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Spectraland Antimicrobial Studies of Alligator Pepper Oil

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Abstract: *The present study is aimed at exploring the spectral and antimicrobial properties of oil extracted from the seeds of alligator pepper (Aframomum melegueta). The spectral properties (FTIR, ^{1}H, ^{13}C and DEPT-135 NMR) of the oven dried and stripped oil extracted from seeds of alligator pepper seeds werestudied. The FTIR, ^{1}H, ^{13}C and DEPT-135 NMR spectra indicates that the major constituents of the oil were triacylglycerols (TAGS) containing unsaturated acyl chains. Prominent peaks in the FTIR include: 3517.98 (carbonyl overtone), 2924.49(CH), 1748.50 (C-O of carbonyl ester) and 1604.54 (C-C cis). The major signals in the proton NMR spectrum were observed between 0.80 and 5.5 ppm. The signals due to allylic (1.961,2.067), bis-allylic (2.65, 2.75, 2.78), vinyl (5.42- and the glyceryl (4.07-4.29 and 5.25-5.30) protons indicate the presence of TAGs in the oil. The ^{13}C NMR and DEPT-135 signals further authenticate the signals of the 1H NMR. Results of the antimicrobial study indicate that the oil is active against three of the studiedmicroorganisms, Proteus mirabilis, Staphylococcus aureus and Escherichia coli but fail to show activity against Streptococcus pneumoniae. The activities were measured by the zones of inhibitions which vary between 1.02 and 3.00 mm.*

Keywords:*Alligator pepper oil, Antimicrobial, FTIR, 1H, ^{13}C NMR Spectra.*

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I. Introduction

Alligator pepper (Zingiberceae *Aframomum melegueta*) is an herbaceous perennial of the ginger family of flowering plants. It is native to swampy habitats along the West African Coast. The fruits appear as pods which contain the seeds. The plant derived its common English name from the peppery nature of its seeds and the bumpy nature of the seeds with the skin reminiscent of an alligator back. According to Sofowora (1993) the seeds have both nutritive and medicinal value. The seeds are used as spice and ingredients in medicinal preparations (Oyegade, *et al*., 1999). The pungency, colour and flavour enhances food taste and in brewing. The seeds are also used for the treatment of infectious diseases both veterinary and traditional medicineand results obtained show that the seeds can be exploited for the development of antimicrobials (Galal, 1996). According to Alaje,*et. al*., (2014) the seeds are rich in various minerals and phytochemicals. This further confirms the nutritional and medicinal importance of the alligator pepper. Chiejina, et. al., (2012) reported antifungal effects of the ethanol extracts of the seeds against fungal diseases of tomato fruits.Doherty, *et. al*., (2010) used ethanol and aqueous extracts of the seeds against various microbes, the ethanol extract being more active. Okwu (2004) demonstrated that ethanol extracts of the seeds showed both antibacterial and antiseptic properties and have been used in the treatment of wounds and prevention of infections. Okoi, *et.al*., (2013) studied the bioactive and antifungal effects of the ethanol extracts of the seeds of alligator pepper and *Ocimum grassitimum* on causative agents of post-harvest decay of carrots. The authors used the ethanol extracts of the seeds of alligator pepper and leaves of *Ocimum grassitimum* on four microbial pathogens: *Rhizopus stolonifera*, *Fusarium culmorum*, *Sclerotinia sclerotiarum* and *Penicillum epansum*. The seed extract of alligator pepper showed higher inhibition of mycelia growth in *Penicillium* (83,33%) and*O.grassitimum* (73.3%). The effect on these microbes were comparable to Dowicide, a known antimicrobial.Available literature indicates that some vegetable oils exhibit antimicrobial activity. Celikel and Kavas (2008), used essential oils obtained from various plants (thyme, myrtle, laurel, sage and orange) to study their antimicrobial activities. The oils showed marked antibacterial and bacteriostatic activity on four microorganisms (*E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Candida albicans* at concentrations of 5-20 micro g /disk and 0.5- 30% v/v. The results show that thyme showed the highest activity while orange the lowest and *L. monocytogenes* showed the least sensitivity towards the oils. Bounatirou, *et al*., (2007), used the essential oils from aromatic and medicinal plants.

II. Materials and Methods

The materials used in this work include: alligator pepper pods, n-hexane, Clinical test organisms, petri dishes, swab stick, thongs, nutrient agar, wire loop, conical flasks, measuring cylinders, autoclave and distilled water.

2.1 Sample Collection

Alligator pepper pods were obtained from local traders in Swali market, Yenagoa and stored in cool dry place until they were used. The pure bacterial isolates used in the study were obtained from the Federal Medical Centre (FMC) Yenagoa. Each organism was maintained on the nutrient agar and sub-cultured/ stored at 4^0C until they were used.

2.2 Methods

2.2.1Oil Extraction

Oil was extracted from the crushed seeds of alligator pepper. The extracted oil was purified and dried according to the method of Angaye, *et al*., (2013), cooled to room temperature and stored in a freezer until used.

2.2.2Inoculation of bacterial Isolates

The pure isolates were freshly grown in nutrient broth and incubated for 24 hours at 37^0C. At the end of the inoculation, a sterile swab stick was used to inoculate the prepared agar plates. A different swab was used to spread the inoculum over the surface of the plate in three directions, rotating the plate approximately at 60^0C.

2.2.3Disc Diffusion Method

The disc diffusion sensitivity method was followed to determine the antimicrobial activity. The discs were soaked in the alligator oil. The five discs were placed using sterile thongs on the surface of the already inoculated agar plates and incubated for 48 hours. At the end of the incubation period, a metre rule was used to measure the zones of inhibition (mm).

2.3 Control

A control experiment comprising inoculums without the study oil was also carried out.

For both the sensitivity test experiment and the control, duplicates were carried out and an average value was obtained.

2.4Spectra analysis

The FTIR of the oil was run on Perkin Elmer Spectrum Version 10.4.3, while the proton, DEPT-135 and carbon-13 NMR spectra of the oil were run on 400 MHz spectrometer.

III. Results

The results of the antimicrobial studies and spectral analysis are displayed in the Table 1 and in Figures 1 to 4 respectively.

Table 1: Results of Antimicrobial Studies

Microorganism	Zone of inhibition	Hexane (control)
Proteus mirabilis	2.5 mm	0.0 mm
Staphylococcus aureus	3.0 mm	0.0mm
Escherichia coli	1.02 mm	0.0 mm
Streptococcus pneumoniae	0.0 mm	0.0 mm

FTIR

The FTIR of the oil (Figure 1) displayed the following signals: 3517.98 (carbonyl ester overtone), 2924.490 (CH), 2854.49(CH_2) ,1748.50 (carbon ester), 1604.54(C-C cis), 1365.46(CH_2), 1464.29, 1357.02(CH_3), 12689.46, 1235.27, 1206.27, 1152.49, 1121.76, 1035.34, 930.04, 850.86,816.90,791.90, 726.56(C-H of CH_3), 661.45 and 606.48 cm^{-1}.

Proton (^1H): NMR

Peaks due to terminal methyl groups:

The proton NMR spectrum displayed signals in the region between 0.80 and 5.5 ppm, the usual region for protons in acyl chains (Figure 2). Two double triplets at 0.886, 0.873, 0.860 and 0.852, 0.846, 0.838 due to terminal methyl protons. The peaks of the methylene envelope were observed between 1.235 and 1.281 ppm.

Protons of methylene group beta to carbonyl group 1.510 and 1.615 ppm The allylic protons due oleic linoleic and linolenic acyl chains were observed at1.961 and 2.067. Methylene protons alpha to carbonyl group were observed between 2.164 and 2.358. The bis-allylic protons due to linoleyl moiety were observed at2.65, 2.75 and 2.78 2.86 ppm. The methylene protons of the glyceryl group were observed between 4.07 and 4.29, while the methine protons of the glyceryl group occur at 5.25 and 5.30 ppm and the vinyl protons of the alkene multiple bond were observed at5.42 and5.424 ppm.

^{13}Carbon NMR

The spectrum showed signals in the region between 13.0 and 220 ppm grouped into four regions (Figure 3). The signals due to the saturated carbons were observed between 13.0 and 60.0 ppm. The terminal methyl carbons at 13.95, 13.81 13.84, 14.00, 14.05; the signals due methylene carbons (27) were observed between 22.34 and 36.47; others between 40.50 and 55.88 ppm. The signals due to the glyceryl carbons were observed at 62.10(TAG 1,3carbons) and 67.71 (TAG-2 carbon). Vinylic (olefinic) carbons were observed between120.76

and 133.12;143.93 and 146. 42.The carbonyl carbons were observed at172.79, 173.20 193.45 and 194.27, then 210, 211.30.

DEPT-135 spectrum.
The DEPT 135 NMR spectrum of the oil is displayed in Figure 4. The signals observed above 170 ppm in the ^{13}Carbon NMR spectrum were absent in the DEPT -135 spectrum (210, 211.30 172.9, 173.20 193.45 and 194.27). The peaks due to methylene carbons (2.164 and 2.358) were observed as negative peaks, while the signals due to methine and methyl carbons were observed as positive signals.

IV. Discussion

The oil was light yellow in colour, pepperish and the yield was low, confirming an earlier study by Adeyeye, et al., (2014) who classified the seeds as low in fatty acids. The presence of polyunsaturated fatty acids (PUFAs) in the oil is confirmed by the presence of allylic and bis-allylic peaks in both the ^{1}H and ^{13}C NMR spectra of the oil. The antimicrobial study shows that the oil extracted from alligator pepper show activity against three of the study organisms: Proteus mirabilis, Staphylococcus aureus and Escherichia coli but fail to show any activity against Streptococcus pneumoniae. The results show that the oil has the potential to inhibit/ inactivate the test organisms and can be used as a constituent of some foods and emollient in ointments for the purpose of inhibiting the growth of microorganisms. This likelihood is against the desire of using natural products for the preservation of food in order to reduce or/ and inhibit the activities of microbials or food borne pathogens compared to the use of synthetic compounds. Also the desire towards green consumption requires the use of fewer synthetic food additives and products with low impact on the environment. This principle has led to the search for new methods of producing safe food that have a natural or green image.

4.1Spectral Analysis

FTIR
The spectrum displayed significant absorptions at 3517.98 (carbonyl ester overtone) 2924.490, 2854.49(symmetrical and unsymmetrical vibrations of CH_3 and CH_2) 1748.50 (ester carbonyl) 1604.54,1514.79,1465.87,1370.90, 1270.81, 1236.82, 1207.85, 1151.84, 1036.85, 936.94,812.01 727.89, 607.85, 573.83 and 508.83 cm^{-1} absorption bands were assigned frequencies by comparison with published literature (Henna-Lu and Tan,2009; Valentina, *et. al.*, 2013). Theseabsorptions show the oil to contain triacylglycerols (Gomez, et. al., 2011).

Proton NMR
The proton NMR spectrum of the oil displayed the following signals: 2 double triplets at 0.886, 0.873, and 0.860; 0.852, 0.846 and 0.838 ppm. Protons of the methylene envelope showed signals between 1.235 and 1.281; methylene protons beta to a carbonyl group were observed between 1.510 and 1.615; signals due to allylic protons between 1.961 and 2.067; methylene protons alpha to carbonyl group were observed between 2.164 and 2.358; Bis-allylic protons between 2.65 and 2.75; 278 and 2.86. The glyceryl protons were observed between 4.07 and 4.29; 5.32 and 5.424, while the vinylic protons were observed between 5.32 and 5.424 ppm.

^{13}C NMR
The^{13}C NMR spectrum displayed signals between 13.95 and 211.30 ppm grouped into four sets of peaks. Saturated carbon atoms of the hydrocarbon chains (methyl and methylene carbons, 13.95 to 55.86 ppm), the allylic carbons were observed at 27.16, 27.21 and 27.76 ppm, while the bis-allylic carbon atoms were observed at 25.09, 25.40 and 25.63 ppm, the glyceryl carbons at 62.10 and 67.71; vinylic carbons were observed between 120.76 and 133.12. (143.93 and 146.42). The signals due to the carbonyl carbons were observed at 172.79, 173.20, 193.45 194.27, 210.50 and 211.3 ppm. The peaks were assigned by comparison with literature (Zamora et. al., 2009). The above spectral assignments were confirmed by the DEPT-135 spectra. The signals above 170 ppm which were absent in the DEPT-135 spectra confirm these peaks to be due to carbonyl (quaternary) carbons (Dawoud, *et al*., 2002).

V. Conclusion

The results from the present work show that the seeds of alligator are low in lipids. Also the oil contains TAGs as indicated by spectral data and the oil can be utilized as a constituent in medicaments for inhibiting microbial activities.

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Appendix

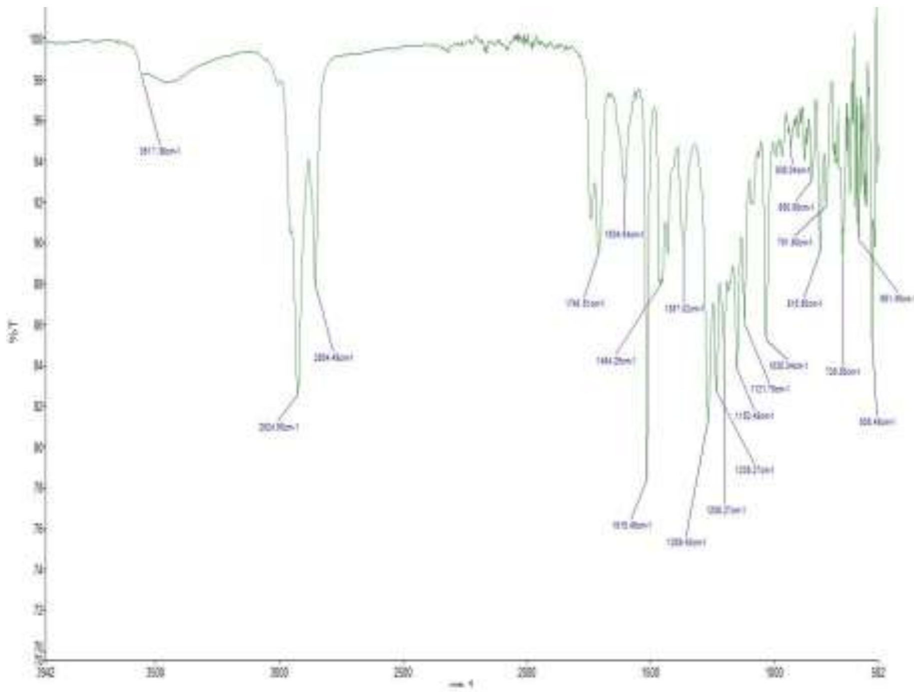

Figure 1: FTIR of Alligator Pepper Oil

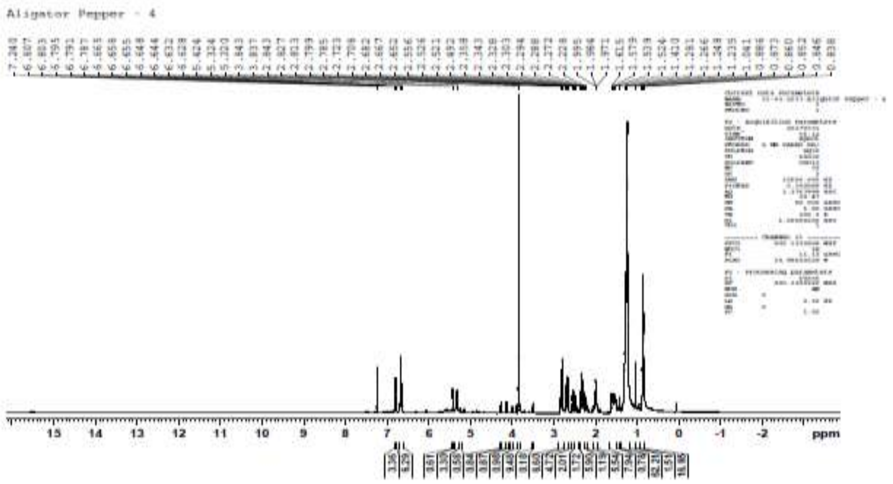

Figure 2: Proton NMR of Alligator Pepper Oil

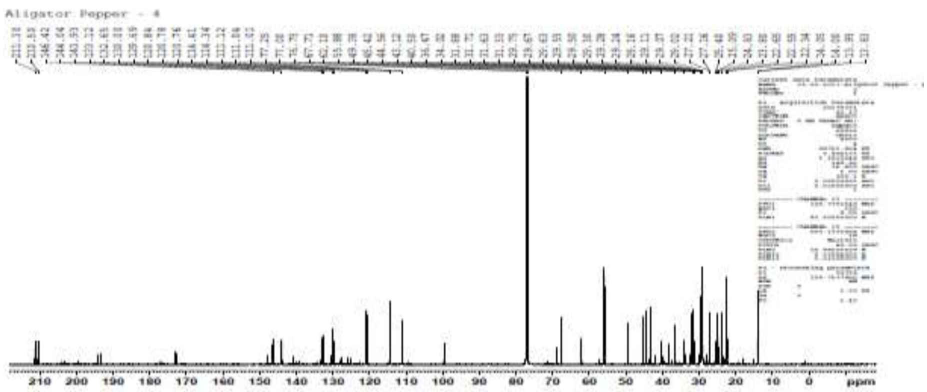

Figure 3: Carbon 13 NMR of Alligator Pepper Oil

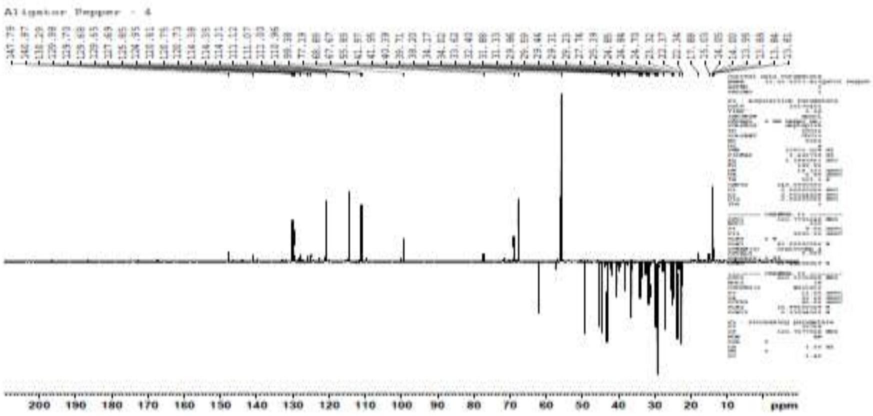

Figure 4: DEPT 135 of Alligator Pepper Oil

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