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Antimicrobial Activities of Essential Oils from *Hura crepitans* (L.), *Monodora myristica* (Gaertn Dunal) and *Xylopia aethiopica* (Dunal A. Rich) Seeds

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Authors' contributions

This study was jointly designed and carried out by the authors. All authors read and approved the final manuscript

Original Research Article

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ABSTRACT

Background: Despite the fact that essential oils are used as food and medicine, oils of rain forest plants remain largely uninvestigated.
Aims: To investigate the physico-chemical properties and antimicrobial activities of essential oils from *Hura crepitans, Xylopia aethiopica* and *Monodora myristica*.
Place and Duration of Study: This study was carried out in the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria between September, 2009 and January, 2010.
Methodology: Standard methods were used to determine the physicochemical properties of the oils while antibacterial and antifungal properties were determined by agar well dilution and poisoned food assay respectively.
Results: *Hura crepitans* had the highest acid value (9.8 mg KOH/g) and free fatty acids (12.15% oleic acid). *Xylopia aethiopica* had the highest activity against the test organisms while *H. crepitans* was the least effective on the fungal isolates. The oils were most effective against *Salmonella typhi* followed by *Enterobacter* sp. *Pseudomonas aeruginosa* showed the highest resistance to the essential oils. At 100mg/ml, the oils inhibited all the

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 # Dr. O. O. Ojo contributed significantly to this study before he passed away in December, 2010.

test bacteria with the diameter of zone of inhibition ranging from 6.0mm-17mm while the mean radial fungal growth was lower. The results indicate that essential oils are promising alternatives to standard antimicrobials.
Conclusion: *Xylopia aethiopica* had relatively better antibacterial and antifungal activities; hence could be formulated in creams and ointments for the treatment of superficial infections and capsules for the treatment of gastrointestinal tract infections. However its toxicity requires further investigation.

Keywords: Essential oils; pathogens; antimicrobial properties; Hura crepitans; Xylopia aethiopica; Monodora myristica.

1. INTRODUCTION

Essential oils are aromatic liquids obtained mostly from plant materials. They exhibit anti-microbial, anti-toxigenic and insecticidal properties [1]. Oil extracts from higher plants have traditionally been used to extend the shelf life of foods as well as in folk medicine by inhibiting bacteria, mould and yeast [2,3]. Essential oils and their components have also been exploited in controlling pathogenic and food spoilage organisms [4,5,6]. They have a great deal of advantages over conventional antibiotics in that they do not induce resistance, a phenomenon commonly encountered with the long-term use of synthetic antibiotics [1]. Apart from genetic and environmental conditions, the extraction methods have been reported to have considerable influence on the chemical composition, organoleptic properties and antimicrobial activity of most essential oils [7,8].

Monodora myristica (Gaertn Dunal) is a tropical tree that belongs to the family Annonaceae. The seeds of the uncultivated plant are usually used as seasoning in West Africa where it is found commonly. The seed of the plant is popular due to its medicinal and nutritional qualities [9]. *Hura crepitans* [L.] is an evergreen tree of Euphobiaceae family. It is commonly known as sand box tree, it is a typical rain forest tree and the seed is very rich in oil [10,11]. *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) is a valuable medicinal plant widely distributed in sub-Sahara Africa [12]. The plant is used for the treatment of dermatitis, gastrointestinal disorder and respiratory infections [13]. The seeds are used as a spice all over the world [14].

The test plants find use in the treatment of gastrointestinal tract infections [13,15]. The aim of this study was to investigate the physicochemical and antimicrobial activities of essential oils from seeds of *H. crepitans*, *M. myristica* and *X. aethiopica*.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Seeds of the plant samples were collected from abandoned farms around Ekiti State University, Ado-Ekiti, Nigeria. The plants: *H. crepitans* (Euphorbiaceae) locally called (in Yoruba) *Egigun odo*, *M. myristica* and *X. aethiopica* [both belong to the family of Annonaceae] are known as *ario* and *eriru* respectively. The plants were identified and authenticated in the Herbarium Unit of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

2.2 Preparation and Extraction of Oils

Seed samples from the test plants were washed, air-dried and milled with an electronic blender. The oils were extracted through hydrodistillation for 4hrs using a Clevenger-like apparatus, dried over anhydrous sodium sulphate and stored at +4ºC until used.

2.3 Determination of Physiochemical Properties of Extracted Oil

The physicochemical properties of the oil samples were determined. The saponification, free fatty acid (FFA) and acid values of the samples were determined using the American Oil Chemists Society (AOCS) official method of analysis [16].

2.4 Source of Test Organisms

Bacterial isolates which included *Escherichia coli, Pseudomonas aeruginosa, Salmonella* Typhi*, Shigella dysenteriae* and *Enterobacter* sp*.* were obtained from the Clinical Microbiology Laboratory, University of Lagos Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria. The organisms were primarily isolated from patients with gastrointestinal infections. Broth cultures of the test organisms used as inocula were standardized by the method of CLSI [17].

2.5 Determination of Antibacterial Activities of Extracted Oil Samples

The antibacterial properties of the extracted essential oils were determined using the paper disc method as described by Oloke [18]. Sterile Whatman No 1 filter paper discs were impregnated with different concentrations of the oils diluted in Tween 80, and allowed to absorb to maximum capacity. Each impregnated and drained disc was aseptically laid on Müller-Hinton agar plates already seeded with a standardized 18hr inoculum of the test organisms. Clear zones of inhibition were measured in millimeters and the diameter of clear zones was used as an indication of antibacterial activity. Each test was carried in triplicate; Tween 80 was used as negative control.

2.6 Determination of Antifungal Properties of Oils using Poisoned Food Assay

A 1:10 dilution of the oil was introduced into sterile nutrient broth to make a varying concentration ranging between 1.25 and 5.00 mg/ml. One milliliter of the diluted oil was introduced into 9 ml of sterile nutrient broth and incubated at 37ºC for 24hrs and observed for sterility. The method of Nene and Thapilyal [19] was used to determine the antifungal activity of the different extracts of the medicinal plants. Sterile extract was mixed with sterilized Potato Dextrose Agar (PDA) (Oxoid Ltd, Basingstoke, Hampshire, England) to achieve a concentration range of 1.25 to 5 mg/ml. The test fungi used in this study included *Aspergillus flavus, Aspergillus niger, Neurospora* sp and *Rhizopus* sp. The test fungi were collected from the stock of the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria. The inoculation was carried out at the center of each plate with a 10mm mycelium block prepared with 6mm sterile cork borer from the advancing edges of a five day old culture of the test fungi on PDA. The blocks were placed at the center of each Petri dish in an inverted position and the inoculated plate was incubated at 25°C for 5 days. The PDA plate without the extract was also maintained in the same condition to serve as control and the experiment was performed in triplicate. The diameter of fungal mycelia was measured in millimeters (mm).

3. RESULTS AND DISCUSSION

Analyses of the physicochemical properties of the essential oils from the seeds of the test plants are shown in Table 1. The results obtained show variation in the antimicrobial properties of essential oils. The saponification values of the three oil samples were within the range of 183.4 - 206.48 mg KOH/g. Except for *X. aethiopica,* the values were within the range reported by Pearson [1976] for most oils from plants. Low saponification value is an index of molecular weight, and of wide industrial applications. Low saponification value indicates large molecular weight and oils and such attributes can be used for the production of candle, soap and lubricants [21].

Table 1. Physicochemical properties of different essential oils from higher plants

Samples	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Free fatty acid (% Oleic acid)
H. crepitans	9.80	183.40	12.15
X. aethiopica	3.24	206.48	6.70
M. myristica	7.10	189.40	4.85

Monadera myristica had the least FFA (4.85% Oleic acid) while *H. crepitans* had the highest values (12.15% Oleic acid). *Hura crepitans* had low keeping quality because high FFA values in seed oils have been reported to cause accelerated auto-oxidation leading to rancidity and browning. It is also indicative of increase in production costs [22]. According to Dart [22] the amount of oil lost during refining is 1.5 x the FFA value. The saponification value of *H. crepitans* in this study (183.4 mg KOH/g) was lower than 210.10 mg KOH/g reported by Adewuyi et al. [23]. The variation in the values may be due to environmental, genetic or some procedural differences as suggested by Owokotomo and Ekundayo [9].

The results from antibacterial assay show that all the essential oils have antibacterial activities against the five clinical bacteria used in this study. As shown in Table 2, *Salmonella* Typhi was the most sensitive to the essential oil from *H. crepitans* while *P. aeruginosa* showed the least susceptibility to the extracts. The zone of inhibition of the pathogens to the test oils was concentration dependent. *Xylopia aethiopica* is known for its nutritional and medicinal purposes in the southern part of Nigeria. It has been reported to have anti-oxidant and anti-proliferative activity against cancerous cells [24,25]. Also, the plant has anti-tumour, anti-asthmatic, anti-inflammatory, anti-hypertensive and antimicrobial properties [26,27]. From the results of this study, *X. aethiopica* had the highest antibacterial effect against the test organisms with the zone of inhibition ranging between 13.5 and 17.00mm at the highest oil concentration tested.

The oils were most effective against *Salmonella* Typhi followed by *Enterobacter* sp. while *P. aeruginosa* showed the highest resistance to the extracts. In contrast to the report of Fleischer et al. [27] *E. coli* was sensitive to the oils from *X. aethiopica* with zones of inhibition of 14.0 mm, 11.0 mm and 9.0 mm at the concentrations of 5.00 mg/ml, 3.75 mg/ml and 2.50 mg/ml respectively. *Monodora myristica* has been reported to be effective in the treatment of stomach-aches, febrile pains, eye diseases and haemorrhoids [9] however, the oil from the plant had the least activity on the test organisms among oils from the tree plants tested. This may be due to the differences in cell wall composition of the organisms and other physiological properties. The variation in the antimicrobial activity may also be ascribed to the different environmental conditions and chemotype of the plants used which often influences biosynthetic pathways [28].

Table 2. Antibacterial activities of essential oil from screened medicinal plants (zone of inhibition in mm)

Pathogens	Tween 80	*H. crepitans* (mg/ml)				*X. aethiopica* (mg/ml)				*M. myristica* (mg/ml)			
		5.00	**3.75**	**2.50**	**1.25**	**5.00**	**3.75**	**2.50**	**1.25**	**5.00**	**3.75**	**2.50**	**1.25**
P. aeruginosa	0	12.5	9.5	8.5	5.5	14.0	11.0	10.0	6.0	14.0	9.0	7.0	6.0*
Salmonella typhi	0	12.5	10.5	10.5	8.5	17.0	13.0	10.0	6.0	13.0	11.5	9.6	7.0
E. coli	0	11.5	9.5	7.5	6.0	14.0	11.0	9.0	8.0	13.5	11.5	8.0	6.0
Enterobacter sp.	0	12.5	9.5	6.5	6.0	15.0	13.0	14.0	12.0	13.0	11.0	9.0	7.0
S. dysenteriae	0	16.4	12.4	9.5	6.0	13.5	11.4	10.5	8.0	13.2	11.2	8.0	6.0

The difference in the activities of the essential oils may have been due to their different physiochemicals properties [8]. Plant essential oil composition is known to differ according to local climatic and environmental conditions. One of the major models of mechanism of anti-fungal properties of oils is to diffuse into cell membranes and cause them to expand, thereby increasing their fluidity or disordering membrane embedded enzymes [29]. Oils that have high phenol contents have a pronounced effect on the membrane transport, nutrient uptake, nucleic acid synthesis and lipase activity [30,31].

The antifungal activity of the tested oil samples vary. This may be as a result of a wide variety of secondary metabolites in the plants [32]. Antifungal potency of the oils is indicated as an increase in the radial mycelia growth with decrease in the concentration of essential oils tested (Tables 3, 4 and 5). Essential oils rich in phenolic compounds possess high levels of antimicrobial activity [30,33] and it is noteworthy that phenolic derivatives cause membrane disrupting activities [31,34]. The lower antimicrobial activity of the oil from *H. crepitans* could be due to the presence of minor active constituents which may have a synergistic effect [35].

This study has found *X. aethiopica* and *M. myristica* to be better candidates for treatment of both bacterial and fungal infections. These could be formulated in creams and ointments for the treatment of superficial infections and capsules for the treatment of gastrointestinal tract infections.

Table 3. Antifungal activities of oil from *X. aethiopica* on pathogenic fungi

Period (hr)	Test fungi	Concentration (mg/ml)				
		5.00	**3.75**	**2.50**	**1.25**	**Control**
	Rhizopus sp	0	0	1.3	1.6	6
	Neurospora sp	1.0	1.6	2.9	3.0	6.4
24	*Aspergillus niger*	0	0	1.4	1.7	2.4
	Aspergillus flavus	0	0	1.0	1.2	3.4
	Rhizopus sp	0	2.0	3.8	5.0	O.P
	Neurospora sp	2.5	2.5	3.5	4.8	O.P
48	*Aspergillus niger*	1.9	2.0	2.2	3.0	O.P
	Aspergillus flavus	1.7	2.0	2.3	3.0	O.P
	Rhizopus sp	3.4	4.2	5.0	O P	O.P
	Neurospora sp	3.4	4.0	4.8	O P	O.P
72	*Aspergillus niger*	2.9	3.2	4.4	O P	O.P
	Aspergillus flavus	3.0	3.0	4.0	4.9	O.P
	Rhizopus sp	5.7	O.P	O.P	O P	O.P
	Neurospora sp	4.8	O.P	O.P	O P	O.P
96	*Aspergillus niger*	3.2	3.7	4.9	O P	O.P
	Aspergillus flavus	4.5	O.P	O.P	O P	O.P

O.P = outgrown plate; radial mycelia growth in mm.

Table 4. Antifungal Activities of oil from *M. myristica* on pathogenic fungi

Period (hr)	Test fungi	Concentration (mg/ml)				
		5.00	**3.75**	**2.50**	**1.25**	**Control**
	Rhizopus sp	0	2.7	3.7	6.0	6.0
	Neurospora sp	0	4.2	5.0	7.0	6.4
24	*Aspergillus niger*	1.3	1.4	1.4	1.5	2.4
	Aspergillus flavus	0	0	1.4	1.6	3.4
	Rhizopus sp	1.5	4.2	O.P	O.P	O.P
	Neurospora sp	1.7	4.2	4.8	5.0	O.P
48	*Aspergillus niger*	2.0	2.2	4.6	O.P	O.P
	Aspergillus flavus	2.0	2.2	2.5	3.0	O.P
	Rhizopus sp	2.8	6.2	O.P	O.P	O.P
	Neurospora sp	2.9	5.0	5.4	O.P	O.P
72	*Aspergillus niger*	3.2	3.5	5.7	O.P	O.P
	Aspergillus flavus	3.2	3.5	3.9	4.4	O.P
	Rhizopus sp	O.P	O.P	O.P	O.P	O.P
	Neurospora sp	O.P	O.P	O.P	O.P	O.P
96	*Aspergillus niger*	5.0	O.P	O.P	O.P	O.P
	Aspergillus flavus	5.8	O.P	O.P	O.P	O.P

O.P = outgrown plate; radial mycelia growth in mm.

Table 5. Antifungal activities of oil from *H. crepitans* on pathogenic fungi

Period (hr)	Test fungi	Concentration (mg/ml)				
		5.00	**3.75**	**2.50**	**1.25**	**Control**
	Rhizopus sp	2.0	3.2	3.8	4.0	6.0
	Neurospora sp	0	3.0	3.6	5.5	6.4
24	*Aspergillus niger*	1.5	1.9	1.5	2.4	2.4
	Aspergillus flavus	1.7	1.5	2.0	2.9	3.4
	Rhizopus sp	3.6	4.2	4.5	5.2	O.P
	Neurospora sp	3.5	4.5	5.0	O.P	O.P
48	*Aspergillus niger*	2.7	2.8	3.0	3.4	O.P
	Aspergillus flavus	2.6	3.0	3.5	4.4	O.P
	Rhizopus sp	5.8	O.P	O.P	O.P	O.P
	Neurospora sp	6.2	O.P	O.P	O.P	O.P
72	*Aspergillus niger*	4.8	5.7	5.9	O.P	O.P
	Aspergillus flavus	O.P	O.P	O.P	O.P	O.P
	Rhizopus sp	O.P	O.P	O.P	O.P	O.P
	Neurospora sp	O.P	O.P	O.P	O.P	O.P
96	*Aspergillus niger*	O.P	O.P	O.P	O.P	O.P
	Aspergillus flavus	O.P	O.P	O.P	O.P	O.P

O.P = outgrown plate; radial mycelia growth in mm.

4. CONCLUSION

The present investigation shows that essential oils of the test plants contain the potential antimicrobial components that may be of great use for the development of pharmaceuticals in industries, as a therapy against various microbial infections and or diseases. They possess significant inhibitory effects against the tested pathogens. The results of the study

support the folklore claim, along with the development of new antimicrobial drugs from the plants, particularly *X. aethiopica* and *M. myristica*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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