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Antimicrobial activity and physicochemical properties of oils from tropical macrofungi

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Nutriceutics potential and physicochemical properties of the oil extract from five species of macrofungi which include *Ganoderma lucidium, Pleurotus tuberregium, Termytomyces robustus, Schizophyllum commune* and *Trametes versicolor* were investigated using standard chemical and microbiological methods. The oil was extracted using Soxhlet method of extraction. Disc diffusion and agar dilution methods were used to test for the antibacterial and antifungal properties of the samples respectively. The extracted oils were tested against five clinical bacterial isolates: *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Serratia marcescens, Enterococcus faecalis* and *Escherichia coli.* The susceptibility of three fungi genera to the oil samples was also tested. The potency of the extracts oils was determined at different concentrations. All the oils extracted from the mushroom were liquid at room temperature. The acid values of the oils ranged between 0.9 and 6.7 mg KOH/g in *T. robustus* and *T. versicolor* respectively. The high iodine values ranged between 39.8 in *T. versicolor* and 127.0 mg I_2/100 g. The saponification value was above 100 mg KOH/g except *T. robustus*. The aromabiogram of the oils from the mushroom had a pronounced effect on the Gram negative bacteria. Oil from *S. commune* has the least inhibitory effect on the bacteria. The antifungal assay of *P. tuberregium* was most effective against *Aspergillus parasiticus*, followed by that of *G. lucidium.* The least effective was oil from *T. versicolor*. The performance of oil from *G. lucidium* was the best out of all the samples. The inhibitory activities of the oils were concentration dependent. The oils tested were good sources of antimicrobials.

Key words: Mushroom, oil, nutriceutics, antimicrobial, pathogens, macrofungi.

INTRODUCTION

Macrofungi (mushrooms) are multicellular structures formed from the differentiation of vegetative mycelial cells (Chang and Miles, 1992). A mushroom is divided into two different tissues: the stipe (stem) and the pileus (cap). Mushrooms are the earliest known fungal organisms used as food for their taste and aroma. They are enjoyed in soups or other food preparations for all season (Hestbjerg et al., 2003; Sasek, 2003). They are very good sources of protein, vitamins, lipids and mineral elements. In searching for new therapeutic alternatives, scientists have studied many kinds of mushrooms and have found variable therapeutic activity such as anticarcinogenic, anti-inflammatory, immuno-suppressor, anti-plasmodium and antibiotic, among others (Asfors and Ley, 1993; Longvah and Deosthale, 1998; Tabata et al., 1981; Kapoor, 2010).

Oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (Buchbauer, 2000). Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure, mitigate or prevent diseases, infections and indispositions by means of inhalation (Buchbauer et al., 1993). This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. Hopefully, this will lead

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Table 1. Physicochemical properties of five Nigeria mushroom.

Parameter	Macrofungi (mushrooms)				
	G. lucidium	***P. tuberregium***	***T. robustus***	***S. commune***	***T. versicolor***
Peroxide value (meq/kg)	7.6	6.4	5.0	4.4	4.0
Iodine value (Mg I_2/100 g)	127.0	159.4	39.8	127.5	51.3
Acid value (mg KOH/g)	5.61	1.1	6.7	2.4	0.9
Saponification value (mg KOH/g)	150.1	108.0	196.4	260.9	67.3
Free fatty acid (% Oleic acid)	2.8	0.6	3.4	1.2	1.0

to new information on plant applications and new perspective on the potential use of these natural products.

Antibacterial-resistance infections are on the high side in the recent time, and oils of plant origin have frequently been reported to be antimicrobial (Tantaoui-Elaraki et al., 1992, 1993; Lattaoui and Tantaoui-Elaraki, 1994). The search for new alternatives, of fungal origin, to antibiotic drugs to prevent the proliferation of pathogenic microbes and infections they caused is essential. There is paucity of information on the antimicrobial activity of oils from the Nigeria macrofungi which informed the objective of this study.

MATERIALS AND METHODS

Collection of samples and extraction of oils

Five macrofungi (mushroom) samples which include (*Pleurotus toberrigium*, *Termytomyces robustus, Schizophyllum commune*, *Ganoderma lucidium* and *Trametes versicolor* were collected from Ado-Ekiti and Iworoko-Ekiti in Ekiti State, Nigeria between September and November, 2010 and identification. Fresh mushroom were randomly divided into three samples of 400 g and air dried. The air dried samples were blended separately to a powdered using an electric blender. The powdered samples were store in different containers and labelled appropriately. Oils were extracted from the macrofungi according to the method of Dusk and Gokel (1987).

Determination of physiochemical properties of extracted oil

The physicochemical properties of the oil samples which include saponification, free fatty acid and acid values were determined using the AOCS official method of analysis (AOCS, 1997).

Source of test organisms

Bacterial isolates which include *Esherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae* and *Enterobacter* sp. were collected from Clinical Microbiology Laboratory, Lagos State University Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria while the fungal isolates were collected from the Department of Microbiology, University of Ado-Ekiti, Nigeria.

Determination of antibacterial activities of extracted oil sample

The bacterial isolates used for this test were standa rdized according to Bauer et al. (1966) while the method of Oloke (2000) was employed to screen the antibacterial and antifungal property of the oil samples.

RESULTS AND DISCUSSION

All the oils extracted from the mushroom were liquid at room temperature with colour varied from light golden yellow to dark brown. The oil yield ranged between 25.02 to 36.03%. This is lower compared with the oil yield of palm fruit (48.65%), groundnut (49.0%), and pumpkin leave (47.4%) (Fagbemi and Oshodi, 1991). The acid values of the oils ranged between 0.9 and 6.7 mg KOH/g in *T. robustus* and *T. versicolor* respectively. The high iodine values ranged between 39.8 in *T. versicolor* and 127.0 mg I_2/100 g. This indicates that the oils could be used for the manufacture of cosmetics (Dawodu, 2009). The oil also has saponification value above 100 mg KOH/g except *T. robustus* with 67.3 mg KOH/g. Except for *T. versicolor* (196.4 mg KOH/g) the saponification values of the oils from the mushroom were not within the values for the oils of most plant origin value reported to fall within 188 to 196 (Akanni et al., 2005). The lower the saponification value the larger the molecular weight (Agatemor, 2006). Peroxide value of the oils samples was highest in *G. lucidium* (7.6) followed by *P. tuberregium* (6.4) the least value was recorded in *T. robustus* 4.0. The values were less than 10 documented for majority of the vegetable oils (Ibrahim and Fagbohun, 2011). This implies that the samples will have a better keeping quality than oils from plants (Kolapo et al., 2007).

Search for natural products for a new antimicrobial agent that would be able to curb the menace of increasing resistance to antibiotics is on the increase. In this study, mushroom oil exhibited varying antibacterial activities against test clinical bacterial isolates. The aromabiogram of the oils from the mushroom is shown in Table 1. Gram positive bacteria were relatively resistant to the oils from mushroom than the Oil from *G. Lucidium,* which was most effective on *Bacillus cereus* which is an opportunist pathogen that causes food poisoning. *P. aeruginosa* exhibited the least resistance to the extract. *Enterococcus faecalis* was entirely resistant to oil from *P. tuberregium* even at the highest tested concentration. Oil from *S. commune* has the least inhibitory effect on the

Table 2. Antibacterial screening of oils from common Nigeria macrofungi on some medically important pathogens.

Samples	Concentration (mg/ml)	Pathogens					
		***Pseudomonas* sp**	***E. coli***	***Serratia* sp**	***S. aureus***	***Bacillus* sp**	***Enterococcus* sp**
G. lucidium	100	8	12	19	20	20	15
	10	7	10	17	12	20	16
	1	7	10	18	11	19	12
	0.1	3	9	11	9	13	12
	0.01	1	1	6	3	12	7
P. tuberigium	100	12	7	19	20	10	0
	10	11	7	15	15	10	0
	1	10	3	19	12	4	0
	0.1	11	1	12	15	1	0
	0.01	3	1	9	12	10	0
Trametes sp	100	25	12	10	20	12	0
	10	20	10	5	10	10	0
	1	11	0	4	10	0	0
	0.1	10	0	2	7	0	0
	0.01	9	0	1	2	0	0
T. robustus	100	24	17	14	15	15	10
	10	20	10	9	15	15	7
	1	15	11	6	10	12	5
	0.1	8	6	6	10	11	3
	0.01	7	2	4	7	5	1
S. commune	100	12	11	6	0	0	0
	10	9	10	0	0	0	0
	1	0	11	0	0	0	0
	0.1	0	7	0	0	0	0
	0.01	0	3	0	0	0	0

pathogens. In that it has little effect on the Gram negative pathogen and no effect on Gram negative bacteria. *P. aeruginosa* was the most sensitive pathogen to Trameter followed by *Staphylococcus aureus. E. faecalis*, on the other hand was completely resistant. Results of the antibacterial activity of the oil however, does not agree with the work of Burt (2004) who reported Gram positive bacteria to be more sensitive to oil than Gram negative bacteria. The antifungal assay was presented on Tables 2 to 6. The relative decrease in the radial mycelia growth was assumed to be as a result of inhibitory effect of the oils. The effect of essential oil on the radial fungal growth was supported by Iwalokun et al. (2007) who observed that the growth of tested fungi isolate was decreased by increasing the oil concentrations.

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 disordering membrane embedded enzymes (Mendoza et al., 1997). Oils that have high phenol contents have a pronounced effect on the membranes transport, nutrient uptake, nucleic acid synthesis and lipase activities (Baydar et al., 2004; Ipek et al., 2005). The comparative low radial mycelia growth in the tested fungi in the presence of varying concentrations of oils from fungi agreed with the report of Iwalokun et al. (2007). This suggests their potential as nutriceutics and pharmaceutics. *P. tuberregium* was most effective against *Aspergillus parasiticus*, followed by *G. Lucidium*, the least effective among the tested oils was oil extracted from *T. versicolor*. *Aspergillus* causes systematic mycoses (Denning, 1996) and its infection is generally symptomatic (Durry et al., 1997). *G. lucidium* inhibited the growth of *Aspergillus niger* best, followed by *P. tuberregium, T. robustus* had the least effect on the fungus. *Rhyzopus* was the most susceptible among the test fungi.

P. tuberregium was the most effective against the

Table 3. Antimicrobial effects of oils of macrofungi on the growth of *Aspergillus parasiticus.*

Hours	Concentration (mg/ml)	Mushroom from where the oils were extracted					Control
		G. lucidium	*P. tuberregium*	*T. robustus*	*S. commune*	*T. versicolor*	
24	100	10.0	5.0	7.0	ND	6.0	13
	10	11.0	5.0	12.0	ND	6.0	
	1.0	13.0	13.0	17.0	ND	6.0	
	0.1	15.0	13.0	17.0	ND	15.0	
48	100	15.0	8.0	21.0	ND	14.0	Out grown
	10	15.0	10.0	20.0	ND	19.0	
	1.0	16.0	10.0	25.0	ND	23.0	
	0.1	17.0	25.0	26.0	ND	34.0	
72	100	21.0	15.0	20.0	ND	17.0	Out grown
	10	22.0	13.0	30.0	ND	25.0	
	1.0	25.0	10.0	32.0	ND	37.0	
	0.1	30.0	34.0	Out grown	ND	Out grown	

ND=Not determined.

Table 4. Antifungal activity of macrofungal oils on *A. niger.*

Hours	Concentration (mg/ml)	Mushroom from where the oils were extracted					Control
		G. lucidium	*P. tuberregium*	*T. robustus*	*S. commune*	*T. versicolor*	
24	100	0	0.0	2.0	ND	2.0	13
	10	2.0	0.0	4.0	ND	1.0	
	1.0	2.0	2.0	3.0	ND	6.0	
	0.1	2.0	8.0	9.0	ND	10.0	
48	100	0.0	5.0	12.0	ND	3.0	Out grown
	10	4.0	5.0	14.0	ND	12.0	
	1.0	8.0	8.0	20.0	ND	15.0	
	0.1	10.0	20.0	22.0	ND	20.0	
72	100	4.0	10.0	20.0	ND	12	Out grown
	10	14	20	23.0	ND	20.0	
	1.0	20.0	26.0	31.0	ND	20.0	
	0.1	30.0	30.0	out grown	ND	28.0	

ND=Not determined.

fungus while *G. lucidium* exhibited the least inhibitory effect. Effect of *G. lucidium* was highest on *Botryodiplodia* sp. Followed by *P. tuberregium. T. versicolor* had the least effect on the fungus. The performance of *G. lucidium* was the best out of all the samples. This justifies its use in folk medicine (Wasser and Weis, 1999). Its effectiveness may be due to its cellular components and secondary metabolites that have been used to treat a variety of disease states (Gan et al*.,* 1998; Chen et al*.,* 1995). Moreover, it is cultivated and consumed for its pharmaceutical value rather than as food (Jong and Birmingham, 1992).

In this study, we demonstrated the antimicrobial activities of oils isolated from different macrofungi. We believe that the activities of these oils are similar to the activities of other antimicrobials from plants. The inhibitory activities of the oils were concentration dependent. We show in this paper that oils from mushroom could lead to novel therapeutic agents with both antibacterial and antifungal attributes.

Table 5. Antifungal activity of macrofungal oils on *Rhyzopus* sp.

Hours	Concentration (mg/ml)	Mushroom from where the oils were extracted					
		G. lucidium	*P. tuberregium*	*T. robustus*	*S. commune*	*T. versicolor*	Control
24	100.0	2.0	0.0	2.0	ND	2.0	13
	10.0	6.0	0.0	5.0	ND	3.0	
	1.0	5.0	3.0	8.0	ND	4.0	
	0.1	12.0	6.0	9.0	ND	5.0	
48	100.0	4.0	2.0	2.0	ND	5.0	Out grown
	10.0	8.0	10.0	6.0	ND	8.0	
	1.0	12.0	6.0	10.0	ND	11.0	
	0.1	25.0	8.0	10.0	ND	14.0	
72	100.0	8.0	6.0	3.0	ND	6.0	Out grown
	10.0	9.0	10.0	8.0	ND	10.0	
	1.0	14.0	8.0	14.0	ND	13.0	
	0.1	25.0	18.0	15.0	ND	14.0	

ND=Not determined.

Table 6. Antifungal activity of macrofungal oils on *Botryodiplodia* sp

Hours	Concentration (mg/ml)	Mushroom from where the oils were extracted					Control
		G. lucidium	*P. tuberregium*	*T. robustus*	*S. commune*	*T. versicolor*	
24	100.0	4.0	10	6	ND	6	10
	10.0	5.0	10	8	ND	10	
	1.0	5.0	10	8	ND	12	
	0.1	7.0	10	12	ND	12	
48	100.0	4.0	15	10	ND	15	Out grown
	10.0	5.0	14	13	ND	14	
	1.0	6.0	15	12	ND	15	
	0.1	5.0	15	14	ND	20	
72	100.0	5.0	20	Out grown	ND	Out grown	Out grown
	10.0	5.0	22	Out grown	ND	Out grown	
	1.0	8.0	23	Out grown	ND	Out grown	
	0.1	15.0	20	Out grown	ND	Out grown	

ND=Not determined.

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