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# Composition and Antibacteria Activity of Steam Distilled Oils from *Xylopia aethiopica* and *Syzgium aromaticum*

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**Abstract:** Essential Oils from dry fruits of *Xylopia aethiopica* and *Syzgium aromaticum* were obtained by steam distillation, using a Clevenger-type system. The composition was determined by means of gas chromatography-mass spectrometry techniques using direct injection. The oils were also screened for antibacteria activity against *E. coli*, *Serratia* sp, *Salmonella typhi*, *Klebsiella* sp, *citrobacter* and *Pseudomonas aeruginosa*. The oil yields were 1.2 and 7.4% for *Xylopia aethiopica* and *Syzgium aromaticum*, respectively. The results showed that twenty three and three constituents representing 74 and 99.9% of the o ils were conclusively identified from *X. aethiopica* and *S. aromaticum*, respectively. β -pinene 13.78%, β-phelladrene, 12.36% gama-terpinene 7.66% and α-pinene 5.56% as the major components of *X. aethiopica* and *eugenol* 93.7% for *S. aromaticum*. The two oils show demonstrated activity against tested organisms with *S. aromaticum* having higher activity than *X. aethiopica* at different concentrations.

**Key words:** Essential oil, distillation, antibacteria

## INTRODUCTION

The antiseptic qualities of aromatic and medicinal plants and their extract, have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s[1,2].

Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast[3,4]. Most of their properties are due to essential oils produced by the plants[5]. Essential oils and extracts from several plant species have been reported to control microorganisms related to skin, dental cares and food spoilage[5-7].

Plant volatile oils are generally isolated from non-woody plant material by distillation methods, usually steam or hydrodistillation and are variable mixtures of principally terpernoids, specifically monoterpenes ($C_{10}$) and sesquiterpenes ($C_{15}$) although diterpenes ($C_{20}$) may also be present and a variety of low molecular weight aliphatic hydrocarbons (linear, ramified, saturated and unsaturated), acids, alcohols, aldehyoes, acyclic esters or lactones and exceptionally nitrogen and sulphur-containing compounds, coumarins and homologues of phenylpropanoids. Terpenes are amongst the chemicals responsible for the medicinal, culinary and frangrant uses of aromatic and medicinal plants. most terpenes are derived from the condensation of branched five carbon isoprene units and are categorized according to the number of these units present in the carbon skeleton.

The antimicrobial properties of plant volatile oils and their constituents from a wide variety of plants have been assessed[8,9] and reviewed[10-15]. It is obvious from these studies that these plant secondary metabolites have potential in medicinal procedures, applications in food, cosmetic and pharmaceutical industries[16-18]. In this studies we have examined the composition and antibacteria activity of essential oils of the fruits of *X. aethiopica* and *S. aromaticum* to correlate the results with the traditional use of these plants and to compare the result with the previous work.

## MATERIALS AND METHODS

**Plant materials:** Ripe fruits of *X. aethiopica* and *S. aromaticum* were obtained from local market in Keffi, Nasarawa State, Nigeria.

**Recovery of essential oil:** The dried fruits were grounded and hydrodistilled using all glass Clevenger apparatus. The oils obtained were dried over anhyrous sodium sulphate. The oil was transferred to a sample bottle and stored in the Fridge until it was sent to a laboratory in Germany (Federal Institute for Geosciences and Natural Resources) for analysis.

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**Identification of the components:** The identification of volatile constituents was conducted by gas chromatography-mass spectrometry techniques using direct injection in the split mode under the following conditions. Hewlett-Packard 6890 equipped with a quartz capillary column: 50m×.25mm i.d×0.1 μm; Helium was used as carrier gas at 1.3 $ML^{-1}$ MM flow rate; oven temperature 30°C (hold 5 min) to 200°C at 8°C $mm^{-1}$ then to 320 at 6°C $mm^{-1}$ injector temperature; 320°C, mass range: 35-600 amu, 1.247 scans $sec^{-1}$; ionization energy; 70 eV. The qualitative identification of different constituents was performed by comparison of their retention times and mass spectra with those of the library.

**Antimicrobial screening:** Screening of the essential oil for activity by agar diffusion disc impregnated method was adopted[19] 50% v/w of the oil was prepared, Whatman paper disc of 7 mm diameter was impregnated and oven dried at 37°C for 1 h to remove the presence of used solvent. $1\times10^6$ CFU $mL^{-1}$ of the test bacteria was prepared and seeded into the solid agar medium. The impregnated paper discs were placed at intervals and incubated for 24 h at 37°C. After 24 h the zone of inhibition was measured against the following microorganisms; *Escherichia coli, Serratia* spp, *Salmonella typhi, Klebsiella* sp., *Citrobacter* sp. *and Pendomonas aeruginosa.*

## RESULTS AND DISCUSSION

After 5 h of hydrodistillation, the essential oil yield was 1.2 and 7.4% for *X. aethiopica* and *S. aromaticum*, respectively. The GC/MS analysis showed that the oil was composed of many compounds and a total of twenty three and three compounds representing 74 and 99.9% of the oils were conclusively identified from the two oils, respectively. Table 1 and 2 show the constituents identified by GC/MS, their retention time and percentage area.

The results shown in table one indicated that the predominant compounds in *X. aethiopica* are; β -pinene 13.78%, β-phellandrene, 12.36% gama-terpinene 7.66% and α-pinene 5.56%. The oil was rich in monoterpene hydrocarbons which constitutes about 70.9% of the total oil. Two compounds were found to be sesquiterpene and the most abundant is Germacrene-D which is 1.02% of the total oil. Also one diterpene was found in the oil which is 0.52%.

Ekong and Ogan[13], were the first to report on the chemical composition of *X. aethiopica* and several publications have appeared subsequently on this subject. A number of diterpenes from the bark, fruits and pericarp of the plant have been reported,[20]; Labunmi and Pieeru[21]; Harrigan *et al.*,[22]. Ekundayo[23] published a review of the volatiles in a number of Annonaceae species among includes *X. aethiopica* and, reported that they consist mainly of mono and sesquiterpenoids with typical constituents being α- and β-pinene, myrcene, P-cymene, Limonene, Linalool and 1,8-ciniole. Elemol and guaiol (among other terpenes) were found in the essential oil of the fruit from the Republic of Benin[24]

Keita *et al.*,[25] reported the constituents of *X. aethiopica* fromMali to contained β -pinene 19.1%, gama-terpinene 14.7% trans-pinocarveol 8.6% and P-cymene 7.3% as the major constituents, also Nianga *et al.*,[26]

Table 1: Identiied chemical constituents in the steam distilled oil of *Xylopia aethiopica*

| Compound | Retention time (min) | Concentration (%) |
|---|---|---|
| Thuj-2-ene | 15.18 | 0.93 |
| α-pinene | 15.37 | 5.57 |
| β-phalladreme | 16.77 | 12.36 |
| β-pinene | 16.86 | 13.78 |
| P-cymene | 18.51 | 1.33 |
| Eucalyptol | 18.74 | 6.90 |
| D-limonene | 18.84 | 1.02 |
| CIS-ocimene | 19.18 | 1.22 |
| Gama-terpine | 19.86 | 7.66 |
| Terpinolene | 20.89 | 0.59 |
| Linalool | 21.16 | 1.22 |
| Sabinone | 22.46 | 1.02 |
| 1,4-cyclohexadiene,3-ethenyl-1,2-dimethyl - | 22.51 | 3.46 |
| Bicyclo [2.2.1] hepten-3-one,6,6,dimethly1-2-methylene | 22.83 | 1.09 |
| Bicyclo [3.1.1] hept-2-ene-2-carboxaldelhyde-6-6-dimethyl- | 23.84 | 4.10 |
| Bicyclo [3.1.1] hept-2-ene-2-methanol, 6,6-dimethyl - | 24.17 | 6.23 |
| 2-cyclohexene-1-ol,2-methyl-5- (1-methylethenyl)- | 24.81 | 0.59 |
| Propanol, 2-methyl-3-phenyl- | 25.24 | 0.59 |
| 2,6-dimethyl-1,3,5,7-Octatetraene, E | 28.05 | 0.47 |
| Eugenol | 28.76 | 0.79 |
| Germacrene-D | 32.77 | 1.02 |
| 4,4-dimethyl-3(3-methylbut-3-enylidene)-2-methylenebic | 36.29 | 0.64 |
| 1H-Napho (2,1-B) pyan, 3-ethenyldo-decahydro-3,4A, 7,7 | 45.42 | 0.52 |

Table 2: Identified chemical constituents in the steam distilled oil of *Syzgium aromaticum*

| Compound | Retention time (min) | Concentration (%) |
|---|---|---|
| Eugenol | 28.88 | 93.70 |
| Caryophyllene | 31.19 | 2.30 |
| Phenol, 2-methoxyl-4-(1-propenyl)-acetate | 33.16 | 3.90 |

Table 3: Antibacterial activity of the essential oils of *Xylopia aethiopica and Syzgium aromaticum*

| | Inhibition | |
|---|---|---|
| Microorganism | Xylopia aethiopica | Syzgium aromaticum |
| *Escherichia* coli | + | ++ |
| Serratia | + | ++ |
| *Salmonella* typhi | + | ++ |
| *Klebsiella* sp. | + | ++ |
| Citrobacter | + | ++ |
| Pseudomonas aeruginosa | + | ++ |

+ = active

reported the constituents of two samples of this plant from Guinea to contained beta pinene 36.2-40.2% α-pinene 13.6-15.4% and sabinene 7.1-7.3%. In he Republic of Benin, Ayedoun *et al.*,[24] revealed that sabinene 36.0%; 1, 8-cineole 12.8%, Linalool 3.9% and terpinen-4-ol 7.0% were the Major compounds in the oil.

Our results showed that the common form of the oil investigated from *X. aethiopica* is different from those from Benin, although they have some constituents in common but the major components found in Benin is different from our results. Similarly the results of oil from Guinea and Mali and our results shows that they all have β-pinene, gama-terpinene and α-pinene as their major monoterpene but their percentages in these regions varies. These variations may be probably due to; geographical origin[27], genetic factors, cultural conditions and environment[28], crop and post crop processing[29] and different chemotypes and nutritional status of the plant as well as other factors that can influence the oil composition.

The oil yield of *S. aromaticum* was 7.4% after 5 h hydrodistillation. Three constituents representing 99.9% of the total oil were conclusively identified with Eugenol having 93.7%, acetate of eugenol, 3.9% and caryophyllene, 2.3%. Raina *et al.*,[30] revealed that dried leaves of this plant from Indian Island of little Andaman gave a yield of 4.8% oil on hydrodistillation and sixteen compounds were obtained with eugenol 94.4% and β-caryophyllene 2.9% as the major components.

Results in Table 3 shows the activity of the essential oils against some pathogenic organisms. The activity is concentration dependent, i.e., the higher the concentration the higher the activity. The activity of *S. aromaticum* against the six organisms used was higher than that of *X. aethiopica*. The activity of the oils would be expected to relate to the respective composition of the plant volatile oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possibly synergistic interactions between components.

The components with phenolic structures such as carvacrol, eugenol and thymol, were known to be highly active against microorganism, members of this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used[31]. The high percentage of eugenol in *S. arometicum* 93.7% than *X. aethiopica* 0.78% shows that the activity is concentration dependent in agreement with Dorman and Dean[9]. The importance of the hydroxyl group in phenolic structure, the presence of an acetate moiety in the structure, aldehyde group stereochemistry had been shown to have some influence in bioactivity[32-34]. All these properties were found in the oil and may be responsible for its antibacterial activity.

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