3.32	-
4	1,3-Cyclohexadiene	13.50	2.35	9.20
5	Tridecane	17.30	1.37	5.39
6	*Alpha*-Cubene	19.30	6.95	7.89
7	Copaene	20.30	15.85	1.71
8	Cyclobuta[1,2,3,4]dicyclopentene	20.60	0.98	-
9	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	20.80	0.98	2.89
10	*Beta*-Caryophyllene	21.90	2.64	5.52
11	*Gamma*-Elemene	22.40	-	2.89
12	1,5-Cyclodecadiene	22.40	1.86	-
13	1,4,7-Cycloundecatriene	23.20	-	2.23
14	*Alpha*-Caryophyllene	23.20	0.98	-
15	1*H*-Cycloprop[e]azulene	23.40	1.86	2.62
16	3-Buten-2-one	24.30	-	1.58
17	3-Hexen-1-ol	27.30	1.76	4.86
18	Isopropyl Myristate	35.60	-	0.52
19	Bicyclo[3,1,1]heptanes	36.00	3.42	-
20	2-Pentadecanone	36.20	2.05	0.53
21	*n*-Heptadecan-1-ol	37.20	3.62	-
22	Cyclotetradecane	37.20	-	3.41
23	Palmitic acid	40.50	0.78	-
24	1-Heneicosanol	43.00	-	3.68
25	3-Eicosene	43.00	2.45	-
26	Phytol	43.90	40.90	4.34
27	1,6-Cyclodecadiene	46.50	2.45	10.12
28	Megastigmatrienone	54.60	3.42	14.98
	TOTAL		99.99	99.99

RT – Retention time

DDPH Antioxidant Assay

The DPPH free radical scavenging activities of essential oils FW and FG are depicted in Fig.-3. This method is widely embraced because it is fast and the procedure is uncomplicated[17]. The activities were measured over a range of concentrations (0.05 to 1 mg/mL). The result obtained indicated that the essential oil from the white leaves (FW) of *Ficus microcarpa* showed higher antioxidant activity than the essential oil obtained from the green leaves (FG). While the standard, ascorbic acid (AA) displayed the highest activity range 15 – 72%, FG had 20 – 39% and FW had narrow activity range of 45 to 55% over the tested concentrations.

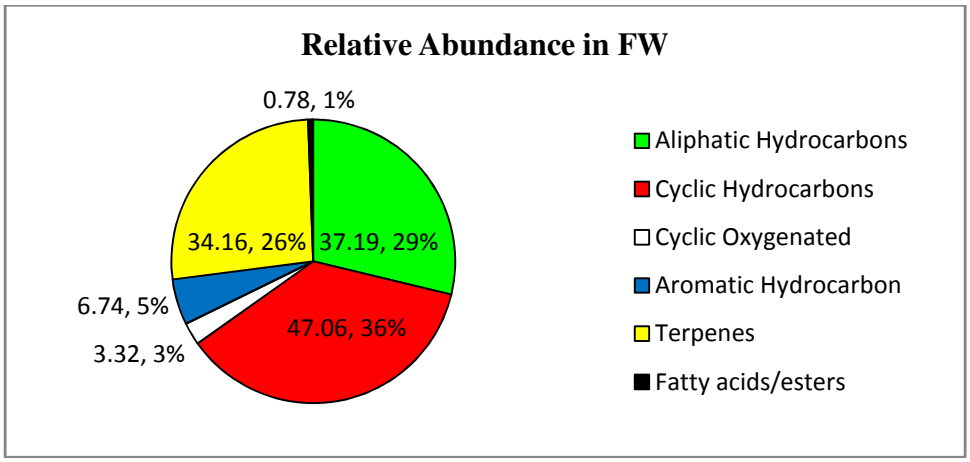

Fig.-1: Distribution of Phytochemicals present in the White Leaf

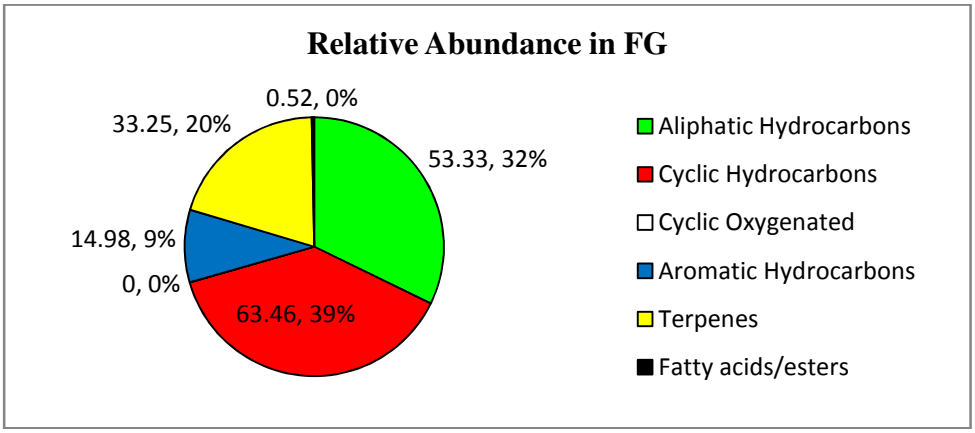

Fig.-2: Distribution of Phytochemicals present in the Green Leaf

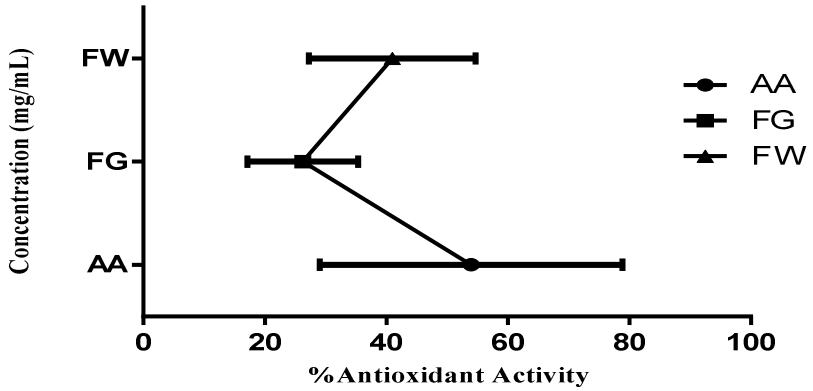

Fig.-3: Antioxidant Capacity of FG, FG and AA

A total of twenty-eight compounds were identified in the total ion chromatogram of both essential oils. Some of these compounds were present in both leaves while others were only present in either the white or the green leaf (Table-1). Phytol was detected as the most abundance compound in the FW and obtained in an insignificant quantity in FG. The reason for this seems elusive at the moment. The phytol has also been detected in the essential oil from the aerial part of *Eupatorium cannabinum* sub sp. Corsicum[18], leaves of *Kigeliapinnata*[19] and also in *Stachysmilanii petrovic* of the family Lamiaceae[20]. Phytol, which

was a major constituent in both FW and FG is a precursor of chlorophyll in the green plant. Phytol is a precursor of vitamin E which helps in retarding cell aging and vitamin K which has found application in the treatment of intestinal illness. The high concentration of phytol in FW may be responsible for the antioxidant activity of the essential oils. Phytol is reported to possess strong antioxidant activities[21]. The presence of the phytol in FW in significant quantity may be responsible for the higher antioxidant activity recorded in the sample. Megastigmatrienone which was present in both samples has also been detected in the volatile oils of Styrax (*Styraxofficinalis* L.) leaves obtained at different phenological stages [22] and also in the flower and stem of the plant obtained from south-eastern France[23]. However, beta-caryophyllene obtained in both samples at low concentrations was a constituent of *Origanum glandulosum* Desf.[24] and *Conyzasumatrensis*[25] essential oils. Although twelve compounds were common to the two essential oils from both leaves, they were however observed in varying quantities. α-cubene, copaene, *β*-caryophyllene and *γ*-elemene are important sesquiterpenes that were detected in both essential oils. They have been reported present in various plants and known to contribute to biological activities such as antimicrobial activities.[26-29]

CONCLUSION

The essential oils obtained via hydro-distillation from the green and white leaves of *Ficus microcarpa* have been analyzed using GC/GC-MS. Although twelve compounds were common to both, a notable variation in the chemical constitution of the leaves was observed. The presence of the phytol in FW in significant quantity suggests that the increased activity may be due to the phytol. The observed antioxidant potential implies that the essential oils from the leaves of the plants may be harnessed for application in food and pharmaceuticals as appropriate[30,31,32]. However, *in vivo* studies are needed to assess the true antioxidant activities of these essential oils and also determine the metabolic pathways involved in their degradation.

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