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Chemical profile, antibacterial, antioxidant and insecticidal activities of essential oils of Nigerian-grown *Ananas comosus* (L) Merr

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Abstract

Ananas comosus is a popular fruit used as a medicine in several native cultures for the treatment of typhoid fever, strangury, helminthiasis, jaundice and for boosting male fertility. Essential oils were extracted from the fruit, peel and shoot of Nigerian-grown *A. comosus* using hydrodistillation method and analyzed using GC-FID and GC-MS techniques. Nine, forty-four and thirty-five constituents accounting for 88.76, 66.08 and 91.51% were identified in the fruits, peel, and shoot oils respectively. The oils had a high percentage of non-terpenes which were made up of esters, fatty acids, alcohols and aldehydes. Major constituents of the fruit, peel and shoot oils were p-xylene (62.43 %), tetradecanoic acid (8.63 %) and p-xylene (29.89 %), respectively. The essential oils were evaluated for their antibacterial, antioxidant and insecticidal activities using Alamar Blue Assay, diphenyl picrylhydrazyl (DPPH) radical scavenging activity and contact toxicity test, respectively. The oils displayed moderate antibacterial potentials to some tested organisms and low radical scavenging activity to DPPH. The insecticidal activity of the peel oil revealed 20% mortality against *Rhyzopertha dominica* and *Callosbruchus analis.* The result from the study is indicative of potential of *A. comosus* essential oil as source of pharmaceuticals.

Keyword. *Ananas comosus*, Essential oil, GC-MS, Alamar Blue Assay, Biological activity

Introduction

Pineapple (*Ananas comosus* (L) Merr), 'the king of fruit' because of its crown of leaves, is the most economically important plant in the Bromeliaceae family [1]. It is an herbaceous perennial which grows to 1.0 to 1.5 meters (3.3 to 4.9 ft) tall, although sometimes it can be taller [2]. The fruit of a pineapple is arranged in two interlocking helices, eight in one direction, thirteen in the other, each being a Fibonacci number [3].

Pineapple has been used as a medicinal plant in several native cultures. It may be consumed fresh, canned, juiced, and are found in a wide array of food stuffs, dessert, fruit salad, jam, yogurt, ice cream, candy, and as a complement to meat dishes. In addition to consumption, in the Philippines the pineapple's leaves are used as the source of a textile fiber called piña, and is employed as a component of wall paper and furnishings, amongst other uses [1,4].

The whole plant is used to treat typhoid fever in Ogun State of Nigeria [5]. Roasted unripe fruit juice is used in India for strangury [6]. The *Garo* tribal community of Netrakona district in Bangladesh uses the fruit juice for fever and leaf juice for helminthiasis and jaundice [7]. The root and fruit are either eaten or applied topically as an anti-inflammatory, digestive and proteolytic agent [8,9]. Pineapple is currently being studied for its effectiveness in preventing heart disease [9]. Pineapple juice has high manganese content and this makes it a good choice for boosting male fertility [10].

More than 280 volatile compounds had been identified in pineapple, whereas only a few volatiles contribute to the aroma of pineapple [11]. It has been reported that esters were the most abundant volatiles in

pineapple, in particular, ethyl hexanoate and methyl hexanoate. [12,13,14,15,16,17,18,19,20]. However, He *et al.*, (2007) reported that hydrocarbons and esters were the main compounds, which could be explained by differences in cultivars, growing conditions, and volatiles extraction methods [21]. Such differences could also justify why methyl butanoate and methyl 2-methylbutanoate were not found in 'Smooth Cayenne' pineapple, despite being the main abundant components in other studies [20]. On the other hand, Berger (1991) reported two minor hydrocarbon compounds, 1-(*E,Z*)-3,5-undecatriene and 1-(*E,Z,Z*)-3,5,8-undecatetraene as the important contributors to fresh-cut pineapple aroma due to their low odor threshold values [22]. Also the results of Takeoka *et al.* (1991) reported many sulfur-containing esters among pineapple volatiles [23].

Currently there is an increasing interest in research related to the aroma volatiles of fruits all around the World, especially in China [24,25,26,27]. For pineapple, volatile compounds of different varieties [27,28], different crop areas [14], different ripening stages [29], fruit development [27,30], storage conditions [30] and flesh position of Gold cultivar [18] (top, middle, and bottom cross-sections along the central axis of the fruit) have been studied recently, but no research work has been done on the volatile components of pineapple from Nigeria.

This study evaluates the chemical constituents, antibacterial and antioxidant activities of essential oil from the peel, shoot and fruit-pulp of Nigerian-grown pineapple.

Materials and methods

Fruit sample

The *Ananas comosus* fruit was purchased from Oje market in Ibadan, Oyo State, Nigeria and authenticated at the Herbarium of Forest Research Institute of Nigeria (FRIN), Ibadan by Mr. Adeyemo, Voucher specimens were duly deposited in the FRIN herbarium (voucher number FHI110498). The fruit peel was separated from the shoot by peeling. The peel and shoot were air dried for two weeks and subsequently pulverized while the fruit was mashed.

Essential Oil Isolation

The mashed fresh fruit, dried peel and shoot of the *A. comosus* were separately subjected to hydrodistillation in an all glass Clevenger apparatus for 4 hrs in accordance with British Pharmacopoeia Method (1980) [31]. The yellowish volatile oils obtained were dried over anhydrous sodium sulphate (Na_2SO_4) and stored at 4 ^oC to prevent loss of oil by volatilization until analyses.

Analyses of the essential oils

Gas Chromatography (GC): The oils were analyzed on an Agilent Model 7890A Gas Chromatography equipped with a HP-5MS fused silica capillary column (30 x 0.25 mm, 0.25 μm film thickness). Analytical conditions were: Oven temperature: 60 ^oC (held for with 2 min), and heated to 280 ^oC at 4 ^oC/min, with final hold time of 10 mins; helium was used as carrier gas at a flow rate of 1 mL/min. Retention indices were determined with reference to a homologous series of normal alkanes analyzed under the same conditions. Percentage composition of each constituent was calculated by electronic integration of the GC peak areas.

Gas Chromatography-Mass Spectrometry (GC-MS): GC-MS analyses were performed on an Agilent Model 7890A Gas Chromatography interfaced to an Agilent 7000 GC/MS Triple Quad. The temperature program used for the GC was the same as described above. The MS was operated in EI mode with ionization voltage 70 eV and ion source temperature, 250 ^oC.

Components identification

The components of the essential oil were identified on the basis of their retention indices. Confirmation of the identified constituents was by comparison of their mass spectra with published spectra [32,33] and those of reference compounds from the Library of National Institute of Standard and Technology (NIST) database (2011) [34].

Antibacterial screening

The essential oils were screened for antibacterial activities against six (6) standard strains of bacteria representing Gram +ve and Gram –ve (*Staphylococcus aureus* (NCTC 6571), *Bacillus subtilis*, *Escherichia coli* (ATCC 2592), *Pseudomonas aeruginosa* (NCTC 10662), *Shigella flexenari* (ATCC 12022) and *Salmonella typhi* (ATCC 700931)). Microplate Alamar Blue Assay was used to determine susceptibility or resistance of the essential oils to selected bacteria strains. Organisms were grown in Mueller Hinton medium and inoculums were adjusted to 0.5 McFarland turbidity index. Stock solutions of the essential oils were prepared in DMSO (1:1 concentration). Media was dispensed to all wells. Essential oils were added to the wells. Essential oils were not added to the well that serves as control. The volume of 96-well plate was made up to 200 µL. Finally $5x10^6$ cells were added in all wells including both control and test. The plate was sealed with paraffin and incubated at 37 ^oC for 18 - 20 hours. Alamar Blue Dye was dispensed in each

well and shaken at 80 rpm in a shaking incubator for 2 – 3 hours. Plates were covered with foil in shaking incubator. Change in color of Alamar Blue dye from blue to pink indicated the growth in bacterial strains. Absorbance was recorded at 570 nm and 600 nm by the ELISA reader (SpectraMax M2, Molecular Devices, CA, USA). All experiments were done in triplicate.

Antioxidant activity: DPPH radical scavenging activity

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of a methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) using the reported method of Yamaguchi *et al.* (1998) [35]. DPPH (*Wako Chemicals* USA, Inc.) solution in methanol was prepared to make 0.3mM. One milliliter of essential oil was added to 1 mL of the 0.3 mM DPPH solution shaken vigorously. The reaction was allowed to progress for 30 min at 37 ^{o}C in the dark and absorbance monitored by multiplate reader, SpectraMax340, Molecular Devices, CA, USA at 517 nm. Upon reduction, the color of the solution fades (Violet to pale yellow). Absolute methanol was used as control.

The activity was determined as a function of the % Radical Scavenging Activity which was calculated using the formula;

$$\% \text{ Radical Scavenging Activity} = \frac{Ac - As}{Ac} \times 100$$

Where: A_C = Absorbance of the control
A_S = Absorbance of the sample

Insecticidal activity

The insecticidal activity was conducted according to the impregnated filter paper method (contact toxicity) as described by Tabassum *et al.* (1997) [36] using three insects; *Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis*.

The filter papers were cut according to the size of glass petri plates (9 cm or 90 mm) and put in the plates. Essential oils were loaded over the filter paper in the plates with the help of micropipette. Ten healthy and active insects of same size and age of each species were put in each plate (test and control) with the help of a clean brush. The plates were incubated at 27 ^{o}C for 24 hours with 50% relative humidity in growth chamber. The survival of the insects was assessed (count the number of survivals of each species).

The Percentage Inhibition or Percentage Mortality was calculated using the formula below:

$$\text{Percentage Mortality} = 100 - \frac{No.of\ insects\ alive\ in\ test}{No.of\ insects\ alive\ in\ control} \times 100$$

Test Control: Positive control contained standard insecticide (Permethrin) at the concentration which is effective against all test insects and test insects. Negative control contained volatile solvent (methanol) and the test insects.

Results and discussion

The essential oils from *Ananas comosus* were yellowish in colour with percentage yield of 0.15, 0.21 and 0.12 for the fruit, peel and shoot, respectively. A total of 9, 44 and 35 constituents, which made up 88.76%, 66.08% and 91.51% of the essential oils were identified in the fruit, peel and shoot, respectively (Table 1).

The major constituents identified in the fruit essential oil were non-terpene compounds; p-xylene (62.43%), ethylbenzene (12.3%) and decane (3.87%). α-Pinene (3.18%) and psi-cumene (2.27%) were the monoterpenes present in the essential oil.

The essential oil of the peel had fatty acids as its major compounds comprising tetradecanoic acid (8.634%), dodecanoic acid (7.77%), 9-octadecenoic acid (2.2%) and 9, 12-octadecadienoic acid (1.96%). The sesquiterpene hydrocarbons and oxygenated compounds constitute about 33% of the identified compounds with the predominance of α-copaene (4.2%), α-muurolene (2.63%), (-) aristolene (2.1%) and (+)sativene (1.18%). Esters which were previously found to be the odor active compounds of pineapple [14-20] were present in the peel oil; methyl nonanoate (0.8%), ethyl trans-4-decenoate (0.62%) and ethyl decanoate (0.26%). An unusual triterpene; squalene (1.01%) was also identified in the oil.

The dominant compounds in the essential oil of the pineapple shoot comprised of non-terpenes (hydrocarbons and fatty acid), p-xylene (29.89%), ethylbenzene (7.64%) and hexadecanoic acid (6.26%). The monoterpenes present were hemimelitene (3.01%), 1,8-cineole (2.13%), α-terpineol (0.72%), cumene (0.6%) and D-limonene (0.5%). Sesquiterpenes make up 4.46% of the total identified components. Ledol (1.62%) and β-caryophyllene (0.82%) constitute most of the sesquiterpenes. Three of the thirty-five compounds identified in the pineapple shoot essential oil are esters which were made up of ethylhexadecanoate (2.5%), methylnonanoate (2.39%) and isopropyl-12-methyldecanoate (0.73%).

The essential oil from the peel had the lowest percentage of monoterpenes (0.84%) and highest percentage of sesquiterpenes (20.87%). Diterpenes are present in only the shoot oil while the peel and shoot oil had triterpene (squalene) present in them

at 1.01 and 4.01%. The oils had a high percentage of non-terpenes which were made up of esters, fatty acids, alcohols and aldehydes.

Although ethylhexanoate is an important pineapple fruit aroma compound [17,19], it was not present in all the studied oils. Some other esters were however observed in the peel (methylnonanoate, ethyldecanoate and ethyl trans-4-decenoate) and the shoot oil (methylnonanoate and ethylhexadecanoate). Earlier reports [14,15,16,17,18,19,20] that esters were the major volatile compounds in pineapple volatile composition was not in agreement with the result from this study, however, the report by He *et al.* (2007) [21] that hydrocarbons and esters were the main compounds agrees to an extent with this study. The differences in chemical composition could however be explained by differences in cultivars, growing conditions and volatiles extraction methods [20]. The high percentage of xylene in the oils could be attributed to the growing condition of the fruit which could be as a result of the proximity of farm to sources of petrochemical fumes or deposition of pesticide by-products.

The compositional pattern of the essential oils from the shoot, peel and fruit-pulp of *A. comosus* from Nigeria are being reported for the first time to the best of our knowledge.

Table **2** presents the results of the antibacterial test of the essential oil from *Ananas comosus* fruit, peel and shoot on the six standard strains of bacteria. The study revealed that *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* strains were resistant to all the oils. *Shigella flexenari* was susceptible to the fruit, peel and shoots with 39.21 %, 20.56 % and 22.74 % inhibition respectively. The peel and shoot oils were also active against *Staphylococcus aureus* strain with 14.97 % and 11.49 % inhibition while *Bacillus subtilis* strain was susceptible to only the fruit oil with 7.36 % inhibition. The percentage inhibition of the oils against all the bacteria strains was low compared to the standard, ampicillin, used as positive control. The notable antimicrobial variations between the oils may be attributed to the fact that the biological activity of an essential oil is linked to its chemical composition and at times to the major chemical constituents [37,38]. Some researchers have reported that a synergistic effect between the minor and major components in essential oils contributes to the antibacterial activity [39,40,41,42].

The three (3) essential oil samples were screened using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The antioxidant activity of the volatile oils was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH. The percentage radical scavenging ability (% RSA) of the volatile oils were calculated based on the absorbance measurement as shown in Table **3**. The essential oil of *A. comosus* shoot, peel and fruit has %RSA values of 8.45, 8.4, and 12.09 respectively. Gallic acid and n-acetyl cystein were used as standards and had 93.13, 95.95 % RSA respectively. The % RSA of the standards used for the study were however high compared to the samples.

The observed low % RSA of the essential oils can be explained by the fact that the oils are not capable of donating hydrogen atom and the low solubility provided by the oils in the reaction medium of the assay because this test utilizes methanol or ethanol as solvent as explained by a report by Mata *et al.* (2007) [43]. Viuda-Martos *et al.* (2009) also cited these factors as the main limitation of this assay for measuring antioxidant activity of lipophilic samples like many essential oils [44].

Ananas comosus peel showed 20% mortality against *Rhyzopertha dominica* and exhibited 20% mortality of *Callosbruchus analis.* The insects were observed to be resistant to the oils used for this study based on the impregnated filter paper method used which is a form of contact toxicity. In contact toxicity stomach poisoning occurs while the insects feed on the whole grains. The weevils have to pick up the lethal dose of treatment from the essential oil to cause toxicity.

Previous studies have shown that the toxicity of essential oils obtained from aromatic plants against storage pests is related to the oil's main components [45]. The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids [46,47,48,49]. Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions [50]. Due to their high volatility, they are fumigant and gaseous and might be of importance for stored-product insects [47]. Various monoterpenes like 1,8-cineole, linalool, α-pinene, terpinen-4-ol, and α-terpinene have been reported to show contact and fumigation toxicity to stored product pests [51,52]. Therefore, the resistance of the essential oils studied for insecticidal activity may be related to the non-dominance of monoterpenes in the identified components in the oils.

The synergistic action between major and minor components of essential oils could also be responsible for the repellent action of the oils to the insects. Plant essential oils are mixtures of different major and minor components and their biological activity is generally determined by their major components or synergism/antagonism among different components [53,54].

Table 1. Essential Oil Components of *Ananas comosus* Fruit, Peel and Shoot

S/N	Compound Name	RI	ACF	ACP	ACSH
1	Ethylbenzene	893	12.3	0.82	7.64
2	p-Xylene	907	62.43	3.09	29.89
3	Nonane	916	1.71	0.34	0.79
4	α –Pinene	948	3.18	-	-
5	S-3-Carene	948	-	0.27	-
6	Cumene	992	-	-	0.6
7	Octanal	1005	-	-	0.4
8	m-Ethyltoluene	1006	-	0.51	2.21
9	Decane	1015	3.87	0.58	1.69
10	D-Limonene	1018	-	0.57	0.5
11	Hemimelitene	1020	2.27	-	3.01
12	α-Terpinolene	1052	-	-	0.52
13	1-Octanal	1059	-	-	0.41
14	1,8-Cineole	1059	-	-	2.13
15	Trans-2-Nonenal	1112	-	0.4	-
16	1,3,5,8-Undecatetraene	1129	-	0.45	-
17	α-Terpineol	1143	-	-	0.72
18	2-methyldecahydronaphthalene	1162	0.99	-	-
19	Methylnonanoate	1183	-	0.8	2.39
20	Dihydrocarveol	1196	0.87	-	-
21	Decanal	1204	-	0.5	0.73
22	(-)α-Copaene	1221	-	4.2	-
23	2-Undecane	1251	-	0.39	-
24	Isoaromadendrene Epoxide	1281	-	0.65	-
25	(+)Sativene	1339	-	1.18	-
26	Ethyldecanoate	1381	-	0.26	-
27	Ethyltrans-4-Decenoate	1389	-	0.62	-
28	p-Eugenol	1392	-	0.24	-
29	(-)β-Elemene	1398	-	0.34	-
30	(-)Aristolene	1403	-	2.1	-
31	Tetradecane	1413	-	0.51	0.38
32	(-)α-Gurjunene	1419	-	0.41	-
33	Geranyl Acetone	1420	-	0.36	0.48
34	γ-Muurolene	1435	-	0.78	-
35	α-Muurolene	1440	-	2.63	-
36	(+)δ-Cadinene	1469	-	1.04	-
37	δ-Guaiene	1490	-	0.8	-
38	α-Himachalene	1494	-	0.99	-
39	β-Caryophyllene	1494	-	-	0.82
40	β-Guaiene	1523	-	1.06	-
41	(+)Ledol	1530	-	-	1.62
42	Epiglobulol	1530	-	2.18	0.71
43	Globulol	1530	-	1.04	-
44	Dodecanoic Acid	1570	-	7.77	-
45	Geranylisovalerate	1583	-	0.49	-
46	Caryophyllene oxide	1599	-	0.38	-
47	Tetradecanal	1601	-	-	0.58
48	β-Bisabolol	1619	-	-	0.45
49	Isopropyl-12-methyltridecanoate	1750	-	-	0.73
50	Tetradecanoic Acid	1769	-	8.63	1.01
51	Tetradecenoic Acid	1777	-	0.78	-
52	Farnesol acetate	1834	-	0.91	-
53	Hexadecanol	1854	-	-	4
54	Pentadecanoic Acid	1869	-	-	0.58
55	Nonadecane	1910	1.14	-	0.68
56	Nonanal	1910	-	2.5	4.73
57	Hexadecanoic Acid	1968	-	1.11	6.26
58	Ethylhexadecanoate	1978	-	-	2.51
59	γ-Palmitoacetone	1980	-	5.57	-
60	Octadecanol	1999	-	2.66	3.02
61	Trans Phytol	2045	-	-	1.79
62	Heneicosane	2109	-	-	1.63
63	9-Octadecenoic Acid	2175	-	2.2	-
64	9,12-Octadecadienoic Acid	2183	-	1.96	-
65	Heptacosane	2705	-	-	1.89
66	Squalene	2914	-	1.01	4.01
	Total		88.76	66.08	91.51
	No. of Compounds		9	44	35
	Monoterpenes		6.32	0.84	7.48
	Sesquiterpenes		-	20.87	4.08
	Diterpenes		-	-	1.79
	Triterpenes		-	1.01	4.01
	Apocarotenes		-	0.91	-
	Non-terpenes		82.44	42.45	74.15

Key: ACF- *Ananas comosus* Fruit, ACP- *Ananas comosus* Peel, ACSH- *Ananas comosus* Shoot RI- Retention Index

Table 2. Percentage Inhibition of Essential Oils of *A. comosus* Plant Parts

Name of Bacteria	Percent (%) Inhibition			
	Drug	ACF	ACP	ACSH
Escherichia coli	72.00	0.00	0.00	0.00
Bacillus subtilis	76.00	7.36	0.00	0.00
Shigella flexenari	65.00	39.21	20.56	22.74
Staphylococcus aureus	79.00	0.00	14.97	11.49
Pseudomonas aeruginosa	80..00	0.00	0.00	0.00
Salmonella typhi	70.00	0.00	0.00	0.00

Key. Drug- Ampicillin, ACF- *A. comosus* Fruit, ACP- *A. comosus* Peel, ACSH- *A. comosus* Shoot

Table 3. Percentage Radical Scavenging Activity of Essential Oils of *A. comosus* Plant Parts

Plant Material		% Radical Scavenging Activity
ACSH		8.45
ACP		8.40
ACF		12.09
Standard	Gallic Acid	93.13
	n-ACETYL CYSTEIN	95.95

Key: ACF- *A. comosus* Fruit, ACP- *A. comosus* Peel, ACSH- *A. comosus* Shoot

Conclusion

This study has shown the composition of the essential oils of Nigerian-grown *Ananas comosus* which comprise of plethora of compounds. The oils had a high percentage of non-terpenes which were made up of esters, fatty acids, alcohols and aldehydes. Major constituents of the oils were p-xylene, tetradecanoic acid, dodecanoic acid, ethylbenzene, hexadecanoic acid and α-copaene. The essential oils were further screened for their antibacterial, antioxidant and insecticidal activities. The volatile oils displayed moderate antibacterial potentials to some tested organisms, low radical scavenging activity to DPPH and the evaluated insecticidal activity of the peel oil revealed 20% mortality against *Rhyzopertha dominica* and *Callosbruchus analis.* The results indicate potentials of *A. comosus* as source of pharmaceuticals.

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