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Research Article

Chemical composition, in vitro antioxidant and antimicrobial activities of essential oil extracted from the dried flower bud of *Eugenia aromatica L*

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Abstract

Plants are the richest sources of bioactive compounds and have been the basis of orthodox medicines since ancient times and has continued to provide cure to diseases of mankind. This study investigated the chemical composition, antimicrobial and antioxidant activities of essential oil extracted from the dried flower bud of *Eugenia aromatica* L. The clove buds essential oil obtained through hydro distillation using the Clevenger apparatus was then analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Later on the antimicrobial assay was carried out using the agar duffusion method. In vitro antioxidant was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power, nitric oxide radical scavenging, total antioxidant capacity and lipid peroxidation assays on the extracted essential oil. The major components of the extracted essential oil of *Eugenia aromatica* L. are Eugenol (76.13%) and Eugenol acetate (19.01%). Results of the antioxidant activities of the essential oil showed promising antioxidant potentials when compared to the positive control (ascorbic acid), strong nitric oxide scavenging activity was observed in the essential oil (IC_{50} of 24.78 µg/mL) than that of the standard drug used (IC_{50} of 34.24 µg/mL). The antimicrobial activities of the essential oil against the most frequently encountered microorganisms which include *Staphylococcus aureus* (ATCC 29213), *Streptococcus mutans, Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) showed significant broad spectrum antibacterial and antifungal activities with zones of inhibition (mm) against *Staphylococcus aureus* (20.00± 0.0), *Streptococcus mutans* (32.00± 1.4), *Escherichia coli* (30.00 ± 0.0) and *Candida albicans* (28 ± 5.7).

1. Introduction

Eugenia aromatica L. commonly known as clove, is most valued for its aromatic flower buds broadly used in cooking, pharmacy, perfumery and cosmetics. The chemical composition of clove oil has been studied widely and a number of mono-sesquiterpenoids has been identified as the major components. These include Eugenol, β-caryophyllene, α-humulene and eugenyl acetate. Essential oil of *Eugenia aromatica* L contains 23 constituents with eugenol (76.8%), β-caryophyllene (17.4%), α-humulene (2.1%), and

eugenyl acetate (1.2%) as the main components [1]. In another work, 22 components of the essential oil obtained from clove buds were identified. Eugenol was the major component (76.23%) [2]. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of clove essential oil revealed the presence of twenty one compounds with eugenol (69.68 %) as major compound [3]. It was also reported that the essential oil of *Eugenia aromatica* L was chemically evaluated by gas chromatograph (GC), the main composition was eugenol (83.13%), β caryophyllene (6.88%), α humulene (2.48%), oxicaryophylene (3.59%), eugenyl acetate (2.41%) [4]. Extraction and GC-MS analysis of the compounds of clove bud oil obtained from Toli-Toli and Bali revealed that the major compounds of clove oil were eugenol (66.37%), caryophyllene (15.38%), α-humulene (1.97%), eugenyl acetate (12.99%) and eugenol (72.34 %), caryophyllene (12.51 %), α-humulene (2.34 %) and eugenyl acetate (5.33 %) respectively.

The reports from Ghana, Italy, Mali, and Pakistan showed the dominance of eugenol in clove oil. The percent ranges from 35.5% to 89.1% [2-3,5]. Other prominent compounds are caryophyllene (6.88-15.38%), α-humulene (1.00- 3.00%), eugenyl acetate (1.2- 5.33%). The unique minor compounds of clove oil from Toli-Toli were (+)-δ-cadinene (0.13 %) and βcaryophylladienol (0.19 %) while in clove oil from Bali were valencene (0.17 %), δ-selinene (0.22 %) and alloaromadendrene (0.24 %) [5]. Down the ages, numerous essential oils extracted from plant materials have been used for their aroma, flavor, bactericidal, preservative and medicinal properties [6]. Since essential oils are a rich source of biologically active compounds, investigating the antimicrobial properties of essential oils extracted from aromatic plants is a growing interest [7].

Clove oil has been reported to have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and is used traditionally as a savoring agent and antimicrobial material in food, it is also used as an antiseptic in oral infections, inhibits Gram-negative and Gram-positive bacteria as well as yeast. The essential oil can be considered as a potential antimicrobial agent for external use [4, 8-10]. Eugenol is primarily responsible for bacteriocidal/bacteriostatic properties, [4]. As part of our ongoing research on Nigerian essential oil medicinal plants, this current work is concerned with the comprehensive analysis of clove oil and the comparative studies with other reports. The aim of this study was to comprehensively analyze the chemical composition, antimicrobial and antioxidant activities of essential oils from the dried flower bud of *Eugenia aromatica* L. and compare with earlier reports for possible chemotype detection. Also to emphasise its possible application in cosmetics as an antimicrobial and antioxidant.

2. Materials and methods

2.1 Plant material and essential oil extraction technique.

The dried flower buds of *Eugenia aromatica* L. (Clove) were collected from Kungu town in Bichi LGA of Kano State, Nigeria in June 2021. The botanical identification and authentication was done by Dr. Nodza George at the Herbarium in Botany Department, University of Lagos, Nigeria with authentication number LUH: 8799. The dried flower buds of *Eugenia aromatica* L. was pulverised by crushing prior to extraction. The essential oil was obtained by hydrodistillation using of 300g of the pulverized clove buds using the Clevenger apparatus for 4 hours [11]. The oil obtained was dried over anhydrous sodium sulphate and stored in a refrigerator prior to analysis.

2.2 GC-MS Analysis of essential oil.

The analysis of the essential oil was carried out using an Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% phenyl methyl siloxane (30m length x 0.32mm diameter x 0.25μm film thickness) (Agilent Technologies). The carrier gas was helium and it was used at constant flow of 1.4871 mL/min and at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. 1μL of the samples were injected in splitless mode at an injection temperature of 300 °C. Purge flow to spilt vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min; gas saver mode was switched off. Oven was initially programmed at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min). Run time was 32.667 min.

with a 5 min. solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was via Scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate).

Relative percentage amounts of the essential oil components were evaluated from the total peak area by apparatus software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with literature data and by computer matching with NIST 2017 and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

2.3 Antioxidant Assay

2.3.1 DPPH Radical Scavenging Assay

The free radical scavenging capacity of the essential oil was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [12]. A solution of 0.1mM DPPH in ethanol was prepared, 1mL of the solution was added to 1 mL of the essential oils at different concentrations (25, 50,75, 100 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Ascorbic acid (4 mg/mL in ethanol) was used as positive control while ethanol was used as negative control. Then the absorbance was measured at 517 nm by using Ultraviolet Visible Spectrophotometer TG 50 Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation

DPPH Scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance of standard (prepared essential oil). The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of DPPH radical formation. The test was repeated as described above for all concentration of each oil in triplicates. Inhibition % was plotted against concentration and the IC_{50} was calculated graphically.

2.3.2 Ferric reducing antioxidant power assay (FRAP)

In the FRAP assay, the method of Pham-Huy et al. [12] was adopted, the absorbance at 700 nm was measured using Ultraviolet Visible Spectrophotometer against a blank. Gallic acid was used as the control. A higher absorbance of reaction mixture indicated greater reducing power. Data was presented as mean and standard deviation for triplicate analysis. The percentage FRAP Scavenging effect was calculated using the equation described in the DPPH assay.

2.3.3 Nitric oxide radical scavenging assay.

The method described by Okoh et al. [13] was adopted. Nitric oxides radicals were generated from a sodium nitroprusside solution; Sodium nitroprusside (1 mL of 10 mM) was mixed with 1 mL of oils to give concentrations of 0.025–0.50 mg/mL in phosphate buffer. The mixture was incubated at 25 °C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent was added. The absorbance was measured at 546 nm using a UV/VIS TG 50 Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Ascorbic acid was used as the positive control. The % inhibition of nitric oxide radical by the oil was calculated using the equation described in the DPPH assay.

2.3.4 Lipid peroxidation

In this assay, 10 µL of essential oil at different concentrations of 25,50,75 and 100 µg/mL or standard solution, (1,1,3,3-tetramethoxypropane, TEP) and 40 µL of 20 mM phosphate buffer (pH 7.0) were added to a test tube on ice bath. In each tube, 50 µL of 3% sodium dodecyl sulfate (SDS), 200 µL of 0.1 N HCl, 30 µL of 10% phosphotungstic acid, and 100 µL of 0.7% of 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100°C for 30 min in water bath. The reaction mixture was mixed with 400 µL of n-butanol and then centrifuged at 3000 rpm for 10 min. Ascorbic acid was used as positive control. Supernatants were collected and pass through an Ultraviolet Visible Spectrophotometer at a wavelengths of 515 nm/555 nm [14]. The percentage of inhibition of lipid peroxide was calculated using the equation described in the DPPH assay.

2.4 Total antioxidant capacity

The method described by Kattamis et al. [15] was adopted and Ultraviolet Visible Spectrophotometer was used to determine the absorbance of the mixture (optical density, OD, of 570 nm). Ascorbic acid was used as control. The percentage of total antioxidant capacity was calculated using the equation described in the DPPH assay.

2.5 Antimicrobial Assay

The antimicrobial activity of essential oil of *Eugenia aromatica* L. was assayed using Agar well diffusion technique [16]. The inocula were prepared from the typed bacterial and yeast cultures of *S. aureus* (ATCC 29213), *S. mutans*, *E. coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) respectively which were maintained in glycerol-peptone water at 4 OC in the pure culture laboratory of Microbiology Department, University of Lagos, Akoka-Yaba, Lagos, Nigeria and were sub-cultured into sterile peptone water in McCartney bottles. The densities of the bacterial suspensions were determined by diluting the broth cultures 1:100 (mixing 0.1mL of the inoculum and 9.9ml of sterile normal saline). These were compared with 0.5 McFarland standards. These suspensions were estimated to 1.0 x106 - 107 CFU/mL

Standard broad spectrum antibiotic discs - Ciprofloxacin and Pefloxacin (10µg –Maxicare Med. Lab, Nig) were used as positive control while Hexane served as negative control.

3. Results and discussion

3.1 Chemical composition

The yield of flower buds of *Eugenia aromatica* L**.** essential oil obtained by hydrodistillation was 1.50 % v/w. The chemical analysis by Gas Chromatography-Mass Spectrometry (GC-MS) identified 22 compounds.

The essential oil of *Eugenia aromatic L***.** was mainly composed of oxygenated monoterpenes (77.26%), as shown in Table 1. The major components of the essential oil are Eugenol (76.13%) followed by Eugenol acetate (19.01%) as shown in Table 2. This result is similar to most results in literature.

Several studies reported that the major components of essential oil of *Eugenia aromatica* L are eugenol, β-caryophyllene, *α*-humulene and eugenyl acetate with varying percentages [1,17-19]. Sidi *et al* [20] reported that the main constituents of *Eugenia aromatica* L were eugenol (78.72%), β-caryophyllene (8.82%) and eugenyl acetate (8.74%).

Nevertheless, some few studies indicated differences in the major components, Sokamte *et al*· [21] reported that eugenol, δ-Cadinene and β-elemene are the major compounds of this essential oil. The content of eugenol in the essential oil and other compounds can be suggested to be directly related to the processing method used to obtain in the oil, which can affect its chemical composition, as distillation and storage conditions are capable of influencing the content of its volatile metabolites [22]. In addition, their geography, season, stage of development, age of the plant, and

Table 1. Oxygenated monoterpenes of essential oil of *Eugenia aromatic L***.**

Compounds	Composition (%)
Eugenol	76.13
Eucalyptol	0.09
Linalool	0.12
cis-Sabinol	0.27
Coniferyl aldehyde	0.24
Estragole	0.41
Total	77.26

Table 2. Chemical composition of the essential oil from dried flower buds of *Eugenia aromatica* L**.**

Compounds	Comp-osition (%)	RIcal		RI
Ethanol, 2-butoxy-	0.17	938		936
Eucalyptol	0.09	1061		1059
Linalool	0.12	**1081**		**1082**
Methyl salicylate	0.23	1285		1281
cis-Sabinol	0.27	1088		1085
Phenol, 4-(2-propenyl)-	0.44	1206		1203
Eugenol	**76.13**	1394		1392
Vanillin	0.11	1406		1403
Caryophyllene	0.67	1492		1494
Humulene	0.13	1577		1579
Eugenol acetate	**19.01**	**1551**		**1552**
Benzene, 1-methyl-3-nitro-	0.15	1187		1189
Butyrovanillone	0.15	1639		1638
Caryophyllene oxide	0.68	1505		1507
Humulene-1,2-epoxide	0.08	1593		1592
Coniferyl aldehyde	0.24	1596		1599
Benzyl Benzoate	0.09	1736		1733
Hexadecanoic acid, methyl ester	0.12	187		1878
9-Octadecenoic acid (Z)-,methyl ester	0.13	2172		2175
Estragole	0.41	1176		1172
Bis(2-ethylhexyl) phthalate	0.49	2703		2704
Dehydrodieugenol	0.09	2794		2791
Total oil content	100%			

The total composition consisting of oxygenated monoterpenes, sesquinterpenes hydrocarbon, oxygenated sesquinterpenes and oxygenated diterpenes. RIcal: Retention index determined relative to n-alkanes (C7-C30) on the HP-5ms column. RI: literature retention indices

climatic conditions can also affect yield and chemical composition [23].

3.2 Antimicrobial Activity

The *Eugenia aromatica L.* essential oil assayed using Agar well diffusion technique exhibited significant in vitro antimicrobial activity with zones of inhibition (mm) against *Staphylococcus aureus* (20.00±0.0), *Streptococcus mutans* (32.00±1.4), *Escherichia coli* (30.00±0.0) and *Candida albicans* (28±5.7) (Table 3). The results compare well with that of the standard drugs. Generally, this study showed that the essential oil of *Eugenia aromatica L.* was effective against both *Gram*-positive and *Gram*-negative bacteria and fungi used and may support its use for the treatment of bacterial and fungal infections as stated by Nuñez *et al* [4] that, Clove essential oil, used as an antiseptic in oral infections, inhibits *Gram*-negative and *Gram*-positive bacteria as well as yeast and may be considered as a potential antimicrobial agent for external use. The essential oil of clove also has been reported to inhibit the growth of molds, yeasts and bacteria [24]. It has been reported that the inhibitory activity of clove is due to the presence of several constituents, mainly eugenol, eugenyl acetate, betacaryophyllene, 2-heptanone [25], and to a lesser extent the lower abundance of α-humulene, methyl salicylate, isoeugenol, methyl eugenol [26].

Table 3. Zones of growth inhibition (mm) of essential oil of *Eugenia aromatica* L. against the growth of microorganisms used.

Microorganisms	Diameters of zones of inhibition (mm)		
	Essential oil	CPX	PEF
Staphylococcus aureus	20.00±0.0	29.00±0.7	25.00±0.0
Escherichia coli	30.00±0.0	27.00±0.0	26.00±0.7
Streptococcus mutans	32.00±1.4	27.00±0.7	27.00±0.0
Candida albicans	28±5.7	29.00±0.0	26.00±0.0

Abbreviations: CPX, Ciprofloxacin; PEF, Pefloxacin. Results are means of duplicate values.

Table 4. Antioxidant assays of the essential oil of *Eugenia aromatica* L.

Antioxidant assays	IC_{50} (µg/mL) for oil	IC_{50} (µg/mL) (Control)
DPPH (%Inhibition)	49.4	29.61
Ferric Reducing antioxidant power (%Inhibition)	9809.8	8466.44
Nitric Oxide Scavenging Activity (%Inhibition)	24.78	34.24
Lipid peroxidation scavenging activity (%Inhibition)	97.87	34.24
Total antioxidant capacity (mg/100)	67	

Control: Ascorbic acid; Gallic acid

3.3 Antioxidant Activity

The IC_{50} of DPPH (49.4 µg/mL), FRAP (9809.8 µg/mL), Nitric Oxide Scavenging activity (24.78 µg/mL) and Lipid peroxidation scavenging activity (97.87 µg/mL) were presented in Table 4. Comparatively, strong nitric oxide scavenging activity was observed in the essential oil (IC_{50} of 24.78 µg/mL) than that of the standard drug (Ascorbic acid) used (IC_{50} of 34.24 µg/mL) while weak antioxidant activity was observed in Lipid peroxidation scavenging activity (IC_{50} of 97.87 µg/mL) compare to the standard drug (Ascorbic acid) used (IC_{50} of 34.24 µg/mL).

The standard drug (Ascorbic acid) used for DPPH and the standard drug (Gallic acid) used Ferric reducing antioxidant power (FRAP) demonstrated higher antioxidant activity than the essential oil. The standard drug with IC_{50} of 29.61 µg/mL and 8466.44 µg/mL for DPPH and FRAP respectively, while the essential oil is with the IC_{50} of 49.4 µg/mL and 9809.8 µg/mL for DPPH and FRAP respectively.

The most abundant component of clove oil is eugenol, to which are attributed majorly the antioxidant properties and antimicrobial activity.

4. Conclusions

The results from this study revealed the dominance of eugenol in the oil of *Eugenia aromatica* L. (76.13%). The essential oil showed potential antimicrobial and antioxidant capacity and this reaffirm the use of the essential oil of *Eugenia aromatica* L. to fight skin infections and as an alternative to current synthetic preservative used in cosmetics and pharmaceuticals.

Authors' contributions

Conceptualization, O.T.A.; Methodology, O.T.A. and J.O.O.; Analysis; O.O.F, I.S.N and J.O.O; Resources, O.O.F; Data Curation, I.S.N; Writing - Original Draft Presentation, O.O.F; Writing- Review & Editing, O.T.A. and I.S.N.; Project Administration, O.T.A.

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Conflicts of interest

Authors have declared that no competing interest exist.

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