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Chemical composition, acetyl cholinesterase inhibitory properties and brine shrimp toxicity of essential oils from *Ficus capensis* Thunb from Nigeria

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ABSTRACT

Essential oils were isolated from leaves, stem barks and root barks of *Ficus capensis* (Moraceae) by hydrodistillation and the chemical constituents identified by means of GC and GC-MS. A total of thirty-seven compounds were characterized in the essential oils. The major constituents were α-pinene, β-pinene, α-cadinol and n-hexadecanoic acid. Toxicity to brine shrimp ranged between 16.38 µg/mL and 43.11 µg/mL while the three oils exhibited acetyl cholinesterase inhibitory activity in the range 11.22 µg/mL and 14.65 µg/mL.

Keywords: *Ficus capensis*, Moraceae, *n*-hexadecanoic acid, α-cadinol, α-pinene.

INTRODUCTION

The genus *Ficus* is a large one with about 1,000 species (Berg, 1989). *Ficus capensis* Thunb (Moraceae) is also known as *Ficus sur* Forssk. It is a tree of variable height (4-9 m). It has spherical crown, often low-branched and widespread in tropical Africa and South Africa of variable species. The tree is believed to have fetish attributes in the promotion of stock and crop increases as it confers fertility (Irvine, 1961).

The volatile compounds of the fig of *F. capensis* growing in France were studied by Grison-Pige et al. (2002). The essential oil was found to contain twelve compounds. The predominant compounds were (*E*)-β-ocimene (40.8%), germacrene D (15.4%) and (*E*, *E*)-α-farnesene (11.4%).

The extract of *F. capensis* exhibited antimicrobial activity against *Vibrio cholerae*, a microbe that causes cholera (Akinside and Olukoya, 1995). In another related study, the aqueous extract of *F. capensis* and other plants were tested *in-vitro* against chloroquine (CQ)-sensitive and resistant strains of *Plasmodium falciparum*. The extract of *F. capensis* proved to be active (IC_{50} < 100 µg/mL) (Muregi, 2003).

Other species of *Ficus* have also been the subject of literature discussion. The analysis of the volatile constituents of *F. exasperata* revealed the presence of thirteen compounds. The major compounds were 1,8-cineole (13.8%), *E*-phytol (13.7%), p-cymene (11.4%), β-ionone (7.5%), 6,10,14-trimethyl-2-pentadecanone (7.0%), cyclooctasulphur

(6.3%) and caryophyllene oxide (5.4%) (Sonibare et al., 2006). The root bark of *F. exasperata* comprises of α-terpineol (33.7%), α-pinene (10.8%) and sabinene (5.6%) as the prominent constituents (Oladosu et al., 2009).

The fig volatile compounds of twenty *Ficus* species were also analyzed and 99 different compounds were identified. These compounds were terpenoids, aliphatic compounds and products from the shikimic acid pathway (Grison-Pige et al., 2002). Proffit and Johnson (2009) compared the volatile organic compounds emitted by receptive syconia of *F. sur* Forssk and *F. sycomorus*.

Sharaf et al. (2002) reported clear differences in the flavonoid profiles of four *Ficus* species: *F. altissima* Blume, *F. nitida* Thunb, *F. carica* L. and *F. pseudosycamonus* Decne. *F. altissima* showed the presence of six methylated flavonoids while no methylated flavonoids were detected in the other three species. The presence of 12 different alkanes in the leaf waxes of some *Ficus* species was reported by Sonibare et al. (2005). Of the twelve alkanes that were identified, it was observed that hentricontane and tricontane occurred as major components in all the twenty four species investigated. Cytotoxic triterpenes have also been isolated from the aerial shoots of *F. microcarpa* (Chiang et al., 2005).

This study was aimed at characterizing the essential oil constituents of *Ficus capenis* and also to investigate the numerous biological activities through the different assays described below.

MATERIALS AND METHODS

Plant material

The plant was collected in Ibadan (Oyo State, Nigeria) around Sabo area in February 2004. The plant sample was identified and a voucher specimen deposited at the Herbarium of the Forestry Research Institute of Nigeria (FRIN), Oyo State (FHI 107415). Air dried samples were ground. Batches of 500 g plant materials were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. The resulting essential oil was kept refrigerated until it was analyzed.

Gas chromatographic analyses

Gas chromatographic analyses (GC) of the oils were performed on an Orion Analytical Micromat gas chromatography fitted with a thermal conductivity detector (TCD). The separation was achieved by capillary columns of different polarities, CPSil-5 (25 m x 0.25 mm i.d, 0.15 µm film thickness), equivalent to OV 101, and CPSil-19 (25 m x 0.25 mm i.d) similar to BP10.

The essential oils were diluted with *n*-hexane at a ratio of 1:5. The diluted oil (0.1 µL) was injected into the GC. The column temperature was programmed from 50 ^{o}C to 230 ^{o}C at 3 ^{o}C/min. The injector and detector temperatures were maintained at 200 ^{o}C and 250 ^{o}C respectively. The carrier gas was hydrogen at a pressure of 0.5 bars and a flow rate of 120 mL/min.

Gas chromatography-Mass spectrometer (GC-MS) analyses of the essential oil samples were carried out on a Hewlett-Packard Gas Chromatography (GC) HP5890A, interfaced with a VG Analytical 70-250s double focusing mass spectrometer, operating at 70 eV, with an ion source temperature of 230 ^{o}C. The GC was fitted with a 25 m x 0.25 mm i.d fused silica capillary column coated with CPSil-5. Helium was used as the carrier gas at 120 mL/min. The GC operating parameters were identical with those of the GC analyses. The mass spectral data were acquired and processed by an on-line desktop computer.

The retention time of the different component from the GC analysis of the essential oils were converted to Kovat indices (KI) using Kovat formula. The mass spectra of each compound from the GC-MS analysis were compared with authentic compounds (Davies, 1960; Adams, 2001).

Toxicological assay

Brine shrimp lethality test

Sea water was collected from the ocean in Lagos, South West, Nigeria. The shrimps

(*Artemia salina*) were purchased from Felimar Aquaculture Centre, Ijebu-ode, Ogun State (produced by Coppens International bv, Helmond, Holland). Sea water (200 mL) was added to the hatching chamber and shrimp eggs added. The hatching chamber was a plastic bowl, partitioned in to two compartments. The partition was perforated such that the nauplii could swim through to the other side after hatching. The eggs were allowed to hatch for 48 h and mature as nauplii at room temperature. The nauplii were then harvested with a pipette after attracting the organism to one side of the vessel with a light source.

The essential oils were prepared in sea water into vials at 1000, 100, and 10 µg/mL (each test in triplicate). The essential oils had been previously dissolved in 2 mL of Dimethylsulfoxide (DMSO) since they are not soluble in water and 0.5 mL each of the dose level was introduced in a test-tube to which 4 mL of sea water added. 10 shrimps per test tube were added to each concentration and made up to 5 ml seawater to make 1000-10 µg/mL of final concentration of extract. After 24 h, the number of deaths over the number of total shrimps (survivors) was counted and recorded (McLaughlin et al., 1973).

The data were analyzed statistically using Finner Computer Programme to determine LC_{50} values. The control experiment was in place without the test solutions (Finney, 1971).

Acetyl cholinesterase enzyme assay

Fresh sheep liver was obtained from a healthy sheep just after being slaughtered. A 1% homogenate w/v of the sheep liver was prepared in double distilled water at 0 ^{o}C.

The acetyl cholinesterase (AChE) inhibitory properties of the test compounds were done by colorimetric method but with the following modifications (Baum, 1971). Three different concentrations (5, 10, 20 µg/0.1 mL of propanone) of the essential oils were prepared separately in test tubes and the solvent propanone was allowed to evaporate. 0.1 mL of the 1% sheep liver homogenate was pre-incubated with 5, 10, 20 µg of the essential oils, separately for 15 minutes at 37 ^{o}C in a thermostatic water bath. A positive control Huperzine A was adopted. The Huperzine A technical grade (obtained from Sigma Co. U.S.A) was also pre-incubated in the same manner as described above with 0.1 mL of 1% (w/v) homogenate at 5, 10, 20 µg amounts to allow inhibition of liver cholinesterase by test compounds.

Immediately after pre-incubation, 0.2 mL of 0.2% fast blue B (a diazotized product of 4-benzoylamino-2,5-dimethoxy aniline-$ZnCl_2$) in water was added followed by 0.1 mL of 0.01 M ethylacetoacetate substrate in acetone and the reaction mixture was again incubated for one minute for enzymatic reaction. The reaction mixture was made up to a total of 1.0 mL with distilled water prior to addition of substrate. The enzymatic incubation time was 1 minute after the addition of substrate. The enzyme activity was stopped at the end of exactly 1 minute by adding 4 mL of glacial acetic acid. The magenta colour developed was read at 540 nm with the aid of a UV spectrophotometer. The control enzyme reaction mixture was done the same way as the samples but without any essential oil. The experiment was conducted in triplicate at each dose level.

RESULTS

The yield of the essential oils were 0.10%, 0.11% and 0.13% for leaf, stem bark and root bark respectively. GC and GC-MS analyses afforded the identification of 27 compounds in the leaf essential oil, 17 in the stem bark and 14 in the root bark (Table 1). The leaf oil composition comprised of 32.3% monoterpenoid which are 21.0% monoterpene hydrocarbon and 11.3% oxygenated monoterpene. The sesquiterpenoid fraction (39.7%) was identified as 21.5% sesquiterpene hydrocarbons and 18.2% oxygenated sesquiterpenes. Other constituents were characterized as 3.1% diterpene, 6.9% fatty acids and 8.4% simple aliphatic.

A high percentage of 33.3% fatty, *n*-hexadecanoic acid was detected in the stem bark essential oil and other principal constituents which were α-pinene (12.2%), β-pinene (8.9%), limonene (4.4%) and α-humulene (4.7%).

The leaf, stem bark and root bark essential oils had LC_{50} values of 16.38 µg/mL, 43.11 µg/mL and 39.40 µg/mL respectively which indicate that the oils are toxic to brine shrimps (Table 2). The three essential oils (leaf, stem bark and root bark) exhibited promising activity of IC_{50} 14.65 µg/mL, 14.11 µg/mL and 11.22 µg/mL respectively against AChE (Table 3).

Table 1: Essential oil constituents of the leaves, stem barks and root barks of *Ficus capensis*.

S/N	Compound	RI*	Leaf (%)	Stem bark (%)	Root bark (%)
1.	α-thujene	933	-	0.2	0.7
2	α-pinene	941	**9.3**	**12.2**	**36.7**
3	camphene	954	-	1.6	0.5
4	β-pinene	981	**4.5**	**8.9**	**14.9**
5	α-phellandrene	1007	-	0.8	1.8
6	*p*-cymene	1021	-	1.5	-
7	*m*-cymene	1023	2.1	-	**5.8**
8	limonene	1030	3.1	4.4	7.9
9	γ-terpinene	1058	2.0	3.7	4.1
10	terpinolene	1088	-	1.9	0.1
11	safranal	1183	1.1	-	-
12	(*Z*)-ocimenone	1222	2.8	-	-
13	*n*-tridecane	1300	1.9	-	-
14	*n*-tetradecane	1397	**6.5**	-	-
15	cyperene	1403	-	1.0	-
16	isocaryophyllene	1406	3.4	0.5	-
17	α-ionone	1408	**6.4**	-	-
18	(*E*)-β-caryophyllene	1420	1.8	-	3.6
19	geranylacetone	1428	1.1	-	-
20	(*E*)-α-bergamotene	1433	-	1.3	-
21	α-humulene	1455	**6.5**	**4.7**	3.2
22	β-ionone	1464	1.0	-	-
23	germacrene	1477	2.4	-	-
24	γ-humulene	1485	0.7	-	-
25	δ-amorphene	1512	1.2	-	-
26	*E*-nerolidol	1544	3.1	-	-
27	3*E*,7*E*-4,8,12-trimethyltri deca-1,3,7,11-tetraene	1561	1.3	-	-
28	spathulenol	1566	3.7	-	-
29	caryophyllene oxide	1570	3.9	-	-
30	α-cadinol	1641	**10.6**	-	-
31	*n*-tetradecanoic acid	1736	-	2.1	-
32	neophytadiene, Isomer I	1821	0.9	-	1.2
33	pentadecanoic acid	1831	-	4.0	-
34	neophytadiene, Isomer II	1841	0.8	-	-
35	neophytadiene, Isomer III	1862	1.4	-	-
36	*n*-hexadecanoic acid	1931	-	**33.3**	**15.5**
37	geranyllinalool	1944	**6.9**	-	-
	TOTAL		**90.4%**	**82.1%**	**96.0%**

RI* = Retention Index (Adams, 2001)

Table 2: Brine shrimp toxicity assay of the essential oils.

Essential oil	LC_{50} (µg/mL)	Lower confidence limit	Upper confidence limit
F. capensis (leaf)	16.38	9.80	25.43
F. capensis (stem bark)	43.11	24.71	72.46
F. capensis (root bark)	39.40	18.17	72.89

Table 3: Acetyl cholinesterase inhibitory properties of the essential oils.

Essential oil	IC_{50} (µg/mL)
F. capensis (leaf)	14.65
F. capensis (stem bark)	14.11
F. capensis (root bark)	11.22
Huperzine A	7.23

DISCUSSION

The high percentage of *n*-hexadecanoic acid in the sample is significant. Hexadecanoic acid has been found in higher amounts (69.1%) in the essential oils of *Cestrum diurnum* (Bhaltacharjee, 2005). Ekundayo et al. (1991) also detected fatty acids from *Psidium guajava*, lauric and myristic acids (34%).

The root bark essential oil comprised of predominantly monoterpenes (72.5%). The major components were α-pinene (36.7%), β-pinene (14.9%), *m*-cymene (5.8%), limonene (7.9%) and *n*-hexadecanoic acid (15.5%). The minor constituents were α-thujene (0.7%) and camphene (0.5%). A diterpene, neophytadiene, Isomer I (1.2%) was also identified in the essential oil. The two sesquiterpenes compounds amounted to 6.8%, consisting of (E)-β-caryophyllene (3.6%) and α-humulene (3.2%). The result suggests that the root bark essential oil can be classified as the α-pinene chemotype. α-Pinene has shown biological activities as antioxidant, antiradical and antimicrobial (Gianni et al., 2005). However, five constituents of varying concentrations were common to the three essential oils. These compounds are α-pinene (9.3-36.7%), β-pinene (4.5-14.9%), limonene (3.1-7.9%), γ-terpinene (2.0-4.1%) and α-humulene (3.2-6.5%).

Thus, analyses of the leaf, stem bark and root bark volatile oils revealed both quantitative and qualitative variations in their composition. There are no literature reports on the essential oil constituents of the part studied.

Toxicity to brine shrimps has been correlated with cytotoxic, pesticidal, antibacterial and anti-tumor properties (McLaughlin et al., 1993).

The activities of the three essential oils compared favourably with the positive control, Huperzine A with IC_{50} of 7.23 µg/mL. Huperzine A was isolated from *Huperzia serrata* (Thunb. Ex Murray) (a traditional Chinese medicinal plant used for the treatment of fever and memory improvement (Patocka, 1998; Shu, 1998). Huperzine A also showed a strong reversible and highly selective inhibitory activity against the enzyme acetyl cholinesterase (Tang and Han, 1999). It has been reported (Huperzine A) to show strong inhibition over other commercially available inhibitor (Patocka, 1998; Xiao et al., 2002; Xu, 1999) thus, the choice as a standard in acetyl cholinesterase enzyme assays. The result of this study is an indication that *F. capensis* can be explored in treatment of cases related to memory improvement, for example Alzheimer's disease (AD).

Furthermore, the observed activities are significant when compared to essential oils from some plants. Dohi et al. (2009) reported the AChE inhibitory activity of some commercial essential oils, of which *Artemisia dracunculus* L showed the most potent inhibitory activity of 58.0 µg/mL. The volatile oil from the bark of *Peltophorum dasyrachis Kurz* ex Baker showed potent inhibitory activity with the IC_{50} value of 83.2 µg/mL (Fujiwarat et al., 2010).

However, the result of this study is a preliminary attempt and also the first at investigating the AChE inhibitory activity and toxicity of these oils. There is the need to deduce the relationship between the constituents and the observed activities.

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