Original Research Article

INFRARED SPECTROSCOPY AND MICROORGANISMS ASSOCIATED WITH AFRICAN NUTMEG (*MONODORA MYRISTICA)* SEEDS SOLD IN A MUNICIPAL MARKET IN IMO STATE, NIGERIA

Abstract

This work evaluated the infra-red spectra of the oil extract from African nutmeg (*Monodora myristica*) seeds, popularly called 'ehuru' in the eastern part of Nigeria – among the Igbos. The microbial analysis of the dried 'ehuru' seeds was also carried out in order to ascertain the prevailing bacteria and fungi on the outer coat of this invaluable seed as sold in the market. *Monodora myristica* oil extract was obtained through soxhlet extractor using ethanol of Analar grade. About 15g of the extract (oil) was obtained after assessing 40g of the grinded dried seeds of the sample. Infrared analysis of the oil extract was evaluated. The IR spectra of the extract indicated that the sample contained the following functional groups: phosphate esters, ketones, amides, amines, amino acids, ammonium salts, alkenes, phenols, alkanes, ether, lactams and carboxyl groups. There is no doubt that the presence of these chemical functional groups in *Monodora myristica* seed conferred to its antimicrobial quality, therapeutic potentials, and its use as food additive (spice). Common microorganisms associated with the dried 'ehuru' seeds were bacteria - *Streptococcus* sp. and *Staphylococcus* sp.; fungus - *mucor* sp. The presence of these microorganisms on the seed might have been introduced as a result of exposure to unhygienic conditions by local handlers and frequent touching by buyers.

Key words: African nutmeg, oil extract, IR spectra, functional group, microorganisms

1. Introduction

African nutmeg (*Monodora myristica*) seed, popularly called 'ehuru' among the Igbo ethnic group in Nigeria is one of the commonly used spices in the eastern part of the country. It has the qualities of aromatic seasoning and flavour. It is only added in foods in small amount but it makes important contribution towards the aroma and flavour of foods due to the presence of the volatile oil (essential oil) and fixed oil. The seed is oblong and pale brown when fresh with a thin seed coat and hard kernel [1]. The oil extract from *Monodora myristica* seed contains significant pharmacological compounds like alkaloids, flavonoids, vitamin A and E as well as many vital lipids. Traditionally, the plant is widely used especially to relieve toothache as well as in the treatment of dysentery. When roasted and ground, the seeds are rubbed on the skin for the treatment of skin diseases [2]. The seed is used by most Ethnic Nationalities in the various regions of Nigeria, as spice in the preparation of pepper soup (a traditional sauce used for health improvement for the healthy, sick and convalescing). Also, it is reported that natives in the Democratic Republic of Congo use it in the treatment of cough, headache, fever, and skin diseases. When grounded to powder, it is used to prepare pepper soup as stimulant to relieve constipation and control passive uterine haemorrhage in women immediately after child birth. Trado-medically, it has been used to cure mild fever and is known to have diuretic properties [3]. The

aromatic stimulating properties of the plant appear to be the reason why it is occasionally used to ease flatulence, vomiting and for the correction of nausea arising from other drugs. *Monodora myristica* has been reported to exhibit antibacterial activity *in vitro* against some human and animal pathogens. This appears to be the bases for the use of the plant in traditional medicine to treat various diseases such as cough, pneumonia, tuberculosis, upper respiratory tract infections, fever and skin diseases [3]. The oily extracts of *Monodora myristica* contain phytochemicals such as tannins, glycosides, sponins and Flavonoids which exert antimicrobial effects against *Bacillus subtilis, Candida albicans and Stahylococcus.aureus* [4]. This work was targeted to ascertain the IR spectra of the African nutmeg oil extract and, thus the possible chemical substances present based on functional group. The research also aimed at finding out the possible microbial flora (bacteria and fungi) on the seed as sold in the market.

2. Material and Methods

2.1 Procurement of raw materials

The dried indigenous spice, *Monodora myristica* (ehuru) seeds were purchased from Ekeonunwa market in Owerri, Imo state of Nigeria. Identification and its authentication were done by Odum, D. C. of Department of Science Laboratory Technology (Biology/Microbiology), Federal Polytechnic Nekede, Owerri, Imo State, Nigeria.

2.2 Sample preparation

The seeds were thoroughly screened to remove the bad ones and stones. The good seeds were prepared by grinding them in a laboratory electric mill. The powdered sample was stored in an air tight, sterile glass container and kept in the refrigerator at 4°C until needed for analyses. Some seeds were kept whole (without grinding) for microbial analysis.

2.3 Extraction of oil

Before extraction, 40g of the powdered sample of *M. myristica* seed from the refrigerator was heated in a hot-air oven at 60ºC (for easy extraction of the oil).The extraction of the oil content of the seed was by soxhlet extractor using ethanol (boiling range 78.0°C-78.5°C). After the extraction, the solvent (ethanol) was removed from the extract through fractional distillation as described by Ababio (1990) [5]. At the end of the extraction, 15g of the extract (oil) was recovered.

2.4 Infrared spectroscopy

The infrared (IR) spectroscopy was carried out using the Philips Scientific, Model No: 40 13-15-17494 equipment at University of Uyo Research Laboratory, Akwa-Ibom State, Nigeria.

2.5 Microbial analysis of the seed

Nutrient agar (NA) medium was used for the isolation of bacteria on the seeds. This was achieved by aseptically rubbing and placing few of the seeds on the NA agar medium in triplicate plates and was

incubated at 30°C for 48 hours. Some seeds were also placed on sabouraud dextrose agar (SDA) in triplicate plates for the isolation of fungi on the seeds. The plates were incubated at 26°C for 4 days. The bacteria isolated were characterized based on cultural characteristics, staining reactions and biochemical reactions as described by Cheesbrough (2000) [6], while morphological characterization of the fungal isolates was through microscopy under lacto-phenol cotton-blue stain.

3. Results and Discussion

The infrared (IR) spectroscopy of the oil extract from *M. myristica* was carried out and the spectra analysed following related authors' guidelines [7, 8]. Figure 1 revealed the IR spectra of the oil extract from *M. myristica* seed while table 1 showed the peak report.

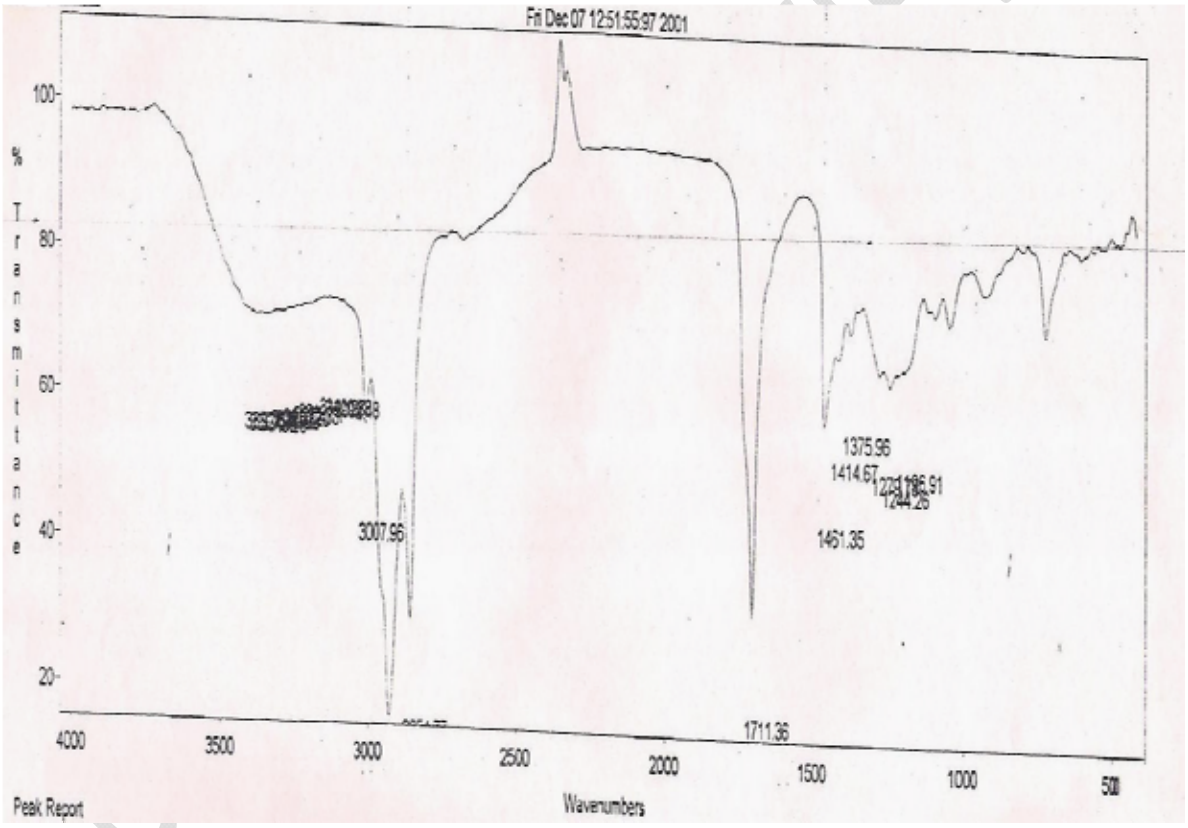

Fig 1: Infrared spectroscopy of oil extract from *Monodora myristica* seed.

Table 1: Analysis of infrared spectra of *Monodora myristica* oil extract

Frequency (cm^{-1})	Transmittance (%)	Frequency range (cm^{-1})	Functional group structure	Inference
1196.91	66.5603	1315 – 1180	p=O	Phosphate ester
1375.96	71.3161	1410 – 1310	O-H	Phenol
1711.36	31.8864	1715 – 1710	C=O	Ketones
3007.96	56.4442	3500 – 3000	N-H	Amines
3141.97	73.4619	3150 – 3050	O-H	Phenol
3266.20	71.8750	3400 – 3200	O-H	Alcohols & Phenols
3345.09	70.8680	3400 – 3200	O-H	Alcohols & Phenols
1244.26	64.2397	1275 – 1200	C-O-C	Ether
1414.67	67.6424	1420 – 1405	C-N	Amides
2854.77	29.7860	3000 – 2850	C-H	Alkanes
3092.48	73.1051	3100 – 2600	N-H	Amino acids
3216.11	72.5560	3300 – 3030	N-H	Ammonium salts
3289.57	71.4642	3300 – 3050	N-H	Amides
3377.59	70.9736	3800 – 3000	N-H	Amines
1279.78	65.8691	1315 – 1180	p=O	Phosphate ester
1461.35	58.5447	1650 – 1450	C=C	Alkenes
2925.85	16.1560	3000 – 2500	O-H	Carboxyl
3120.88	73.3944	3150 – 3050	O-H	Phenol
3232.38	72.1722	3300 – 3050	N-H	Amides
3306.22	71.4238	3400 – 3200	N-H	Amides
3392.04	71.2208	3400 – 3200	N-H	Amides & lactams

The microbial flora of *Monodora myristica* seed was studied to investigate the bacterial and fungal diversity associated with the sample as obtainable in the market environment. The result obtained was as shown in table 2.

Table 2: Microorganisms isolated from the *Monodora myristica* seeds

Bacteria	Fungi
Streptococcus sp.	*Mucor* sp.
Staphylococcus sp.	

DISCUSSION

The results obtained from the infrared spectroscopy revealed that *M. myristica* seed contains important organic substances. Phenolic compounds possess a broad spectrum of biochemical activities including antioxidant and radical scavenging properties, and their antioxidant potential is

believed to be conferred on them by the presence of a hydroxyl functional group (-OH), which is bonded directly to an aromatic hydro-carbon (phenyl) ring. This makes them readily donate electrons to free radicals and stabilize them before they destroy living cells [9]. Due to the presence of the phelolic compounds, McCue and Shetty (2004) [10] and Etouchi *et al*., (2010) [11] reported that *M. Myristica* extract has the ability to exhibit anti-amylase activities which may be potentially useful in the treatment and management of diabetes mellitus and obesity. Also, the bactericidal and/or bacteristatic effects of this seed as reported by Odoh *et al*., (2004) [12] and Ogu *et al*., (2011) [13], based on the findings of this study is as a result of the presence of these phenolic compounds. It has been confirmed that *M. myristica* possesses vermicidal property [14]. This property can be attributed to the presence of phenol and phenolic compounds present in the seed (Table 1).

The study showed that this naturally endowed seed contains ketones (C=O). It has been reported that exogenous ketone supplements may provide benefits which include evident weight loss, athletic performance enhancement, anti-inflammatory properties and cancer prevention [15]. In a study to investigate how african nutmeg (*monodora myristica*) lowers cholesterol and modulates lipid peroxidation in experimentally induced hypercholesterolemic male wistar rats by Nwozo *et al*. (2015), they reported that the extract was able to control the increase in body weight, thus observed reduction in body weight [16]. It is believed that these aromatic ketones (e.g. gingerol and paradol) that give the seed its pungent and peppery taste [17]. Therefore, the African nutmeg seed can be harnessed as an exogenous ketone source for the benefit of mankind directly or indirectly.

Amines (N-H) are very essential in life because they are involved in creating amino acids. Amino acids are the building blocks of proteins in human body. Many vitamins are also made from amino acids. Amines are mostly used in pharmaceutical industries as analgesics (pain killers), decongestant (agent that relieves nasal congestion), and anesthetics [18]. Thus, the oil extract from this organic seed can be refined and processed for amine production to be used in orthodox medicine.

The IR spectroscopy on the experimental seed revealed that it contains amino acids (N-H). As has been earlier said, amino acids are essential monomers in protein synthesis. It has also been reported that food supplements containing amino acids are good for children's growth, boost immune system, protect the liver from toxic agents, regulate blood pressure, and accelerate tissue repair [19]. Significant reduction in the levels of serum total cholesterol and triglycerides in hypercholesterolemic rats treated with *M. myristica* oil extract has been reported [16]. This indicates that the extract possesses cholesterol lowering potential. These outlined benefits confirmed its use as therapeutic agent in trado-medicine as reported by Cimanga *et al*. (2002) [3].

Ether (C-O-C) was another component of the oil extract from African nutmeg. Ether is a highly volatile and flammable liquid that has the ability to depress cerebral activity when used in anaesthetic dosage. Although in some individuals, ether causes irritation to the upper airway, it is a bronchodilator and it can be useful in treating bronchospasm resistant to other drugs [20]. This confirmed the use of *Monodora myristica* as a curative substance for respiratory tract maladies [3].

Alkane group (C-H) was another observable substance identified in the oil extract of the African nutmeg seed. An investigation study by Olga *et al.* (2017) [21] involving the utilization of n-alkanes with an odd number of carbon atom as carbon sources by three yeast strain revealed high biomass yield, accumulation of high amount of lipids, and their ability to produce nutritionally important fatty acids. A study with similar alkane group from the oil extract of the seed under investigation may likely produce the same result. Thus, studies to investigate the use of this oil extract to promote and/or achieve desirable traits and characteristics in organisms like fungi and bacteria should be encouraged.

The reason for the isolation of very few microorganisms from the outer seed coat of *M. myristica* seed is not farfetched (Table 2). This is because an antimicrobial screening done by Adewole *et al*. (2013) [22], using the oil extract from *M. myristica* seed showed that the oil had antimicrobial action against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. It was recorded that the antimicrobial activity exhibited by the oil extract competed favourably with the action of the antibiotic, streptomycin against these bacteria. A similar antimicrobial action on *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli and Salmonella typhi* was observed using ethanolic extracts of *M. myristica* seed [23]. The identified microorganisms might have been introduced on the seeds of 'ehuru' as a result of exposure to unhygienic conditions by handlers and frequent touching by buyers. It is therefore necessary that the seeds be properly cooked before consumption. There is no doubt that the presence of these chemical functional groups in *M. myristica* seed conferred to it therapeutic, antimicrobial and industrial uses.

4. CONCLUSION

Having confirmed that the oil extract from *Monodora myristica* seed is of great value, large scale production of the seed should be encouraged so that its products like the oil extract can be channeled towards the production of pharmaceutical and personal health care products. Pharmaceutical industries are hereby encouraged to harness the great potentials of this nature's gift to mankind. Also the oil extract from this invaluable seed can be used in the right proportion as dietary supplements to promote healthy living. Therefore, the use of African nutmeg seed oil extract sold as spice, food supplement and health booster should be encouraged. Having seen the various chemical functional groups contained by *Monodora myristica* seed, it is very important that scientists, pharmacist, and dieticians utilize this natural product for antimicrobial, therapeutic, and nutritional purposes.

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