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Studies on Essential Oil Extracted from Hippocratae Velutina Leaves in Relation to Chemical Composition, Antioxidant and Antimicrobial Activities

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Abstract: Plants and their constituents have been used as medicines for so many centuries. *Hippocratae velutina* essential oil was analyzed to determine its chemical content, antioxidant activity and antibacterial activity. *H. velutina* was found to contain 0.3% essential oil. By using gas chromatography, one hundred and ninety-five (195) components were discovered to have fourteen major components, with terpenes, making up 75.36% of those components. Beta ocimene has 26.16%; Farnesene, 14.60%; and others (9%), n-alkanes (7.15%), fatty acid-derived substances (6.87%), green leaf volatiles (2.35%), and Shikimate metabolites (9%). With an IC_{50} of 7.14±1.45mg/ml, which is significantly lower than those of the two standards employed, *H. velutina's* aerial parts demonstrated outstanding free radical scavenging activity outcome with DPPH. With MIC values between 0.15 and 0.23 mg/ml for eight out of all ten microorganisms examined, the essential oils of *H. velutina* leaves demonstrated antibacterial activity against selected strains of Gram-negative and Gram-positive bacteria as well as antifungal activities, with a range in the sensitivity of the microorganisms to the oil. According to the findings, the extracted oil from *H. velutina* has bioactive antioxidant phytochemicals capable of inhibiting a wide range of microbial growth. This confirms its use as a folkloric drug against several ailments.

Keywords: *Chemical composition, antimicrobial, antioxidant, H. velutina, essential oil.*

1. Introduction

Essential oils (EOs) are an aromatic blend of active ingredients with a potent aroma that is derived from aromatic plants (Mohamed and Alotaibi, 2023), obtained as a volatile mixture of chemical compounds with a strong aroma. In general, the major compounds found in essential oils determine their bioactivity properties. They have been extensively used for applications such as bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, and medicinal. It is possible to compare the biological activity of the oils to that of pharmacological preparations made synthetically. Therefore, essential oils are hopeful natural extracts that require additional research to determine their potential for use as supplements, preservatives, or antioxidants in the food or pharmaceutical industries (Santana de Oliveira *et al*., 2020). The ability to smell is stimulated by the sense organ and essential oil helps in this regard when inhaled. Phytochemical analysis of these oils revealed the presence of beneficial compounds like terpenes, antioxidants, and esters that help boost the immune system (Elshafie *et al*., 2017). Due to their sweet fragrance, essential oils are used to make fragrances to creams, manufacture perfumes, hand and body washes and lotions. Other uses of essential oils include the treatment of sinus infections, cold sores, sore muscles, wounds, and skin rashes.

H. velutina is from the family of Celastraceae, predominantly in subtropical and tropical Africa and America. There are approximately 1,200 species in the family of Celastraceae, distributed across 100 genera, and they can be found in temperate regions in both hemispheres, with tropical and subtropical regions having the greatest diversity with trees and shrubs predominately, but lianas make up about 30% of species. The Hippocrateoideae and Salacioideae subfamilies (formerly known as the Hippocrateaceae family) are predominately composed of climbers, whereas, the genus Celastrus is the only member of the Celastraceae stricto that is found in the New World. There are just over 100 liana species in 13 genera, mostly found in forest habitats, in the Neotropics, (Biral, 2017).

The plant has long thin leaves which are arranged opposite each other and small flowers. Its leaf seeds act as a medicine for pain relief. The young branches are useful as a binding substance that provides structural stability to the material in use. In Sierra Lone, the leaves are dried and boiled, then, applied to the body to relieve soreness and inflammation. The Igbo of South Nigeria use the plant as a mechanism to keep termites and beetles away from yams, to avoid holes being dug in the yam crop. In Senegal, the plant seeds are used in the management of headaches and fever.

Objective of the Study

The objective of this study is to establish and/or ascertain the medicinal application of extracted essential oil via the examination of the chemical makeup, antioxidant properties, and antibacterial properties of *H. velutina*.

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Essential oils' chemical content

Depending on the oil, an essential oil might have hundreds to thousands of chemical components. Terpenoids and phenylpropanoid derivatives are the major ingredients in essential oils. Most plants' essential oils are composed primarily of terpenoids. The flavor, smell, and harsh taste of this compound are due to the availability of phenylpropanoid derivatives. From primary metabolites, essential oils from fruits and leaves are said to have biological activities such as antimicrobial and antioxidant activity (Gertrude *et al*., 2022)

2. Experimental Methods

Hippocratea velutina leaves were collected from the botanical garden of the University of Ibadan, Oyo State, Nigeria, and was identified at the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. The plant was verified with the herbarium number ABU030479 by Prof. B.Y. Abubakar. Before extraction, the young leaves were separated from the stalk and cleaned. The leaves of the *H. velutina* were identified using the gas chromatography-mass spectrometry (GC-MS) technique. The hydrodistillation technique was employed in the study to extract essential oil from *H. velutina* leaves. According to the British Pharmacopoeia's guidelines, the oil was extracted using the Hydro distillation process on a Clevenger-type apparatus for 3 hours (Kowalska *et al.,* 1995). Before analysis, the oil production was determined in relation to the matter after the essential oils were collected in n-hexane and kept in the refrigerator at 4^{o}C. The constituents of the essential oils were verified based on their retention variables, which were calculated for a homologous series of n-alkane, and by comparison of fragmentation patterns (NIST data/base/chem station data system) in the mass spectra as reported by (Nicholas *et al.*, 2022).

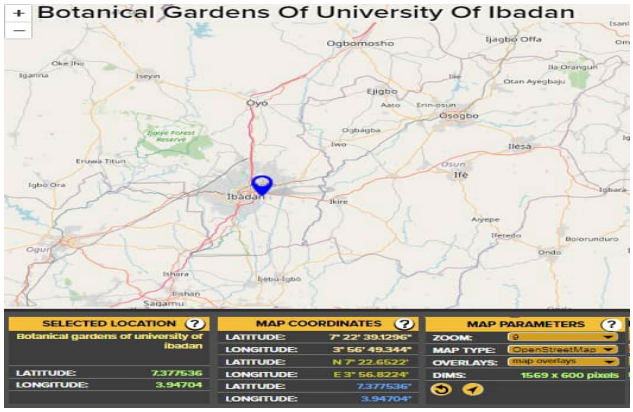

Figure1. Map of the botanical Garden, University of Ibadan

Gas Chromatography Mass Spectrometer (GC-MS) Analysis of the Essential Oil

The GC-MS was a split/split-less injector interfaced with mass-selective detector running at 70 eV. It was used in addition to an Agilent 7809A gas for the analysis of the essential oil. The ion source's temperature was set to 200 °C at a scan rate of 1428 amu/sec, with a mass spectral range of m/z 50–700. The GC employed measures 0.25 mm internal diameter, 30 m long, and 0.25 m film thickness (HP-5MS) column. The following temperature settings were set for the oven: an initial temperature of 80 degrees Celsius for 2 minutes, followed by 240 °C for 6 minutes after, increasing by 10 °C/min. A constant flow rate of 1 mL/min of helium was used as the carrier gas. At 1 L, 362 cms^{-1}, and $5.62x10^{4}$ Pa, respectively, the linear velocity, injection volume, and pressure were changed. The temperature of the oven was fixed at 60 °C which was maintained for 1 minute.

Antioxidant Assay

A modified version of the Bruits method was used to authenticate the ability of the essential oil to possess scavenging activity using diphenylpicryl hydrazine (DPPH). The volatile oil was measured in five different concentrations ranging from 0.015 to 1 mg/mL in test tubes and was vigorously shaken. Incubation was carried out for 30 minutes at room temperature. The absorbance of the treated essential oil samples and blank DPPH solution (control) was thereafter measured at 517nm using a UV/Visible (GS-UZ12) spectrometer. Ascorbic acid and Butylated hydroxyl anisole (BHA) which are established antioxidants served as standards. The analysis was repeatedly done in triplicate and the mean absorbance was calculated. The activities of samples were analyzed using the equation below;

$$\%\text{Inhibition} = \frac{Ab - As}{Ab} \times 100$$

Where the blank's absorbance is Ab, and the sample's absorbance is As.

Antimicrobial Assay

Antimicrobial analysis was determined by the use of the Kirby-Bauer disc diffusion method as reported by Hudzicki (2009) with some adjustments. The microorganisms in use here are as follows; six bacteria which are *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*, *Salmonella typhi,* and *Staphylococcus aureus,* with four fungi*: Aspergillus niger, Candida albicans, Penicillium notatum, and Rhizopus spp*. The microorganisms used were obtained from and identified in the Department of Pharmaceutical Microbiology, University of Ibadan, Oyo State, Nigeria.

Preparation of Sample Solution

In the preparation, 1000 µg/mL of the oil sample was equivalent to 1 mL. Therefore, 500 µg/mL of the essential oil is equivalent to 0.5 mL. More serial dilutions gave different concentrations such as 62.50 µg/ml, 125 µg/ml, and 250 µg/ml, respectively. The test tubes labelled number six (6) and seven (7) were negative control containing (DMSO) while the test tube labelled number eight (8) was the negative control containing n-hexane, positive controls; Gentamycin for bacteria and Tioconazole for fungi, respectively.

Percentage Yield Analysis Result

In this study, the essential oils from *H. velutina* leaves were evaluated for their physical characteristics and percent yield

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(Table 3.1). There is only a 0.3% essential oil output, which is lower than the levels noted by Niko *et al.* (2009) on different plant materials. The method and duration of extraction of essential oils are key factors that greatly influence the quality and quantity of essential oil yields (Samadi, 2022). Interestingly, the chemical makeup of essential oils and percentage yield are usually correlated (Niko *et al.,* 2009).

3. Results and Discussion

Table 1. Appearance and Yield of *H. velutina Essential Oil*

Weight of Sample packed for Extraction (g)	350
Weight of Essential Oil Extracted (g)	0.9
Percentage Yield (%)	0.3
Colour	Colourless
Odour	Herbal light

Gas chromatography Mass Spectrometry Analysis Result

GC-MS was employed in determining the chemical makeup of the essential oils' most potent ingredients of *H. velutina* leaves. A total of one-hundred and ninety-five (195) components were determined from the GC analysis (see Appendix II). From these, fourteen major components amounting to 75.36% contain terpenes (Beta-ocimene, ≥26.16%; Farnesene, ≥14.60%, and others ≥9.24%), *n*-alkanes (≥7.15%), fatty acid-derived compounds (≥6.87%), green leaf volatiles and Shikimate metabolites (≥2.35%) were seen. The structures of these fourteen major components are indicated in Figure 3.1. The Gas Chromatograph for all 195 components present in *H. velutina* leaves essential oil is shown in Appendix I. The presence of different terpenoid compounds has been recorded as the main active components of essential oils, giving such oils high antimicrobial and antioxidant properties.

Table 2. Chemical Composition *H. velutina* Leaves Essential Oil

Compound	Retention Time (minutes)	Percentage Composition (%Area)
Major Components (%Area≥1.00)		
Beta-ocimene	4.6970	26.16
Farnesene	10.800	14.60
Nonacosane	23.229	7.15
n-hexadecanoic acid	15.752	6.87
9,12,15-octadecatrienoic acid, (z,z,z)-	17.373	6.47
Methyl salicylate	6.684	2.35
2-hexadecen-1-ol,3,7,11,15-tetramethyl,acetate, (R-R*,R*-(E))	17.065	2.29
3,7,11-trimethyl-,(s-(z))-1,6,10-dodecatrien-3-ol,	11.424	2.00
(6E,10E,14E,18E)-3-bromo-2,6,10,15,19,23-hexamethyltetracosa-6,10,14,18,22-pentaen-2-ol	22.732	1.43
3-hexen-1-ol,benzoate,(z)-	11.520	1.38
1,8,9-triazabicyclo(4.3.0)nona-6,8-diene	11.585	1.35
2-methyl-5-(1,1,5-trimethyl-5-hexenyl) Furan	10.009	1.14
3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol	16.300	1.11
3,7-dimethyl-(E)-1,3,6-octatriene	4.478	1.06
Minor Components (%Area<1.0)	-	24.64
Total		100.00

Antioxidant assay analysis results

The *H. velutina* leaves essential oil has not been reported of any antioxidant activity. The *H. velutina* leaves scavenging activity of its essential oils using DPPH free radical is shown in Figure 3.2 (a, b, and c) and Tables 3.3 and 3.4 as compared with two known standards (ascorbic acid and butylated hydroxyanisole). The change in absorbance produced by reduced DPPH was used to evaluate the ability of *H. velutina* leaves essential oils to act as free radical scavengers (Table 3.3). Results in Table 3.4 as represented graphically in Figure 3.2 (a, b and c) showed that the scavenging effect of *H. velutina* leaves essential oils is excellent with an IC_{50} of 7.14±1.45 mg/ml which is considerably lower and thus more active than both standards (ascorbic acid: 16.24±1.59 mg/ml; and BHA: 17.60±1.76 mg/ml). A wide variety of foods and medicinal plants contain natural antioxidants. These natural antioxidants, particularly the carotenoids and polyphenols, have a variety of biological effects, including those that are anti-inflammatory, anti-aging, anti-atherosclerosis, and anticancer (Xu *et al.,* 2017). The effect of the combination of the trio compounds tends to be synergetic and thus provides improved results than the isolated compounds. The findings of this antioxidant study support the folkloric usage of the plant as a preventive and curative agent in some diseases. The provided data can enrich a possible comprehensive data of the antioxidant activity of *H. velutina* in the future.

Table 3. Essential Oils' Antioxidant Potential of *H. velutina* Leaves and Standards

Concentration (mg/ml)	Absorbance		
	Essential Oil of	Ascorbic Acid	Butylated Hydroxyanisole (BHA)
1.000	0.01±0.00	0.03±0.01	0.04±0.01
0.500	0.04±0.04	0.04±0.00	0.05±0.01
0.250	0.03±0.00	0.05±0.01	0.06±0.00
0.125	0.1±0.01	0.06±0.01	0.07±0.01

*Values are mean ± standard deviation of triplicate determinations

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Table 4. The % Inhibition of the Various Concentrations of *H. velutina* Leaves Essential Oils and Standards

Concentration (mg/ml)	% Inhibition Essential Oil of *H. velutina*	Ascorbic Acid	Butylated Hydroxyanisole (BHA)
1.000	99.37±0.19	97.98±1.06	97.22±1.41
0.500	96.94±1.28	96.97±1.41	96.46±1.40
0.250	97.75±0.87	96.46±1.76	95.45±2.11
0.125	92.17±3.45	95.20±2.11	94.95±2.11
$IC_{50\ (DPPH)}$	7.1412±1.45	16.235±1.59	17.599±1.76

*Values are mean ± standard deviation of triplicate determinations

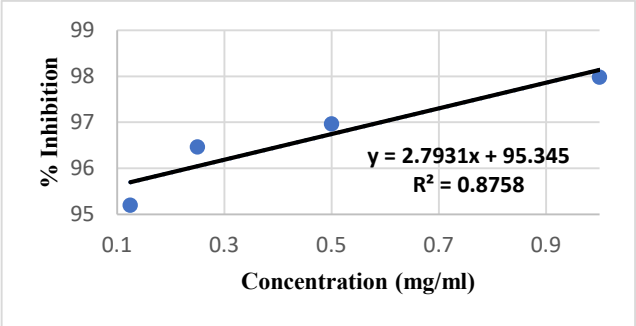

Figure 1. Percentage (%) Inhibition of Ascorbic Acid

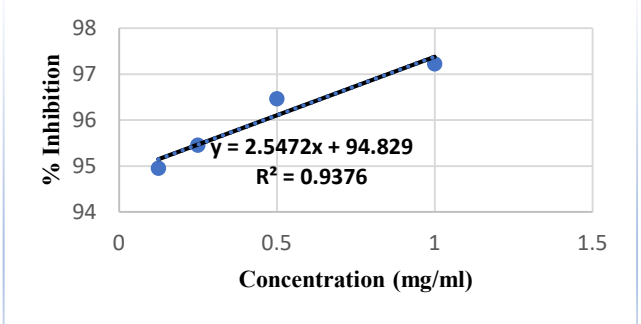

Figure 2. Percentage (%) Inhibition of Butylated Hydroxyanisole (BHA)

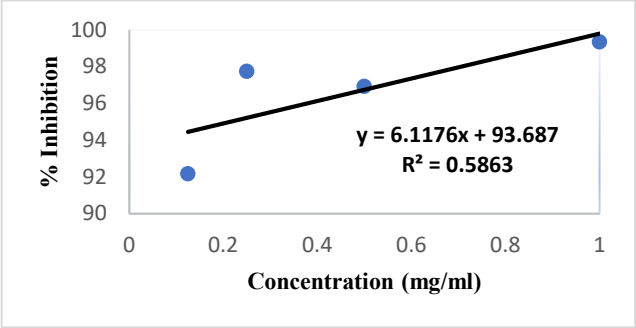

Figure 3. Percentage (%) Inhibition of Essential Oils of *H. velutina*

Antimicrobial Assay Results

Using linear regression graphs based on the measured diameters of the inhibition zones, the minimum inhibitory concentration (MIC) of *H. velutina* leaves essential oil was calculated from the zones of inhibition (Table 3.6). Table 3.5 shows the different isolate concentrations of *H. velutina* leaves essential oils zones of inhibition compared to the control drugs. Three categories of microorganisms were used, with isolates representing each category: gram-negative and gram-positive bacteria, as well as fungi. The results showed that essential oils of *H. velutina* exhibited remarkable antimicrobial properties against some of the tested organisms. Specifically, the oils inhibited the growth of all the gram-positive (*Bacillus subtilis*, *S. aureus*) and gram-negative bacteria (*E. coli*, *K. pneumonia*, *S. typhi,* and *P. aeruginosa*), as well as *C. albican* but were inactive against the other Filamentous fungi (*Penicillumnotatum* and *Rhizopussp*). Observably, the *H. velutina* leaves essential oils exhibited good antimicrobial activities at high concentrations of 100 mg/ml. However, these antimicrobial activities decreased at reduced concentrations as indicated by the varying zones of inhibition (Table 3.5). At a low concentration of 25 mg/ml, the *H. velutina* leaves essential oils could no longer prevent the two fungi species from growing (*Aspergillus niger* and *Candida albicans*) which hitherto inhibited. *H. velutina* leaves essential oils at 12.5 mg/ml concentration could not stop *Bacillus subtilis* and *Salmonella typhi from growing*. *H. velutina* leaves essential oils were not able to prevent the growth of any of the tested microorganisms at 6.25 mg/ml concentration and below. The oil presents the best action as antibacterial, in which *Staphylococcus aureus* was the most susceptible bacteria across all tested concentrations. The *H. velutina* leaves essential oils revealed MIC values for eight out of the ten microorganisms tested, with the MICs ranging from 0.15 and 0.23 mg/ml (Table 3.6). There are no available scholarly reports on the antimicrobial analysis of *H. velutina* leaves, but the anti-bacterial and antifungal properties exhibited by *H. velutina* may be due to some bioactive phytochemicals common to essential oils. Specifically, some terpenes, eugenol, terpineol, carveol, and citronellol have demonstrated quick bactericidal responses against *Salmonella enterica, S. aureus strains*, and *E. coli* respectively (Guimarães *et al*., 2019) with synergetic effects in combination with other phytochemicals (Ayaz *et al*., 2019). Moreover, the activity of aldehydes and their derivatives against pathogenic bacteria for future use in the clinical setting has been reported by (Aljaafari *et al*., 2022). From the antimicrobial results obtained, it could therefore be inferred that the essential oils of *H. velutina* leaves possessed antibacterial activity against Gram-positive/negative bacteria as well as antifungal activities with a varying sensitivity of the microorganisms to the oil. We can therefore suggest that the essential oils of *H. velutina* leave a potentially broad-spectrum antimicrobial agent.

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Table 5. *H. velutina* Essential Oil Zones of Inhibition Compared to the Control Drugs

Concentration (mg/ml)	Zone of inhibition* (mm) Pathogenic bacterial species						Pathogenic fungal species			
	SA	EC	BS	PA	KP	ST	CA	AN	PN	RS
100	18.0±0.0	17.0± 0.0	15.0± 0.0	17.0± 0.4	17.0±0.4	15.0± 0.4	13.0± 0.4	13.0± 0.0	--	--
50	16.0± 0.0	15.0± 0.4	13.0± 0.4	15.0± 0.4	15.0±0.4	13.0± 0.4	10.0± 0.0	10.0± 0.0	--	--
25	14.0± 0.0	13.0± 0.4	10.0± 0.0	12.0± 0.0	13.0± 0.4	10.0± 0.0	--	--	--	--
12.5	11.0± 0.5	13.0± 1.4	--	10.0± 0.0	10.0± 0.0	--	--	--	--	--
6.25	--	--	--	--	--	--	--	--	--	--
3.125	--	--	--	--	--	--	--	--	--	--
Negative control**	--	--	--	--	--	--	--	--	--	--
Positive control***	39.0± 0.5	37.0± 0.4	37.0± 0.4	38.0± 0.0	39.0± 0.0	38.0± 0.0	28.0± 0.0	28.0± 0.0	27.0± 0.0	23.0± 1.4

*Values are mean ± standard deviation of triplicate determinations**Negative control: n-Hexane (for Bacteria and fungi); ***Positive Control: Gentamicin (10µg/ml) for bacteria, Tioconazole (30%) for fungi.

AN=*Aspergillus niger*; BS= *Bacillus subtilis*; CA= *Candida albicans;* EC= *Escherichia coli*; KP=*Klebsiella pneumoniae;* PA= *Pseudomonas aeruginosa*; PN=*Penicillum notatum*; RS=*Rhizopus sp;* SA= *Staphylococcus aureus;* ST=*Salmonella typhi;*

Table 6. Antimicrobial Activity of *H. velutina* Leaves Essential Oils Showing MIC.

Microorganisms		Zone of Inhibition* (mm)	MIC** (mg/ml)
Gram Positive			
	Bacillus subtilis	10.0± 0.0	0.18±0.00
	Staphylococcus aureus	11.0± 0.5	0.23±0.01
Gram Negative:			
	Klebsiella pneumoniae	10.0± 0.0	0.22±0.00
	Escherichia coli	13.0± 1.4	0.22±0.02
	Salmonella typhi	10.0± 0.0	0.18±0.00
	Pseudomonas aeruginosa	10.0± 0.0	0.22±0.00
Fungi (Yeast):			
	Candida albicans	10.0± 0.0	0.15±0.00
Filamentous Fungi (mold):	*Aspergillus niger*	10.0± 0.0	0.15±0.00

*Values are of triplicate determinations of standard deviation ± mean

4. Conclusion

Thus, *H. velutina*'s aerial portions were used to extract essential oils, with a 0.3% yield as a result. The existence of major terpenes, fatty acids, and n-alkanes, a crucial element of essential oils, was discovered through chemical elucidation. *H. velutina leaves essential oil* was also subjected to antioxidant as well as antimicrobial analysis. The results indicate that *H. velutina* leaves essential oil have bioactive antioxidants capable of inhibiting a wide range of growth. This confirms the use of the plant as a folkloric drug against several ailments.

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