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Chemical Composition and Antibacterial Activity of Steam Distilled Oil of Ashanti Pepper (*Piper guineense*) Fruits (Berries)

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Abstract: The composition of the volatile oil from the fruits (berries) of Ashanti pepper (*Piper guineense*) was investigated using gas chromatography-mass spectrometry techniques. Out of 29 peaks (representing 99.2% of total oil), 27 components were identified representing 96.40% of the total oil composition. The major components were (1s)-(-1)-β-pinene (43.9%), D-Limonene (7.7%), caryophyllene (6.9%), car-2-ene (5.4%) and 1,6,10-dodecetrien-z-ol, 3, 7, 11-trimetyl (2.9%). The oil was also screened for its antibacterial activity against *Escherichia coli*, *Serratia*, *Salmonella typhi*, *Klebsiella* sp., *Citrobacter* and *Pseudomonas aeruginosa*. It was found out that it exhibited no activity against any of these organisms.

Key words: Volatile oil, Ashanti pepper, antibacterial, organism

INTRODUCTION

Ashanti or West Africa black pepper (*Piper guineense* Schum and Thom) is a herbaceous climber commonly found in the African tropical forest zones. The fruits or berries and leaves are usually sold in Nigerian markets as condiments and for food flavouring. The fruits are widely used in Nigerian traditional medicine as a remedy for rheumatism and bronchitis (Onyenekwe *et al*., 1997). *P. guineense* belongs to the group of pepers called false cubebs and the berries are often used as substitutes for the edible black pepper (*Piper nigrum*). Some degree of nutritive value was earlier associated with the plant in Nigeria (Mwinuka *et al*., 2005). *P. guineense* berries, like other types of pepper, yield an aromatic essential oil on steam distillation. Nothing is know about the chemical composition of the volatile oil of this plant in Nigeria until 1988 when Ekundayo *et al.* (1988) first reported chemical composition of this plant. It was reported that phenylpropanoid-rich essential oil was comprised of myristicin, sarisan, safrone and elemicin as a dominant components and fifty-one mono and sesquiterpenoids as a minor component, also preliminary toxicity of the volatile oil was briefly evaluated.

Report have it that under prevailing production and handling conditions, most spices, including Ashanti pepper, herbs and other vegetable seasonings contain a large number of micro-organisms capable of causing spoilage or more often human disease (Uzuegbu and Emifoniye, 1984; Obuekwe and Ogbimi, 1989). On this basis, Onyenekwe *et al*. (1997) carried out chemical constituents of the volatile oil of *P. guineense* berries using gamma radiation of different dosages to remove the natural microflora without adversely affected the flavour quantities. In his report forty-five compounds were identified with β,-caryophellene, germacrene-D, α-ylangene, Limonene and Myrcene as the dominant constituents.

Since geographical factor (Lawrence, 1988), genetic factors, culture conditions and environment (Charles and Simon, 1990), crop and post crop processing (Paakkonen *et al*., 1990) and different chemotypes and nutritional status of the plant as well as other factors that can influence the oil composition. Therefore, in the present study, we have investigated the chemical composition and antibacterial activity of *P. guineense* found in Ekiti State in comparism with the one found in Imo State as reported by Onyenekwe *et al.* (1997) and the one found in Oyo State all in Nigeria as reported by Ekundayo *et al.* (1988).

MATERIALS AND METHODS

Plant materials: Ripe fruits (berries) of *P. guineense* were collected from Isinbode, Ekiti State, Nigeria.

Recovery of essential oil: The dried fruits were grounded with mortal and pistle in the laboratory and 150 g were subjected to hydrodistillatron according to AOAC (1980) using the clevenger apparatus for 5 h. The oil obtained were dried over anhydrons 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 carried out using 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: 50 m×0.25 mm i. d×0.1 μm; Helium was used as carrier gas at 1.3 mL mm^{-1} flow rate; oven temperature 30°C (hold 5 min) to 200 at 8°C min^{-1} then to 320 at 6°C min^{-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 (Smith *et al*., 2002). Fifty percent 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. 1×10^{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 microorganism; *Escherichia coli*, *Serratia*, *Salmonella typhi*, *Klebsiella* sp., *Citrobacter* and *Pseudomonas aeruginosa.*

RESULTS AND DISCUSSION

After five hour of hydro distillation, the essential oil yield was 0.83% v/w. Out of 29 peaks, representing 99.2% of total oil, twenty seven components were identified, this represent 96.4% of the total oil component. Table 1 shows the constituents identified by GC/MS, their retention time and percentage area.

The oil was rich in monoterpene hydrocarbons which constitute about 73.3% of the total oil with (1s)-(-1)- pinene 45.9%, D- limonene 7.7% and -car-2-ene 5.4% as the major monoterpene and also sabinene, p-cymene and trans-beta-ocimene as the minor monoterpene hydrocarbons. Onyenekwe *et al.* (1997) reported that myrcene, limonene and β-pinene were present as the major monoterpene hydrocarbon. Furthermore, 19.4% was found to be present as sesquiterpene hydrocarbons with caryophyllene, 6.9% β-sesquiphellandrene, 2.6% and (+) -epi-bicydosesqui-phellandrene as the major components of sequiterpene hydrocarbon. β-caryophyllene, germacrene and himachelene were reported to be the major sesquiterpene hydrocarbon present by Onyenekwe *et al.* (1997). There were only two oxygenated terpenes which accounted for 3.4% of the total oil i.e., 3-ally-6-methoxyl phlenol, 0.5% as monoterpenoid and 1, 6, 10-do decatrien-3-ol, 3, 7, 11-trimethoyl, 2.9% as sesquiterpenoid. No diterpene has been found so far from the essential oil composition of this plant. The result showed that there were variations in the chemical composition of this plant as the components varied, some compound were present as found in Imo State and not found in Ekiti State and Oyo State vice versa but some of the main components like caryophylene, pinene and limonene were still found as one of the major components of the oil in both regions.

Table 2 shows that the oil showed no antibacterial activity on various microorganisms tested. It has been reported that Ashanti pepper can be contaminated with microorganisms and this is capable of causing spoilage or

Table 1: Identified chemical constituents in the steam distilled oil of *P. guineense*

S/No.	Compound	Retention time (Min)	Concentration (%)
1	α-Pinene	15.36	2.3
2	Sabinene	16.76	0.5
3	β-Pinene	16.84	6.7
4	β-Myccene	17.53	0.8
5	β-Phellandrene	17.91	0.9
6	Car-2-ene	18.18	5.4
7	Para-cymene	18.50	0.6
8	D-Limonene	18.81	7.7
9	CIS-Ocimene	19.16	1.7
10	Tran-beta-ocimene	19.54	0.5
11	(1s)-(-1)-Pinene	21.17	43.9
12	γ-terpinene	23.60	1.8
13	Limonene	23.97	0.6
14	3-ally-6-methoxyl phenol	28.75	0.5
15	Copaene	30.04	0.8
16	Cyclohexane, 1-ethyl-1-methyl-2, 4-bis (1-methyl-ethenyl).	30.40	1.3
17	Caryophyllene	31.17	6.9
18	α-humulene	32.05	1.4
19	α-amorphene	32.64	0.7
20	(+)-epi-bicyclosesquiphellandrene	32.76	1.9
21	Naphthalene, Decahydro-4 A methyl-1-methylene-7-(1-methyle)	32.90	1.5
22	β-sesquiphellandrene	33.86	2.6
23	β-elemene	34.32	0.7
24	1.6,10-dodecatrien-3-ol, 3,7,11-trimethyl.	34.76	2.9
25	D1-epi-α-cedrene	35.42	0.6
26	Trans-α-bisabolene	35.81	0.5
27	9, 10-dihydro-isolongifolene	36.28	0.5

Table 2: Antibacterial activity of the essential oil of *Piper guineense*

Microorganism	Inhibition zone
Escherichia coli	NA
Serratia	NA
Salmonella typhi	NA
Klebsiella spp.	NA
Citronacter	NA
Pseudomona aeruginosa	NA

NA = No Activity

more often human diseases (Uzuegbu and Emifoniye, 1984; Obuekwe and Ogbimi, 1989). Present finding is in agreement with the study of these researchers. Dorman and Deans, (2000) showed that composition of the plant volatile oils, the structure configuration of the constituents, their functional group and the relative percentage composition in the plant oils has effect on the antimicrobial activity on the organism. It is a known fact that components with phenolic structures such as carvacrol, eugenol and thymol, were highly active against microorganism, they are known to be either bactericidal or bacteriostatic agent depend on the concentration (Pelczar *et al*., 1988). None of these phenolic compounds were found in the oil, the only phenolic component found was 3-ally- 6-methoxyl phenol and its concentration was so small about 0.5% and this may hinder its effect.

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REFERENCES

AOAC, 1980. Official Methods of Analysis 13th Edn. Association of Official Analytical Chemists, Washington, DC.

Charles, D.J. and J.E. Simon, 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. J. Am. Hortic. Sci., 115: 458-462.

Dorman, H.J.D. and S.G. Deans, 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Applied Microbiol., 88: 308-313.

Ekundayo, O., I. Laakso, R. Adegbola, B.M. Oguntimein, A. Sofowora and H. Raimo, 1988. Essential oil constituents of ashanti pepper (*Piper guineense*) fruits (Berries). J. Agric. Food Chem., 36: 880-882.

Federal Institute for Geosciences and Natural Resources, Hanover, Germany.

Lawrence, B.M., 1988. In: Developments in Food Sciences Flavours and Fragrances: A World Perspective. Lawrence, B.M., B.D. Mookheyee, B.J. Willis (Eds.). Elseview: Amsterdam.

Mwinuka, N.M., G.O. Ibeh and G.I. Ekeke, 2005. Proximate composition and level of some toxicants in four commonly used spices. J. Applied Sci. Environ. Manage., 9: 150-155.

Obuekwe, C.O. and A.O. Ogbimi, 1989. Prevalence of *Bacillus cereus* and some other gram-positive bacteria in Nigerian dried food condiments. Nigeria Food J., 7: 11-19.

Onyenekwe, P.C., G.H. Ogbadu and S. Hashimoto, 1997. The effect of gamma radiation on the microflora and essential oil of Ashanti Pepper (*Pipper guineense*) berries. Posthavest Biol. Technol., 10: 161-167.

Paakkonen, K., T. Malmsten and L. Hyvonen, 1990. Drying, packaging and storage effects on quality of basil, marjoram and wild marjoram. J. Food Sci., 55: 1373-1382.

Pelczar, M.J., Chan and E.C.S. Krieg, 1988. Control Microorganisms, the Control of Microorganisms by physical agents. In: Microbiology. New York: McGraw-Hill International, pp: 469-509.

Smith, S.I., K.S. Oyedeji, B. Opere, B.A. Iwalokun and E.A. Omonigbehin, 2002. The effect of some Nigerian Herbs on *Helicobacter pylori*. Afr. J. Clin. Exp. Microbiol., 3: 10-14.

Uzuegbu, J.O. and A.T. Emifoniye, 1984. Postharvest fungal spoilage of some Nigeria fruits and vegetables. Nig. Food J., 2: 153-155.