



# International Journal of Advanced Chemistry Research

ISSN Print: 2664-6781  
 ISSN Online: 2664-679X  
 IJACR 2023; 5(1): 110-114  
[www.chemistryjournals.net](http://www.chemistryjournals.net)  
 Received: 02-01-2023  
 Accepted: 10-02-2023

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## Comparison of the chemical profile of *Zanthoxylum zanthoxyloides* leaf and stem bark essential oils and their possible therapeutic potentials

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DOI: <https://doi.org/10.33545/26646781.2023.v5.i1b.150>

### Abstract

*Zanthoxylum zanthoxyloides* has many health benefits in Nigeria traditional medicine especially in the treatment of sickle cell anemia, microbial infections and cancer. The medicinal properties of plants are related to their phytochemicals and among them is essential oil which shows multiple biological activities due to its complex nature. Therefore, the aim of this study is to investigate the chemical profiles of the essential oil of *Zanthoxylum zanthoxyloides* leaves and stem bark for their chemical constituents with a view to providing basis for future pharmacological research on the essential oils of the two plant parts. The oils were extracted by hydrodistillation using Clevenger and analyzed using Gas Chromatography coupled with Mass Spectrometry. The leaf and the stem bark consist 11 and 46 constituents equivalent to 88.4% and 99.5% of the total oil, respectively. Caryophyllene oxide (27.0%),  $\alpha$ -pinene (16.0%) and humulene epoxide II (8.0%) were the dominant constituents in the leaf oil while  $\alpha$ -pinene (38.5%) and pellitorine (13.9%) in the stem bark. Both oils showed some similarities in their composition, although the stem bark oil contains more constituents. Pellitorine, caryophyllene oxide, and  $\alpha$ -pinene, which are detected in appreciable quantity in the leaves and stem bark essential oils possess diverse biological activities, which include anticancer, antioxidant, and antimicrobial. The chemical profile of the essential oils from the leaves and stem bark of *Zanthoxylum zanthoxyloides* suggests that the oils could provide a cure to many ailments.

**Keywords:** *Zanthoxylum zanthoxyloides*, essential oils, medicinal properties, Caryophyllene oxide,  $\alpha$ -pinene, pellitorine

### Introduction

Essential oils are complex mixture of plants' volatile and semi volatile compounds, which determine specific flavor and fragrance of the plants. They also possess multi-medicinal properties including anti-inflammatory, antiseptic, insecticidal, spasmolytic, analgesic, sedative, anesthetic, anthelmintic, antiviral, antibacterial, antifungal, and anticancer <sup>[1]</sup>. These properties make them to have various applications in the food, cosmetics, and pharmaceutical industries. A number of reports have connected the bioactivities of essential oils to the effect of synergy among their chemical constituents, especially the most abundant <sup>[2]</sup>. Moreover, essential oils have shown very good activities when compared with the synthetic drugs in many *in vitro* bioassays reported in the literature <sup>[3]</sup>. Therefore, knowledge of the chemical profile of essential oil could help to predict the likely therapeutic properties of the oil and thus guide in the bioassays it can be subjected to, in addition to the ethnobotanical use of the medicinal plant from which it was extracted. Since the interest in studying chemistry and biological activities of medicinal plants due to rise in the case of toxicity and drug resistance in the treatment of many life threatening diseases has increased, essential oils which have shown multi-medicinal activities among plants' phytochemicals will be of great benefit to pharmaceutical industries.

*Zanthoxylum zanthoxyloides* Lam. (syn *Fagara zanthoxyloides*) of Rutaceae family and genus *Zanthoxylum* is a small tree or shrub commonly found in dry forest vegetation and savannah areas. The local name is "IGI ATA" in the Southwest of Nigeria (Yoruba tribe). There are seven species of genus *Zanthoxylum* Linn. In Nigeria and all having close similarities and relationships.

The major characteristic of these species is the covering of leaf stalks, branches, branchlets, inflorescence axes and trunk with spines [4]. Ethno pharmacological uses of the plant include analgesic, anticancer, antihelmintic, antimalarial, antituberculosis, and treatment of oedema, whooping cough, gonorrhoea, urethritis, eye infection, dysentery, and paralysis. It helps to reduce pain of migraine, neuralgia, and labour. The root extracts are applied externally to haemorrhoids, swellings, snake bites, wound, leprosy, abscesses, arthritic and rheumatic pain [5]. Phytochemical screening revealed the presence of alkaloids, sterols, carbohydrate, lignans, coumarins, aliphatic and aromatic amides [4]. Several studies have shown that some of *Z. Zanthoxylum* (and all *Zanthoxylum* species) phytochemicals exhibited anticancer properties. Other pharmacological activities of the plant include antiplasmodial, molluscicidal, antihelmintics, anti-convulsant, anti-bacterial, anti-sickling, anti-inflammatory, insecticidal, fungicidal, antiparasitic, anaesthetic, anti-hypertensive and anticancer [4, 6-9]. Previous reports showed that some isolated compounds in *Z. zanthoxyloides* stem bark extract was genotoxic and cytotoxic and even caused death when administered to mice at high concentrations [8]. The leaves, seeds, and fruits essential oils of the plant and their bioactivities had also been reported [6, 10-12].

Despite the large number of reports on the plant, there is dearth of information on the chemistry and bioactivities of essential oils extracted from the plant's stem bark. Therefore, this study was designed to evaluate the chemical constituents of the leaves and stem essential oils of *Z. zanthoxyloides*, observe the variation in their compositions, and use their chemical profile to predict their possible pharmacological activities in order to provide basis for future pharmacological research on the essential oils from the two plant organs. The stem bark essential oil from Nigeria is being reported for the first time.

## Materials and Methods

### Collection of plant and authentication

*Zanthoxylum zanthoxyloides* leaves and stem bark were collected at Saunders road, University of Ibadan, Oyo State Nigeria (7° 23' 0.2" N/3° 54' 28.8" E) and authenticated at the Forestry Research Institute of Nigeria where a voucher specimen was deposited.

### Essential oil extraction

Essential oil was extracted from the leaves and stem bark of *Z. zanthoxyloides* using the procedure described previously [13]. The plant parts were air-dried and pulverized before subjecting a known weight to hydrodistillation for 3 h in an all-glass Clevenger-type apparatus. The extracted oils were

then dried over anhydrous sodium sulphate to remove moisture and weighed before they were kept in a sealed glass vials under refrigeration at 4 °C prior to analysis. The percentage yield was calculated using the formulae;

$$\% \text{ yield} = \frac{\text{Mass of essential oil}}{\text{Mass of plant}} \times 100$$

### Gas chromatography/mass spectrometry (GC-MS) analysis

The essential oils were analyzed by Shimadzu GC-MS-QP2010 operated in the electron impact (EI) mode of 70 eV, scan rate 3.0 scan/s, scan range 40-400 atomic mass units, and GC-MS solution software v. 4.20 (Shimadzu Scientific Instruments, Columbia, MD, USA). The GC column was a ZB-5 fused silica capillary column (Phenomenex, Torrance, CA, USA) with a (5% phenyl)-polymethylsiloxane stationary phase using helium as carrier gas with a column head pressure of 552 kPa and flow rate of 1.37 mL/min. The initial temperature of injector was 50 °C, which increased at a rate of 2 °C/min to 260 °C. A 5% w/v solution of the sample in CH<sub>2</sub>Cl<sub>2</sub> was prepared and 0.1 μL was injected with a splitting mode of 30:1. Identification of the essential oil constituents was based on comparison of their retention indices with respect to a series of *n*-alkanes, and comparison of their mass spectra fragmentation patterns with the ones in the literature [14] and those stored in the home data library [15].

## Results

### Extraction results

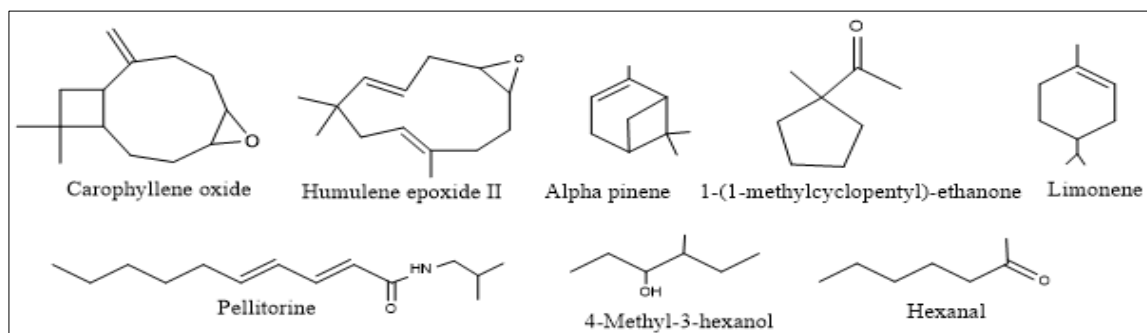
The colour and percentage yields of the leaves and stem bark essential oils investigated in this study are given in Table 1.

**Table 1:** Percentage yield and colour of *Z. zanthoxyloides* essential oils

Essential oil	percentage yield (w/w)	Colour
Leaf	0.36	Colourless
Stem bark	0.18	Colourless

### *Zanthoxylum zanthoxyloides* Essential Oils Chemical Constituents

The chemical constituents identified in the essential oils of the leaves and stem bark of *Zanthoxylum zanthoxyloides* as eluted from HP-5 MS column were reported in Table 2. The leaf essential oil comprised of 11 components equivalent to 88.4% of the total oil while 46 compounds corresponding to 99.5% of the whole oil were identified in the stem bark oil. The structure of the most abundant constituents is given in Figure 1.



**Fig 1:** Chemical structure of most abundant constituents in the *Z. zanthoxyloides* essential oils

**Table 2:** Chemical constituents of *Zanthoxylum zanthoxyloides*

Chemical constituents	RI	Percentage compositions (%)	
		Leaves	Stem bark
(Z)-1, 2-dimethylcyclopentane	716	-	0.8
Methylcyclohexane	718	-	5.2
Ethylcyclopentane	726	-	0.3
3-methyl-3-pentanol	744	-	0.2
2-methylheptane	754	-	0.2
Toluene	760	-	1.7
3-methylheptane	763	-	0.2
(Z)-1, 3-Dimethylcyclohexane	777	-	0.3
(E)-1, 3-Dimethylcyclohexane	780	-	0.2
Hexanal	799	-	9.7
(Z)-1, 4-Dimethylcyclohexane	807	-	0.2
Ethylbenzene	856	3.7	0.4
<i>o</i> -Xylene	865	4.3	0.3
1-Hexanol	862	-	0.2
<i>p</i> -Xylene	867	-	0.2
Heptanal	901	-	0.3
4-Methyl-3-hexanol	905	8.5	-
$\alpha$ -Pinene	930	16.0	38.5
Camphene	947	-	0.2
1-(1-Methyl-cyclopentyl)-ethanone	961	11.2	-
Sabinene	970	-	0.4
$\beta$ -pinene	975	-	1.3
6-Methyl-5-hepten-2-one	981	-	0.3
2-Pentylfuran	987	-	0.9
1-Octanal	1002	-	0.4
2-Ethylhexanol	1025	-	0.3
Limonene	1026	5.2	1.4
(2E)-Octenal	1055	-	0.2
Terpinolene	1083	-	0.2
Linalool	1097	-	0.2
Nonanal	1103	-	1.3
Naphthalene	1182	-	0.4
Decanal	1204	2.0	0.4
Bornyl acetate	1281	-	0.3
$\alpha$ -Terpinyl acetate	1343	-	0.8
$\beta$ -Caryophyllene	1416	1.6	2.4
$\alpha$ -Humulene	1452	0.9	2.5
Ethyl-(E)-cinnamate	1464	-	0.3
Germacrene D	1477	-	0.8
$\delta$ -Cadinene	1514	-	0.8
Germacrene-D-4-ol	1573	-	0.1
Caryophyllene oxide	1575	27.0	0.3
Humulene epoxide II	1602	8.0	-
Cedrol	1605	-	2.4
Pellitorine	1929	-	13.9
Palmitic acid	1954	-	6.2
1-Octadecanol	2079	-	0.3
Oleic acid	2129	-	1.0
Oxacyclonadec-6-en-2-one	2133	-	0.6
Monoterpene hydrocarbons		21.2	42.0
Oxygenated monoterpenes		-	1.3
Sesquiterpene hydrocarbons		2.5	6.8
Oxygenated sesquiterpenes		35.0	2.8
Non-terpenes		29.7	46.6
Number Identified		11	46
Percentage identified		88.4	99.5

RI - Retention Indices

### Discussions

The essential oils hydro-distilled from the leaves and stem bark of *Z. zanthoxyloides* were Colourless. However, the leaves gave higher yield than the stem bark. The disparity in the essential oil yields could be attributed to the amount of volatile oils stored in the specialized plant cell where they

are secreted such as the secretory hairs or trichomes, internal secretory cells, epidermal cells, and the secretory pockets [16]. The above result did not agree with the account given by Régis *et al*, for the fresh leaf oil of the same species harvested in 2017 at Bamena. The leaf yielded 0.04% light yellow essential oil [10]. Likewise, the fresh leaf from Côte

d'Ivoire yielded 0.03% yellow essential oil [17]. These variations also could be connected to the age of plant, nature of the plant sample (dry/wet), time of harvest, and geographical locations of the plant [16].

Compounds of varying classes were detected in the leaf oil. The prominent constituents in the oil were two oxygenated sesquiterpenes, Caryophyllene oxide (27.0%) and humulene epoxide II (8.0%); two monoterpene hydrocarbons,  $\alpha$ -pinene (16.0%) and limonene (5.2%); a ketone, 1-(1-methyl-cyclopentyl)-ethanone (11.2%); and an alcohol, 4-methyl-3-hexanol (8.5%). Contrary to the previous reports from different geographical regions including Nigeria, major components reported were absent in the present work. Leaf oil from Benin City, which contained only monoterpene hydrocarbons (98.2%) was dominated by myrcene (30.0%) and (*E*)- $\beta$ -ocimene (31.9%), which were not detected in the present report as well as  $\alpha$ -pinene (26.5%) [18]. Côte d'Ivoire leaf oil was characterized largely of sesquiterpene and monoterpene hydrocarbons with the high percentage of germacrene D (10.2%) and myrcene (10.0%) [17]. The disparity in the chemical constituents of the *Z. zanthoxyloides* leaf volatile oils from different regions in Nigeria could be attributed to many factors like period of harvest, extraction procedure, geographical and genetic factors [19]. Most of the constituents identified in the leaf essential oil in this study are of economic importance. Caryophyllene oxide is known to possess anticancer and analgesic activities. Its ability to accumulate in the cells in addition to its anticancer potential enable it to find application in chemotherapy.  $\alpha$ -pinene has biological activities such as antioxidant, antimicrobial, anti-seizure, anti-inflammatory, anticancer, anticoagulant and also reduce the danger of cardiovascular disease besides its vital role in the fragrance and flavor industry [3]. Humulene epoxide II is a good scent compound, with an antimalarial potential [20]. Limonene has many health benefits which include antioxidant, anticancer, antidiabetic, antiviral, antinociceptive, gastro protective, and anti-inflammatory [21]. It is also used in the industries as flavor and fragrance ingredient. It had earlier been reported that the leaf essential oil from *Zanthoxylum* species are rich source of antioxidant volatile compounds [8].

The stem bark oil comprised of high percentage monoterpene hydrocarbons (42.0%), mainly  $\alpha$ -pinene (38.5%), a good scent compound with multiple bioactivity as explained before. Although, most of the minor components in the oil were good fragrant ingredients. They include 2-pentyl furan (0.9%), bornyl acetate (0.3%), ethyl-(*E*)-cinnamate (0.3%), dermacrene-D-4-ol (0.1%), limonene (1.4%) and linalool (0.2%). Other dominant constituents were pellitorine (13.9%), hexanal (9.7%), palmitic acid (6.2%), and methyl cyclohexane (5.2%). Hexanal is a perfume ingredient and when applied externally, it slows down aging process. It helps to facilitate preservation of perishable fruits under storage [22]. Pellitorine is an unsaturated secondary amide that exhibited insecticidal, antiplasmodial, antibacterial, anticancer, and anti-sickling activities [4, 23-25]. It is likely one of the components responsible for the peppery taste of the stem bark as reported by Bowden and Rose [26], it was isolated from the bark of the stem and root of *Z. zanthoxyloides* [24]. It had also been isolated from other *Zanthoxylum* species [4, 26]. On the contrary, the stem bark essential oil of Côte d'Ivoire origin was dominated by linalool (49.5%) and E-nerolidol

(11.3%) [17]. The formal was detected as minor constituent in this study while the later was absent.

Comparing the chemical constituents of the leaf and stem bark oils, they both showed some similarities in their composition, although the stem bark oil contains more constituents. All the compounds in the leaf oil were also found in the stem bark oil beside 4-methyl-3-hexanol, 1-(1-methyl-cyclopentyl)-ethanone and humulene epoxide II which were among the main constituents in the former.

## Conclusion

The leaves and stem bark essential oils of *Zanthoxylum zanthoxyloides* showed some similarities in their chemical profile but the stem bark oil contains more chemical constituents. Most of the major constituents in the oils are of economic importance. Pellitorine, caryophyllene oxide and  $\alpha$ -pinene, which are detected in appreciable quantity possess good anticancer, antioxidant, and antimicrobial agents among many others. Therefore, since there is continuous search for new drugs from natural products to overcome the challenges associated with synthetic drugs, essential oil of *Z. zanthoxyloides* is a promising natural products which could exhibit numerous bioactivity due to their chemical profiles if there is no antagonistic interaction among their chemical constituents. This study recommends that the oils should be subjected to bioassays using their chemical profile as a guide.

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