American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AkiNik

AJEONP American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2020; 8(4): 01-05

Received: 03-08-2020
Accepted: 23-09-2020

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Chemical constituents and antifungal properties of the essential oils from the stem bark of *Mitragyna ciliata* Aubrév. & Pellegr. and leaves of *Cynometra vogelii* Hook. f. from Nigeria

Moses S Owolabi, Lanre Akintayo Ogundajo, Balogun Olaoye Solomon, Logunleko Olatunde, Ambika Poudel, Chelsea N Powers and William N Setzer

Abstract
In this study, documentation on the essential oils extracted from the stem bark of *Mitragyna ciliata* and leaf of *Cynometra vogelii* were chemically characterized and quantified using GC-MS. The major components in the stem bark of *M. ciliata* were found to be non-terpenoid derivatives: benzaldehyde (28.3%), hexanal (4.0%), along with monoterpenoids: furanoid (*cis*-linalool oxide (12.4%) and *trans*-linalool oxide (7.9%)), 2,5-dimethyl-*p*-cymene (4.4%), and the fatty acid: palmitic acid (12.5%), while the leaf oil of *C. vogelii* was dominant with sesquiterpene hydrocarbons: β-caryophyllene (28.6%), α- and β-selinene (11.3% and 9.4%), and the fatty acid ester: isopropyl palmitate (19.2%). The oil of *M. ciliata* displayed marginal antifungal activity against *Aspergillus niger* (MIC = 625 µg/mL) and *Cryptococcus neoformans* (MIC = 313 µg/mL) but no antifungal activity against *Candida albicans.* Leaf essential oil of *C. vogelii* displayed good antifungal activity against *C. neoformans* (MIC = 80 µg/mL) but no antifungal activity against *A. niger* or *C. albicans*. The synergistic combination of essential oil constituents and the recent trends in the use of these oils obtained from aromatic plants as antifungals well as their biocidal activities can facilitate their application to overcome the problem of multidrug-resistant micro-organisms in our society.

Keywords: *Mitragyna ciliata, Cynometra vogelii,* GC-MS, antifungal, monoterpenoids, sesquiterpenes

1. Introduction
The genus *Mitragyna* consists of 17 species in the *Rubiaceae* family found in the tropical and subtropical regions of Asia and Africa. *Mitragyna ciliata* Aubrév. & Pellegr. is a large tree, with a trunk diameter of 12 to 15 meters, cylindrical, and without thickening at the base but sometimes with low rounded buttresses [1, 2]. *M. ciliata* locally is called 'abura' in Yoruba land and is commonly used in traditional medicine for the treatment of fever, vomiting, general weakness, hypertension, dysentery, gonorrhea, amenorrhea, leprosy, colds, chest pain, food poisoning and sterility, to ease childbirth, and as an anthelmintic and diuretic [3-8]. Studies have shown that *M. ciliata* has many pharmacological properties including antimalarial activity [9-11], trypanocidal activity [12], anti-inflammatory activity [9], antioxidant activity, and cardioprotective activity [13, 14].
Cynometra (Fabaceae family) is a genus of tropical forest trees with 85 species distributed among tropical regions in Africa, in Asia and in the Americas [15]. *Cynometra vogelii* Hook. f. is an evergreen non-climbing tree with a wide, spreading crown up to 20 m tall with bole up to 100 cm in diameter, occurring from Senegal east to Nigeria. It produces a reddish brown, hard wood, used locally for tool handles and firewood. It is commonly called '*Patapara*' in Ivory Coast and 'Egi' in Yorubaland south west of Nigeria. Traditionally, *C. vogelii* is used in wound healing. It has also been reported that *C. vogelii* bark possesses laxative, narcotic, analgesic and antimicrobial activities [16-18]. In continuation of characterization of chemical constituents of medicinal plants in Nigeria, we present the chemical compositions and antifungal activities of essential oils from the stem bark of *Mitragyna ciliata* and the leaf of *Cynometra vogelii* growing in southwestern Nigeria. As far as we are aware, there have been no previous reports on these essential oils.

2. Materials and Methods

2.1 Plant Materials and Identification

Steam bark of *M. ciliata* and leaf of *C. vogelii* were collected separately from Ashipa Area, Badagry local government area in Lagos state, Nigeria (6° 24′ 54.07″ N, 2° 52′ 52.75″ E). The plants were taxonomically identified and authenticated by curators, with a voucher specimen LUH 8076 and 7793, respectively, at the Herbarium of the Department of Botany of the University of Lagos. The stem bark of *M. ciliata* and the leaf of *C. vogelii* were air-dried in the shade to a constant weight and then pulverized using a blender.

2.2 Hydrodistillation of Essential Oils

For each plant, the plant materials (450 g) were introduced into a 5-L flask and distilled water was added until it covered the sample. Hydrodistillation was carried out for 3 to 4h in an all-glass modified Clevenger apparatus according to the British Pharmacopoeia. The essential oil obtained was extracted with *n*-hexane and transferred to a pre-weighed amber sample bottle; dried with anhydrous sodium sulphate to eliminate traces of water. The oils were kept under refrigeration at (4 ^{o}C) until ready for analysis. The yields of the oils were 1.75% and 0.85%, on dry weight basis for *M. ciliata* and *C. vogelii*, respectively.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

Each of the essential oils was analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70eV), scan range = 40-400 atomic mass units, scan rate = 3.0 scans/s and GC-MS solution software. The GC column was a ZB-5 fused silica capillary column (30m length × 0.25mm inner diameter) with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25µm. The carrier gas was helium with a column head pressure of 552kPa and flow rate of 1.37mL/min. Injector temperature was 250 °C and the ion source temperature was 200 °C. The GC oven temperature program was programmed for 50 °C initial temperature, temperature increased at a rate of 2 °C/min to 260 °C. A 5% *w/v* solution of the sample in CH_2Cl_2 was prepared and 0.1µL was injected with a splitting mode (30:1). Identification of the constituents of the volatile oil was achieved based on their retention data (retention indices) determined with reference to C_{10}-C_{40} *n*-alkane homologous series, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [19] and stored on the MS library [NIST database (G1036A, revision D.01.00)/Chem Station data system (G1701CA, version C.00.01.08)]. The chemical compositions of the essential oils of *M. ciliata* and *C. vogelii* respectively are summarized in Table 1.

2.4 Antifungal Screening

Essential oils from *M. ciliata* and *C. vogelii* were assessed using micro-dilution method [20, 21] against *Cryptococcus neoformans* (ATCC 24607), *Candida albicans* (ATCC 18804) and *Aspergillus niger* (ATCC 16888). Briefly for *C. neoformans* and *C. albicans*, 100 µL of Roswell Park Memorial Institute (RPMI) medium buffered with 3-(N-morpholino) propanesulfonic acid (MOPS) pH 7.0 were placed inside each well of micro plate. Also, 100 µL of diluted essential oil (1% in DMSO) or the positive (100 µM amphotericin B in 100% DMSO) and negative (100% DMSO) controls were placed inside the first row of the plate after which each well was serially diluted (1:1) down the column except the negative control. Afterward, 100 µL of inoculum in MOPS buffered RPMI (4×10^3 cells/mL) were added to each well, to obtain a final concentration of 2×10^3 cells/mL. Plates were incubated at 37 °C for 48 or 72 hours for *C. albicans* or *C. neoformans*, respectively while and 25 ^{o}C for 24 hours the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Antifungal activity of oils from *M. ciliata* and *C. vogelii* against *Aspergillus niger* (ATCC 16888) was determined as described above using PDA inoculated with *A. niger* hyphae, the solution was the diluted to a McFarland turbidity of 1.0. Minimum inhibitory concentrations (MICs) were determined in triplicate with potatoes dextrose broth (PDB) in a 96-well microplate using serial dilution (1:1). The plates were incubated at room temperature for seven days. Amphotericin B was used as a positive control, while DMSO and RPMI media alone were used as negative controls. Inhibition was determined as the lowest concentration without turbidity by comparing the growth of the positive and negative controls with the samples.

3. Results and Discussion

Hydrodistillation of the stem bark *M. ciliata* gave a pale-yellow essential oil while leaves of *C. vogelii* produced a colorless essential oil, respectively. A total of 20 constituents, 97.8% of *M. ciliata* and 8 constituents, 96.1% of *C. vogelii* volatile oils were identified by GC-MS, respectively (Table 1). Major non-terpenoid constituents of *M. ciliata* were benzaldehyde (28.3%) and hexanal (4.0%). *Cis*-linalool oxide (furanoid) (12.4%), *trans*-linalool oxide (furanoid) (7.9%), 2,5-dimethoxy-*p*-cymene (thymohydroquinone dimethyl ether) (4.4%), linalool (3.4%) and geranyl acetone (3.6%) were the predominant monoterpenoid constituents present in stem bark *M. ciliata*. The fatty acids palmitic acid (12.5%) and linoleic acid (2.4%) were also revealed as part of the constituents of the essential oils. The gas chromatogram of *M. ciliata* with labeled peaks is shown in Figure 1. Studies have shown that the methanolic extract of the stem bark of *M. ciliata* possesses anti-inflammatory, analgesic, antitumor and wide pharmacological activities [13, 22]. The leaf essential oil of *C. vogelii* was dominated by sesquiterpene hydrocarbons, namely β-caryophyllene (28.6%), α-selinene (11.3%), β-selinene (9.4%), caryophyllene oxide (6.7%), and α-humulene (5.9%), along with the non-terpenoid isopropyl palmitate (19.2%). The gas chromatogram of *C. vogelii* with labeled peaks is shown in Figure 2. Studies have shown that phytochemical investigation of the volatile oils from the root of *C. megalophylla* revealed the presence of: *p*-cymene, γ-terpinene, β-phellandrene and terpinen-4-ol as the major monoterpenoid constituents [23]. However, the non-volatile fractions of other species including *C. hankei*, *C. mannei* and *C. lujae* have been investigated for their alkaloid contents [24-26]. As mentioned earlier, there is paucity of information on the volatile constituents of these aromatic plants. The present study may be the first kind of reporting the essential oil constituents of these species. *M. ciliata* and *C. vogelii* essential oils were screened for antifungal activity (Table 2). The broth microdilution assay revealed *C. vogelii* to have a weak antifungal activity against *Aspergillus niger* and *Candida albicans* (MIC = 1250µg/ml) respectively but stronger activity against *Cryptococcus neoformans* (MIC = 80µg/ml). Oil of *M. ciliata* was less effective against *A. niger* (MIC = 625 µg/ml) and displayed a marginal activity towards *C. neoformans* (MIC = 313µg/ml). But a weak antifungal activity against *C. albicans* (MIC = 1250 µg/ml). The

antifungal activity of *C. vogelii* can be attributed to the high concentrations of β-caryophyllene, α- and β-selinene, α-humulene and caryophyllene oxide in the oil. Sesquiterpene hydrocarbons such as β-caryophyllene, α-humulene, and α-cadinene have shown moderate antifungal activity against several microorganisms [27–29]. The low concentrations of monoterpenoid derivatives in *M. ciliata*: *trans*- and *cis*-linalool oxide and 2,5-dimethoxy-*p*-cymene are likely responsible for the observed marginal and weak antifungal activities. The biological activity of essential oils depends on the several chemotypes and their concentrations [30-35]. In conclusion, this investigation has presented the composition of essential oils from stem bark of *Mitragyna ciliata* and the leaves of *Cynometra vogelii* for the first time, and has shown that the stem bark *M. ciliata* oil to have marginal antifungal properties against *A. niger* and *C. neoformans*, while *C. vogelii* leaf oil has shown promising antifungal activity against *C. neoformans.* This activity is in agreement with the uses of these plants in traditional medicine. Several essential oils have shown notable antifungal activities against opportunistic fungal pathogens. These readily available species may add to treatment options for pharmacological usage.

Table 1: Chemical composition of the stem bark of *Mitragyna ciliata* and leaf of *Cynometra vogelii* essential oils

Compounds	**RI_{cal}[a]**	**Percentage compositions (%)**	
		M. ciliata	***C. vogelii***
Butyl acetate	781	1.7	-
Octane	800	0.4	-
Hexanal	801	4.0	-
(2*E*)-Hexenal	851	0.8	-
1-Hexanol	863	3.2	-
α-Thujene	931	0.6	-
Benzaldehyde	962	28.3	-
6-Methyl-5-hepten-2-one	983	1.7	-
Myrcene	987	-	9.2
Octanal	1004	0.4	-
Benzyl alcohol	1034	0.8	-
cis-Linalool oxide (furanoid)	1068	12.4	-
trans-Linalool oxide (furanoid)	1085	7.9	-
Linalool	1098	3.4	-
Nonanal	1104	4.6	-
Methyl salicylate	1192	-	9.8
Myrtenal	1195	1.5	-
2,5-Dimethoxy-*p*-cymene (= Thymohydroquinone dimethyl ether)	1410	4.4	-
β-Caryophyllene	1417	-	28.6
Geranyl acetone	1445	3.6	-
α-Humulene	1454	-	5.9
β-Selinene	1487	-	9.4
α-Selinene	1493	-	11.3
Caryophyllene oxide	1580	-	6.7
Methyl palmitate	1922	3.2	-
Palmitic acid	1955	12.5	-
Isopropyl palmitate	2019	-	19.2
Linoleic acid	2135	2.4	-
Total Identified		97.8	96.1

[a]Retention indices calculated on ZB-5 capillary column.

Table 2: Antifungal activities of *Mitragyna ciliata* bark essential oil and *Cynometra vogelii* leaf essential oil

Samples	**MIC, µg/mL**		
	A. niger	***C. albicans***	***C. neoformans***
C. vogelii	1250	1250	80
M. ciliata	625	1250	313
Amphotericin B	1.22	0.61	0.61

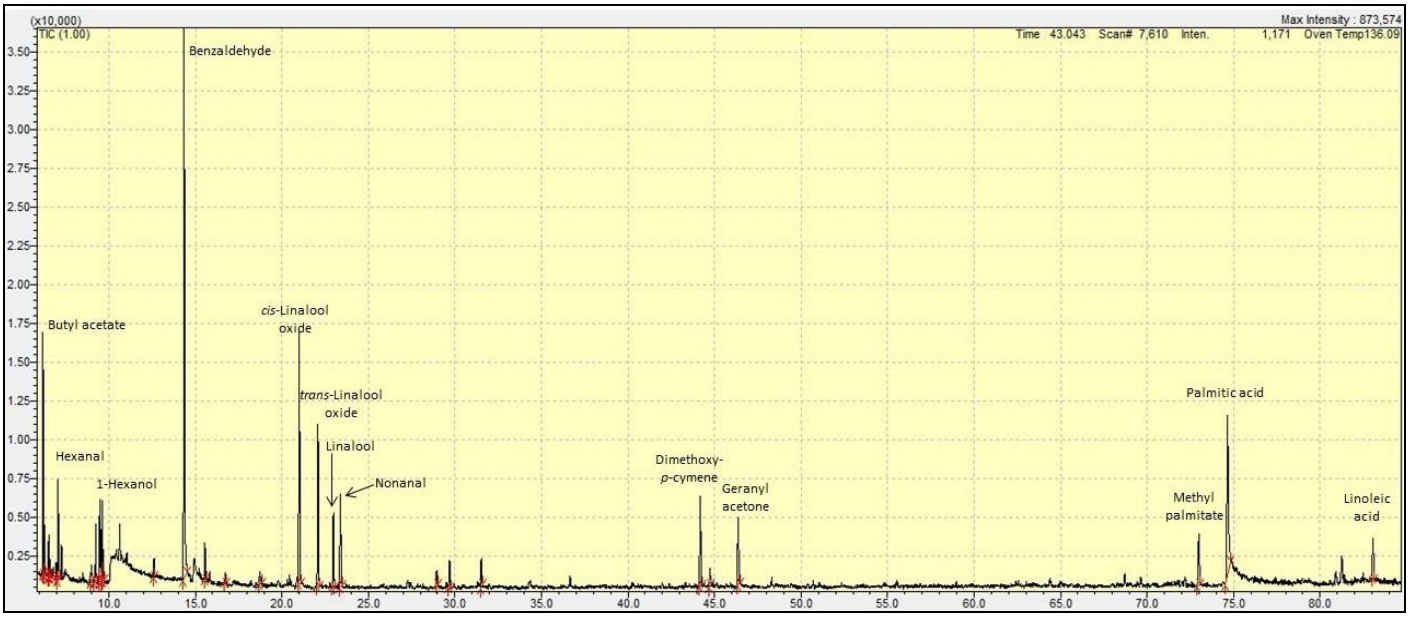

Fig 1: Gas chromatogram of *Mitragyna ciliata* stem bark essential oil

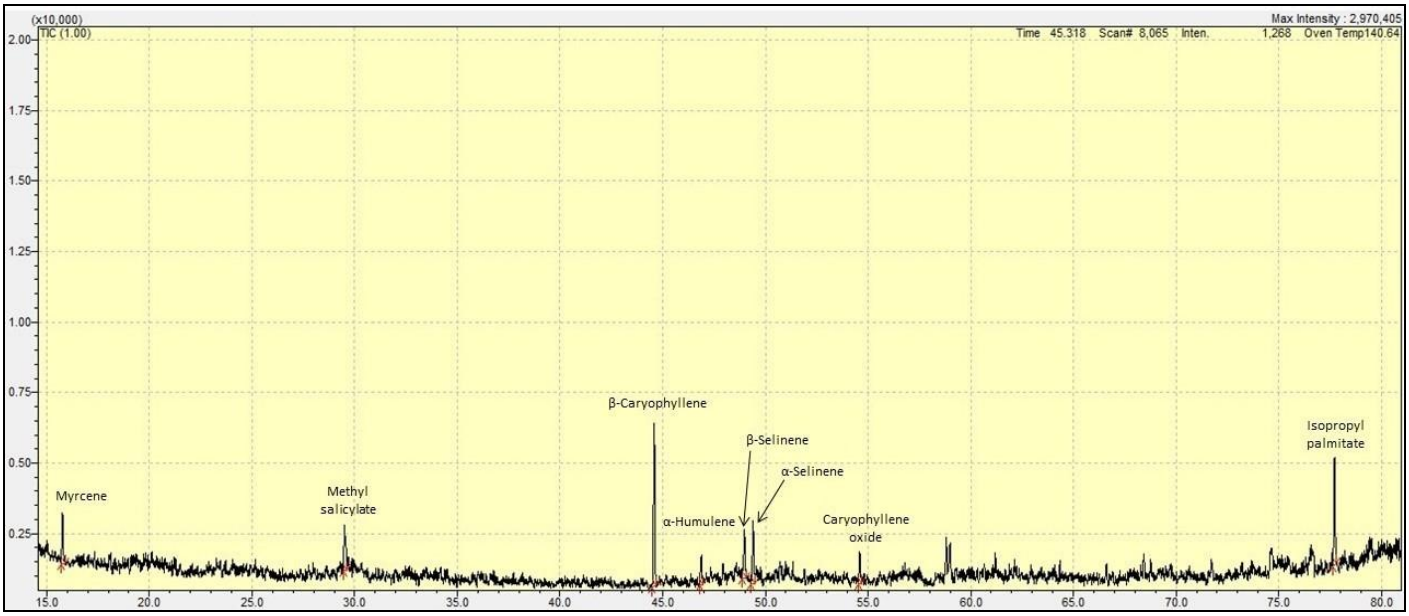

Fig 2: Gas chromatogram of *Cynometra vogelii* leaf essential oil

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