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Comparison of the Chemical Constituents, Antimicrobial and DPPH free Radical Scavenging Activities of the Fresh and Air-dried Leaves Essential Oils of *Hildegardia barteri* (Mast.) Kosterm

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ABSTRACT

Due to the ethnomedicinal use of *Hildegardia barteri* in treating stomach disorders and some skin issues, we set out to investigate and compare the biological activities of essential oils from the fresh and air-dried leaves of the plant. Essential oils were extracted by hydrodistillation using an all-glass Clevenger apparatus and analysed by GC/MS. Antioxidant activity was by the DPPH scavenging method while antimicrobial activity was by the agar diffusion method. Essential oils (EOs) from fresh 0.34% (w/w) and air-dried 0.28% (w/w) leaves of *Hildegardia barteri* were colourless with an herbal smell. Both oils had ten (99.99%) and seventeen compounds (100%) from the fresh and air-dried leaves respectively. The fresh leaves EO comprised mainly 58.21% unsaturated fatty acids (oleic acid (34.29%), cis, cis linoleic acid (22.10%), and derivatives (1.82%)), and 38.81% saturated fatty acids (palmitic acid (24.85%) and stearic acid (13.96%)). The air-dried leaves EO was mainly 59.15% unsaturated fatty acids (α-Linolenic acid (41.56%), oleic acid (13.14%), and derivatives (4.45%)), and 30.43% saturated fatty acids (palmitic acid (21.41%), stearic acid (6.97%), and derivatives (2.05%)). Antimicrobial activities were tested on *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pleisomonas shigelloides, Bacillus cereus, Proteus vulgaris, Candida albicans,* and *Candida tropicalis* at 6.25-50 mg/mL. The EO from the fresh leaves at 50 mg/mL for some of the organisms tested gave zones of inhibition from 7.3±1.33-11.3±2.73 mm and MIC (0.39-6.25 mg/mL), while the air-dried leaves gave from 8.0±2.00-15±4.73 mm with MIC (0.39- 6.25 mg/mL). Antioxidant activities at 6.25-200 µg/mL of both oils and ascorbic acid were investigated. At 200 µg/mL, the fresh and air-dried oils gave the same activity of 66.41% and 66.42%, comparable to ascorbic acid's 69.23%. The unsaturated and saturated fatty acids in both oils could contribute to the plant's antimicrobial and antioxidant activities and its usage in managing stomach issues and various skin problems.

1. INTRODUCTION

Essential oils extracted from medicinal and aromatic plants have numerous therapeutic properties and applications, which has motivated research into these substances (Swamy and Sinniah, 2015). Some essential oils have found applications in drugs, sanitary products, dentistry, pharmaceuticals,

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cosmetics, natural remedies, and food industries (Swamy *et al*., 2016; Mahmoudi, 2017). An increase in population globally will result in a greater prevalence of bacterial infections and diseases which will be a serious threat to human health particularly as antibiotic resistance occurs (Ahmad and Beg, 2001; Hall-Stoodley *et al*., 2004; Swamy *et al*., 2016). Therefore, there is a need to explore alternative methods and develop new molecules against human pathogenic bacteria as the high concentration of synthetic antibacterial drugs employed may sometimes become toxic with opposing side effects (Galvao *et al*., 2012). Thus, essential oils from plants have been proven to exhibit a good potential against both Gram-positive and Gram-negative bacteria (Edris 2007; Lang and Buchbauer 2012; Hassanshahian *et al*., 2014; Teixeira *et al*., 2013). Also, the effective antimicrobial properties of essential oils may help overcome the problems of high bacterial resistance to current drugs and the extreme costs of recent generations of antibiotics (Soliman, *et al*., 2017).

Different studies have shown that essential oils exhibit significant antioxidant activity due to the presence of secondary metabolites such as flavonoids, phenolic compounds, and terpenoids which can be used to combat oxidative stress that is implicated in some lifestyle-related diseases like cardiovascular diseases, diabetes, cancer, Parkinson and Alzheimer's diseases (McCord, 2000; Ruan *et al*., 2008; Miguel, 2010; Cavar *et al*., 2012; Andrade *et al*., 2013; Aleksic and Knezevic 2014).

Hildegardia barteri (Mast.) Kosterm, mostly grown for its lovely blossoms belongs to *Hildegardia* a genus of trees with thirteen species in the family Malvaceae (Hildegardia Notes, 2009). It is distributed across West Africa, East Africa, Madagascar, southern India, the Philippines, Indonesia, northern Australia, and Cuba. The Yoruba people in Nigeria call it 'okurugbedu', the Hausa call it 'Kariya', while in Ibo it is called 'Ufuku eso'. The fruit is eaten fresh or used in culinary practices. In contrast, different parts of the plant are used in traditional medicine for treating stomach issues and skin problems (Hildegardia. (2021, September 22), in *Wikipedia*. https://en.wikipedia.org/wiki/Hildegardia_(plant)). The extract of the entire plant of *Hildegardia populifolia* is used to treat dog bites and malaria (Saradha and Paulsamy, 2013). There are some scientific works on the seed oil of the nuts of *Hildegardia barteri* but data on the essential oil profile of the leaf is scarce, so we set out to extract the essential oils from both the fresh and air-dried leaf samples, compare their constituents, antimicrobial, and antioxidant activities to see how they correlate and thus provide more information on the essential oil data of the plant.

2. MATERIALS AND METHODS

2.1. Plant collection and preparation

The leaves of *Hildegardia barteri* were collected inside the campus of Olabisi Onabanjo University Ago-Iwoye. Authentication was done at the Forest herbarium of the Forestry Research Institute of Nigeria (FRIN) in Ibadan, Oyo State where a voucher specimen with FHI number 112841 was deposited. The fresh leaves were chopped into smaller bits and the essential oil was extracted immediately, while the air-dried sample was obtained by air-drying the fresh leaves under shade for three weeks before being pulverized, and the essential oil was subsequently extracted.

2.2. Extraction of the Essential oils

The prepared fresh and air-dried leaf samples (500 g) each of *Hildegardia barteri* were subjected to hydrodistillation in an all-glass Clevenger-type apparatus for 3 h following

the established procedure (British Pharmacopoeia, 1980). The oils obtained were dried over anhydrous sodium sulphate (Na_2SO_4), stored in separate vials, and kept inside a refrigerator until ready for analysis.

2.3. Analysis of the Essential oils

Gas Chromatography/Mass Spectrometry (GC/MS)

The GC/MS analyses were performed on an Agilent model 5975C GC-MSD system with split/splitless automated injection interfaced to a 5973-mass selective detector operated at 70 eV with a mass range of m/z 50 -500. The oven temperature was programmed from 75-250℃ at the rate of 4℃/min. Helium was used as the carrier gas at a 1 mL/min flow rate, and the volume of oil injected was 1.0 µL. The separated compounds' relative percentage quantities were estimated using FID chromatograms.

Identification of the Constituents of the Leaf Essential Oils

The constituents of the oils were identified using retention indices (RI) measured by co-injection with a homologous sequence of n-alkanes under identical experimental conditions.

Additional identification was carried out by comparing their mass spectra with those from the National Institute of Standards and Technology (NIST) (Database 69), the home-made MS library assembled from pure substances and known essential oil components, and their retention indices with values reported in the literature (Adams, 2007).

Antimicrobial activity and Minimum Inhibitory Concentration (MIC) of the Essential oils of Hildegardia barteri

The following bacteria: *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pleisomonas shigelloides, Bacillus cereus, Proteus vulgaris,* and fungi: *Candida albicans* and *Candida tropicalis* were maintained on nutrient agar and potato dextrose agar (PDA) respectively. Two to three colonies of the test organisms were suspended in 3 mL normal saline in test tubes and then standardised with 0.5 McFarland solution. Sterile swab sticks were used to touch the suspension of the test organisms and then to swab the surface of Muller Hinton Agar plates for bacteria and PDA for fungi. A sterile cork borer of 6 mm diameter was used to bore holes in each plate and different concentrations (6.25-50 mg/mL) of essential oils were introduced into the different holes in triplicate. The plates were incubated uprightly for 24 h at 35℃ for bacteria and at 27±2℃ for fungi.

The MIC was determined by microdilution broth susceptibility as described by Kalemba and Kunicka (2003). A stock solution of the essential oil was prepared in 10 % DMSO, and serial dilutions were prepared to yield concentrations of 0.20-12.50mg/mL. Nutrient broth, essential oil of the leaves, and the test organisms were prepared in test tubes and made up to the final volume of 2 mL. There were two more controls: a negative control (essential oil but no inoculum) and a positive control (inoculum but no essential oil). Following a gentle shake and an incubation period of 24 hours at 35℃ for bacteria and 48 hours at 27±2℃ for yeast, the MIC was ascertained visually by examining the tubes.

The lowest concentration that inhibited the growth of the organism was taken to be the MIC.

Antioxidant activity (AA) of the Essential oils of Hildegardia barteri

Antioxidant activity was evaluated according to Ebrahimzadeh *et al*. (2010) with slight modification by measuring the radical scavenging activity of the essential oils from the fresh and air-dried leaves of *Hildegardia*

barteri on stable 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH). A 4 μM solution of DPPH in methanol was prepared and a stock solution of sample (1 mg/mL) in methanol was prepared under dark room conditions. Various concentrations (6.25 – 200 μg/mL) were added to 1.0 mL (4 μM DPPH) and the final volume was made to 3.0 mL with methanol. After giving the mixture a good shake, it was left in the dark for half an hour. The absorbance of the mixture was measured at 517 nm on a spectrophotometer, with a decrease in absorbance indicating an increase in DPPH-radical scavenging activity. The experiment was done in triplicate with ascorbic acid as standard reference. Percentage inhibition was calculated as follows:

$$I(\%) = 100 \times \frac{A_{blank} - A_{sample}}{A_{blank}}$$

where A_{blank} is the absorbance of the control (containing all reagents except the sample), while A_{sample} is the absorbance of the test sample.

3. RESULTS AND DISCUSSION

3.1.Presentation of Results

The essential oil from the fresh leaves yielded 0.34% (w/w) while the air-dried leaves was 0.28% (w/w). Both oils were colourless and had an herbal smell.

Table 1: Essential oil constituents of the fresh and air-dried leaves of *Hildegardia barteri*

S/N	Retention Time (min)	Compounds	% in Fresh leaves	% in Air-dried leaves	Chemical formula of compounds
1.	4.78	1-(3,5-dimethyl-1 adamantonoyl) semicarbazide	-	0.79	$C_{14}H_{23}N_3O_2$
2.	13.94	Neophytadiene	-	3.47	$C_{20}H_{38}$
3.	14.14	Cyclohexane,1-methyl-4-(1-methylethenyl)-, trans	-	1.40	$C_{10}H_{18}$
4.	14.30	3,7,11,15-Tetramethyl-2-hexadecen 1-ol	-	2.06	$C_{20}H_{40}O$
5.	14.57	Pentadecanoic acid, 14-methyl-methyl ester	0.95	2.05	$C_{17}H_{34}O_2$
6.	14.88	Palmitic acid	24.85	21.41	$C_{16}H_{32}0_2$
7.	15.93	Linolelaidic acid, methyl ester	0.87	1.86	$C_{19}H_{34}0_2$
8.	16.00	Acetamide,2-cyano	0.62	-	$C_3H_4N_2O$
9.	16.01	Oleic acid, methyl ester	-	1.26	$C_{19}H_{36}O_2$
10.	16.15	l- Guanidino succinimide	-	0.36	$C_5H_7N_3O_2$
11.	16.24	Cis, cis- Linoleic acid	22.10	-	$C_{18}H_{32}O_2$
12.	16.29	α-Linolenic acid	-	41.56	$C_{18}H_{30}O_2$
13.	16.32	Oleic acid	34.29	13.14	$C_{18}H_{34}0_2$
14.	16.52	Stearic acid	13.96	6.97	$C_{18}H_{36}0_2$
15.	16.58	Allantoic Acid	1.16	-	$C_4H_8N_4O_4$
16.	16.87	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-		0.64	$C_{11}H_{17}NO_2$
17.	17.98	dl-phenylephrine	0.57	-	$C_9H_{13}NO_2$

18.	17.97	n-propyl 11-octadecenoate	-	1.33	$C_{21}H_{40}O_2$
19.	18.96	Bis(2-ethylhexyl) phthalate	-	0.48	$C_{24}H_{38}O_4$
20.	20.06	γ-sitosterol	0.62	-	$C_{29}H_{50}O$
21.	20.32	Supraene	-	0.85	$C_{30}H_{50}$
22	20.99	2-Methylaminomethyl-1,3-dioxolane	-	0.38	$C_5H_{11}NO$
		TOTAL	**99.99%**	**100%**	

The fresh leaves had 10 compounds representing 99.99% of the oil, composed mainly of 58.21% unsaturated fatty acids and their derivatives (oleic acid (34.29%), cis, cis-linoleic acid (22.10%), derivatives (1.82%)) and 38.81% of saturated fatty acids (palmitic acid (24.85%) and stearic acid (13.96%) amidst compounds like allantoic acid 1.16% and γ-sitosterol 0.62%. The air-dried leaves had 17 compounds representing 100% of the oil, composed mainly of 59.15% unsaturated fatty acids and their derivatives (α-Linolenic acid (41.56%), oleic acid (13.14%), and derivatives (4.45%)) and 30.43% of saturated fatty acids and their derivatives (palmitic acid (21.41%), stearic acid (6.97%) and derivatives (2.05%)) alongside different hydrocarbons, their derivatives, and some heterocyclic compounds in trace amounts.

Table 2: Antimicrobial activity of the essential oils from the fresh and air-dried leaves of *Hildegardia barteri*

Test org	6.25 mg/mL		12.50 mg/mL		25.00 mg/mL		50.00 mg/mL		MIC mg/mL	
	FLO	ALO	FLO	ALO	FLO	ALO	FLO	ALO	FLO	ALO
SA	0.00	0.00	0.00	0.00	0.00	0.00	7.3±1.33	0.00	3.13	3.13
ST	0.00	0.00	0.00	0.00	0.00	0.00	8.0±2.00	11±3.67	3.13	0.78
EC	0.00	7.3±1.34	0.00	8.0±2.00	0.00	9.3±3.34	0.00	15±4.73	6.25	0.39
PS	6.7± 0.66	0.00	7.3±1.34	0.00	8.7±1.34	7.0±1.00	9.3±1.77	8.0±2.0	3.13	3.13
BC	0.00	0.00	0.00	0.00	7.0±1.00	0.00	10±2.19	0.00	3.13	6.25
PV	0.00	0.00	0.00	0.00	7.3±1.33	0.00	9.7±0.85	0.00	3.13	6.25
CA	7.7±1.67	0.00	8.7±1.34	0.00	9.7±1.91	11±2.40	11.3±2.73	10±2.97	0.20	1.56
CT	0.00	0.00	0.00	7.7±1.67	0.00	9.7±1.86	0.00	13±1.34	3.13	0.39

Key: FLO= Fresh leaves oil, ALO= Air-dried leaves oil, MIC = Minimum inhibitory concentration
Bacteria: SA=*Staphylococcus aureus,* ST= *Salmonella typhi,* EC=*Escherichia coli,* PS= *Pleisomonas shigelloides,* BC= *Bacillus cereus,* PV= *Proteus vulgaris*
Fungi: CA =*Candida albicans,* CT = *Candida tropicalis*

Table 2 displays the antibacterial activity of the oils at different concentrations and their MIC values. Where there was activity, it was found to increase with concentration. At 50 mg/mL, FLO was found to have the highest zones of inhibition of 7.3±1.33, 9.3±1.77, 10±2.19, and 9.7±0.85 mm for *Staphylococcus aureus, Pleisomonas shigelloides, Bacillus cereus,* and *Proteus vulgaris* respectively with all having an MIC of 3.13 mg/mL. In contrast, ALO had the highest zones of inhibition of 11±3.67 and 15±4.73 for *Salmonella typhi* and *Escherichia coli* with MIC values 0.78 and 0.39 respectively. For the fungi tested, *Candida albicans* had activities at all concentrations tested for FLO with the highest zone of inhibition of 11.3±2.73 mm

while *Candida tropicalis* had 13±1.34 mm at 50 mg/mL with MIC values of 0.20 and 0.39 mg/mL respectively for the ALO. The ALO had no activity at the concentrations tested for *Salmonella typhi, Bacillus cereus,* and *Proteus vulgaris*.

Table 3: Antioxidant activity (AA) of the essential oils from the fresh and air-dried leaves of *Hildegardia barteri*`

Conc. (µg/mL)	Fresh leaves essential oil AA (%)	Air-dried leaves essential oil AA (%)	Ascorbic acid AA (%)
6.25	23.33	26.66	60.10
12.5	23.07	43.58	64.30
25	64.10	57.45	58.35
50	65.89	58.97	60.50
100	66.00	61.50	65.40
200	66.41	66.42	69.23

Values are mean of n=3

The essential oils from both the fresh and air-dried leaves were tested for free radical scavenging activities on DPPH at different concentrations (6.25-200 µg/mL). They both had activities at 200 µg/mL: fresh leaves oil at 66.41 % and air-dried oil at 66.42% comparable to the 69.23% of ascorbic acid used as the standard.

3.2 Discussion

The fresh and air-dried leaves' essential oils of *Hildegardia barteri* were compared for their chemical compositions, antimicrobial, and antioxidant activities. From Table 2, both oils were found to have some compounds in common while other compounds existed in the fresh leaf oil and not in the air-dried oil and *vice versa*. Oleic acid found in the fresh leaf oil was 34.29% and in the air-dried leaf oil was 13.14%, palmitic acid in the fresh and air-dried leaf oils was 24.85% and 21.41%, while Stearic acid was 13.96% and 6.97% respectively. Cis, cis linoleic (22.10%) was present only in the fresh leaf oil while α-Linolenic acid (41.56%) was present only in the air-dried leaf oil. Some of the aforementioned compounds were detected in trace amounts (linolenic acid (0.8%, leaf essential oil), palmitic acid (2.2% leaf oil and 0.3% stem oil), linoleic acid (0.9% and 0.5% in the leaf and stem) in the previous work carried out by Balogun *et al*., (2017). It is interesting to note that while hexahydrofarnesyl acetone, a significant component in all of the leaf, stem, and root oils in Balogun *et al*.'s work, was absent from both of the oils extracted for this investigation, oleic and stearic acids present in this study was absent in theirs.

Studies on the seed oil of *Hildegardia barteri* showed that the seed oil contained saturated and unsaturated fatty acids such as myristic (23.74%), lauric (23.32%), palmitic (29.38%), stearic (23.74%), linoleic (0.03%), linolenic, 1.43% and oleic (21.50%) (Ogunsina *et al*., 2011; Ibironke and Fawale, (2015) some of which were also found in both oils under study.

Table 2 shows the antimicrobial activities of the fresh and air-dried leaf oils against the bacteria and fungi tested. The fresh and air-dried oils were found to have no activity against *Staphylococcus aureus* and *Salmonella typhi* except at 50.00 mg/mL. The fresh leaf oil was not active against *Escherichia coli* at any of the tested concentrations. However, the air-dried leaf oil was active in

a concentration-dependent manner, giving the highest zone of inhibition of 15±4.73 mm at 50 mg/mL and MIC = 0.39 mg/mL. Both oils were found to be mildly active against *Pleisomonas shigelloides* with zones of inhibition ranging from 6.7± 0.66 to 9.3±1.77 mm and MIC of 3.13 mg/mL. The fresh leaf oil was only active at 25 – 50 mg/mL for *Bacillus cereus* and *Proteus vulgaris* while the air-dried leaf oil was inactive. The fresh leaf oil at all concentrations was active against *Candida albicans* of the two fungi used, while the air-dried oil was active only at higher concentrations. Still, for *Candida tropicalis* FLO was inactive while ALO was active from 12.50-50 mg/mL with zones of inhibition ranging from 7-13 mm and MIC of 0.39 mg/mL. However, in the literature, the seed oil of *Hildegardia barteri* had no zones of inhibition for *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* (Musa *et al*., 2023).

Palmitic acid found in both oil samples is known to possess a range of pharmacological activities, including anti-inflammatory, antiviral, analgesic, and regulation of lipid metabolism (Mayneris-Perxachs *et al*., 2014; Librán-Perez *et al*., 2019). Linoleic acid from plant sources has been known to have anti-inflammatory and acne-reductive properties (Diezel *et al*., 1993; Letawe *et al*., 1998). In this investigation, we found that the essential oil from air-dried leaves was more effective against Gram-negative bacteria than Gram-positive bacteria.

Table 3 gives us the antioxidant activity profile of both oils which were found to compare well with ascorbic acid used as a standard. Antioxidants have been reported to protect cellular organs from oxidative deterioration by hunting free radicals (Firuzi, 2011), however, natural antioxidants are now being favoured as opposed to synthetic ones like butylated hydroxy anisole (BHA) and butyl hydroxytoluene (BHT) because of the issue of toxic effect (Lanigan, 2002). Palmitic acid also known as hexadecenoic acid present in both oils is known to possess antioxidant, hypocholesterolemic, nematicidal, pesticide, antiandrogenic, hemolytic, and 5-alpha reductase activities (Xian *et al*., 2006). The high percentage of α-Linolenic acid (41.56%) in the air-dried leaf oil could also have contributed to the antioxidant activity of the oil because α-Linolenic acid extracted from plant sources has been shown to act as anti-inflammatory and antioxidant agent (Alam *et al*., 2021).

4. CONCLUSION

Our comparison of the essential oil constituents of the fresh and air-dried leaves showed that both oils had some compounds in common while others were found in either the fresh leaf oil or the air-dried leaf oil. The two oil samples had different responses to the Gram-positive, Gram-negative, and fungi tested, while they showed similar antioxidant profiles. The chemical constituents of both oils could be the reason why the plant is used in treating stomach issues and skin-related problems.

Conflict of Interest

The authors declare no conflict of interest.

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