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Full Length Research Paper

Comparative studies of the crude extracts of sesame against some common pathogenic microorganisms

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Concern about the rising prevalence of antibiotics resistant strains pathogenic microorganisms has been expressed in the last three decades. However, intensive studies on extracts and biologically active compounds isolated from medicinal plants have equally doubled in the last decade. The ethanolic, methanolic and aqueous extracts of *Sesame radiatum* and its combination with S*esame indicum* leaves were studied for their *in-vitro* antimicrobial activity against tested pathogenic micro-organisms. The gas chromatography-mass spectrometry phytochemical screening showed the presence of essential oils mainly the phenolic and carboxylic acids groups. Ethanolic extracts of *Sesame* leaves have no inhibitory effects on S*taphylococcus aureus* but strongly effective against S*treptococcus pneumoniae* and mildly effective against *Candida albicans*. However, the methanolic extract exhibited a mild antimicrobial activity against *Staph. aureus* and *C. albicans,* but no inhibitory effect on S. *pneumoniae*. *C. albicans* was mildly inhibited by the aqueous extract. However, there was a strong antimicrobial effect against *S. pneumoniae* and *Staph. aureus* in the combination of aqueous extracts of both species. The *Sesame* leaves extracts are equally effective against tested micro-organisms as the standard antibiotics.

Key words: Pathogenic micro-organisms, anti-microbial, sesame leaves.

INTRODUCTION

The search for components with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Davis, 1982). However, there has also been a rising interest in the research for natural products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades and in recent times (Dapkevicus et al., 1998; Wang et al., 1998; Nascimento et al., 2000; Rios and Recio, 2005). Moreso, many of these plants have been known to synthesize active secondary metabolites such as phenolic com-pound found in essential oils with established potent antimicrobial activities, which indeed have formed the basis for their applications in the pharmaceuticals, alternative medicine and natural therapies (Reynold, 1996; Lis-Balchin and Deans, 1997; Rios and Recio, 2005). Santo et al. (1998) remarked that the World Health Organization has indeed recognized medicinal plants as the best source of obtaining variety of synthetic drugs. No doubt, the study on medicinal plants will allow for the demonstration of their physiological activity and also catalyze many pharmacological studies leading to the development of a more potent drugs with nil or minimal toxicity and high sensiti-

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vity especially towards the emerging microbial agents (Ebana et al., 1991; Fabricant and Farnsworth, 2001).

Sesame belongs to the family Pedaliaceae and genus *Sesamum* (Purseglove, 1974). The genus consists of about 36 species of which 19 species are indigenous to Africa (Weiss, 1983; Uzo, 1998). *Sesame* is reputed in folk medicine in Africa and Asia. All parts of the plant are useful however in the South-Western Nigeria, decoction of the leaves is used for the treatment of bruised or erupted skins, catarrh and eye pains. Warm water leaves infusion is used to gargle and treat inflamed membranes of the mouth. But the decoction of both leaves and roots have been found to be effective against chicken pox and measles (anti-viral) and used as hair shampoo for *Taenia capitis* (antifungal properties) (Gill, 1992).

Sesame seed oil has been used as healing oil for thousands of years and also enjoyed by humans since the dawn of civilization. In Nigeria, three species, which include *Sesame alatum* (Thonn), *Sesame indicum* L. and *Sesame radiatum* Schum and Thonn are widely cultivated for different purposes (Dabir 2000). However, in Tiv and Idoma areas of Nigeria's Benue state, two breeds of *Sesame*; the *S. radiatum* and *S. indicum*, are usually cultivated mainly for their seeds and leaves (Agboola, 1979). They also constitute the staple food consumed locally in these areas and also especially in South-West and Middle Belt areas of Nigeria where it is richly cultivated by local subsistence farmers. Extensive study has been carried out on the seed and oils but there is paucity of knowledge on the antimicrobial activity of the leaves especially that of the synergistic affect of both *S. radiatum and indicum* in comparison with other known standard antibiotic and antifungal, hence the basis of this study.

METHODS

Preparation of extracts

Sesame plants (*S. radiatum*, Schum and Thonn - Pedaliacaea family) were bought from a vendor in Agege market, Lagos, after being identified in May 2005. The plant was authenticated by the herbarium section of Forestry Institute of Research (FRIN) with FHI # 107513 on the 5th of August, 2005 (Shittu, 2006) .Voucher specimens were deposited to Botany Department of University of Ibadan and Lagos State University respectively. The leaves having been separated from the rest of the plants were air dried for 2 weeks and later grounded into powdery form using a grinder. For the aqueous extract, 100 g of the powdered leaves was added to 1.0 litre of distilled water at a ratio of 1:10 in a beaker and allowed to boil to boiling temperature after intermittent stirring on a hot plate for one hour. The decoction was filtered using white sieve clothing and the filtrate evaporated at 50°C to dryness in a desiccator to produce a black shinning crystal residue form with a yield of 83% (w/w) of the extract. The crude extract was kept in the fridge (4°C) before being reconstituted and later used for the *in-vivo* study

In order to prepare the methanolic and ethanolic-extracts, the plant parts were air-dried. Each dry powdered plant material (100 g) was extracted with 500 ml of 80 % methanol and 98% Ethanol for 72 h with Soxhlet equipment using modified method of Alade and Irobi (1993). The extract was filtered using Whatman filter paper no. 1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at – 20°C.

Phytochemical screening

Screening of crude *Sesame* leaves ether extracts were analyzed by gas chromatography-mass spectrometry (GC/MS). GC analysis was performed using a Hewlett Packard gas chromatograph (model 6890) equipped with a flame ionization detector and injector MS transfer line temperature of 230°C respectively. A fused silica capillary column HP-InnoWax (30 in x 0.25 mm, film thickness 0.25 (mu) m) was used. The oven temperature was held at 50°C for 5 min holding time and the temperature was raised, from 50 - 230°C at a rate of 2°C /min. Helium was the carrier gas at a flow rate of 22 cm/s. 1 ml of extract mixed with methanol (80%), at a split ratio of 1:30 was injected (Shimoda et al., 1996; Shittu et al., 2006). GC/MS analyses were carried out on a Agilent Technologies Network mass spectrometer (model 5973) coupled to H.P. gas chromatograph (model 6890) equipped with NBS 75 K Library Software data. The capillary column and GC conditions were as described above. Helium was the carrier gas, with a flow rate of 22 cm/s. Mass spectra were recorded at 70 eV /200°C. The scanning rate of 1 scan/s and the run time was 90 min. Compound identification was accomplished by comparing the GC relative retention times and mass spectra to those of authentic substances analyzed under the same conditions, by their retention indices (RI) and by comparison to reference cornrounds

Antimicrobial screening

Staphylococcus aureus (clinical), *Streptococcus pneumonia* (clinical) and *Candida albicans* (clinical) were the micro organisms used and they were obtained from the Microbiology Laboratory of the Lagos State University Teaching Hospital (LASUTH). These microorganisms were identified and confirmed at the Microbiology Department of the Drug Quality Control Laboratory, LASUTH, Ikeja, Lagos. Standard strain of *Staph. aureus* (ATCC 29213) of oxoid Culti-loop (Oxoid Ltd., Hampshire, England) was also used. A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 h except for *C. albicans* which was incubated at 25ºC for 24 - 48 h. Each of the 24 h old pure culture was suspended in a Roux bottle containing 5 ml of physiological saline. Each suspension of microorganisms was standardized to 25% transmittance at 560 nm using a UV-visible spectrophotometer.

Antimicrobial activity of one standard antifungal (Clotrimazole) and one standard antibiotic (Ofloxacin) were tested in parallel against the test microorganisms. The modified Collin et al. (1995) agar-well diffusion method was employed to determine the antimicrobial activities for both ethanolic and aqueous extracts. Various concentrations of each of the extracts was made by diluting 1 ml of each reconstituted extract in 2, 4, 6, 8, 10 and 0.1ml in 10, 20, 30, 40 and 50 ml of sterile distilled water respectively. The Mean Inhibitory Concentration (MIC) of the extracts against the tested microorganisms was obtained. Using modified Collins et al., (1995) method, approximately 10 ml of sterile Muller-Hinton Agar (MHA) was poured into sterile culture plates and allowed to set. About 10 ml of the antibiotic medium No 2 seeded with 0.5 ml of a 24 h old culture of bacteria isolates was layered onto the MHA and allowed to set. The seed medium was then allowed to dry at room temperature for about 30 min. In the case of *C. albicans*, Sabouraud Dextrose Agar (SDA) seeded with a 24 h old *C. albicans* was layered on the MHA. With the aid of a sterile cork borer, wells of about 8 mm in diameter were punched on the plates. About 0.5 ml

Table 1. Sensitivity of micro-organisms to ethanolic extracts of *Sesame radiatum* leaves.

Micro-organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
Staphylococcus aureus	–	–	–	–	–
Streptococcus pneumoniae	+++	–	–	–	–
Candida albicans	++	+	–	–	–

(+) Susceptibility (inhibition zone ≥ 10 mm).
(-) Absence of susceptibility.
The MIC of *Streptococcus pneumoniae* was at full concentration = 76.2 µg/ml, while for *Candida albicans* at 1:2 = 28.2 µg/ml.

Table 2. Sensitivity of micro-organisms to aqueous extracts of *Sesame radiatum* leaves.

Micro-organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
Staphylococcus aureus	–	–	–	–	–
Streptococcus pneumoniae	–	–	–	–	–
Candida albicans	+	+	+	+	–

(+) Susceptibility (inhibition zone ≥ 10 mm).
(-) Absence of susceptibility.
The MIC for *Candida albicans* at 1: 6 = 31.0 µg/ml.

Table 3. Sensitivity of micro-organisms to methanolic extracts of *Sesame radiatum* leaves.

Micro-organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
Staphylococcus aureus	+	+	–	–	–
Streptococcus pneumoniae	–	–	–	–	–
Candida albicans	–	–	–	–	–

(+) Susceptibility (inhibition zone ≥ 10 mm).
(-) Absence of susceptibility.
The MIC for the staphylococcus aureus at 1: 2 = 39.3 µg/ml.

of each dilution of the extracts was dispensed into the wells and the plates were incubated at 37ºC for 24 h except for the plates seeded with *C. albicans* which were incubated at 25ºC for 24 - 48 h. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

The zones of inhibition of the tested microorganisms by the extracts were measured using the Fisher-Lilly antibiotic zone reader model 290 with serial number 003n007 (U.S.A). The diameter sizes in mm of the zone of inhibition measured were shown in the respective tables. The MIC for each microorganism used was determined using micro dilution method by Eloff (1998) as the last concentration/dilution (lowest) of the extract that inhibited the growth of the tested pathogenic micro-organisms. The various MIC are shown in the respective tables.

RESULT

The results obtained in Table 1 showed that ethanolic extract had a very strong antimicrobial effect against *S. pneumoniae* at full concentration while a strong and mild antimicrobial effect on *C. albicans* at full and 1:2 dilution of the extracts respectively. Ethanol extracts of *Sesame* leaves have no inhibitory effects on *Staph. aureus*. Table 2 revealed that the methanolic extract exhibited a mild antimicrobial activity against *Staph. aureus* at both the full concentration and 1:2 dilution of the extract and no inhibitory effect on both the S. *pneumoniae* and *C. albicans.* Table 3 showed that *C. albicans* was mildly inhibitted by the aqueous extract at full concentration, 1:2; 1:4 and 1:6 dilutions of the extracts. No antimicrobial effects were observed for the remaining tested micro-organisms. The results obtained in Table 4 showed that ethanolic extract of the two combined *Sesame* sp. had a very strong and mild antimicrobial effect against *S. pneumo-*

Table 4. Sensitivity of micro-organisms to ethanolic extracts of two combined *Sesame* species leaves.

Micro-organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
Staphylococcus aureus	–	–	–	–	–
Streptococcus pneumoniae	++	–	–	–	–
Candida albicans	+	–	–	–	–

(+) Susceptibility (inhibition zone ≥ 10 mm).
(-) Absence of susceptibility.
The MIC of *Streptococcus pneumoniae* at full concentration = 70.0µg/ml, while for *Candida albicans* at 1:2 = 36.6µg/ml.

Table 5. Sensitivity of micro-organisms to methanolic extracts of two combined *Sesame* species leaves.

Micro-organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
Staphylococcus aureus	+	+	–	–	–
Streptococcus pneumoniae	++	–	–	–	–
Candida albicans	+	+	+	+	–

(+) Susceptibility (inhibition zone ≥ 10 mm).
(-) Absence of susceptibility.
The MIC for *Staphylococcus aureus* at 1:2 = 39.3 µg/ml, and that for *Candida albicans* at 1: 6 = 28.2 µg/ml.

Table 6. Sensitivity of micro-organisms to aqueous extracts of two combined *Sesame* species leaves.

Micro-organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
Staphylococcus aureus	+ + +	+ + +	+ + +	+ + +	–
Streptococcus pneumoniae	++	+ +	+ +	+ +	–
Candida albicans	+	+	+	+	–

(+) Susceptibility (inhibition zone ≥ 10 mm).
(-) Absence of susceptibility.
The MICs for the *Staphylococcus aureus* at 1:6 = 60.7 µg/ml, *Streptococcus pneumoniae* at 1:6 = 47.6 µg/ml and *Candida albicans* at 1:6 = 36.6 µg/ml.

niae and *C. albicans*, respectively, at full concentrations and no antimicrobial activity against *Staph. aureus.* Table 5 revealed that the methanolic extract of two combined specie exhibited a mild antimicrobial activity against *Staph. aureus* at both the full concentration and 1:2 dilution of the extract and also had mild inhibitory effect against *C. albicans* at full concentration and all dilutions of the extracts used. However, there was no inhibitory effect on *S. pneumoniae.*

Table 6 showed that the two combined aqueous extracts had a very strong inhibitory effect against *Staph. aureus* at the full concentration, 1:2 dilution and strong inhibition at 1:4 and 1:6 dilutions of the extracts. There was strong antimicrobial effect against *S. pneumoniae* at full concentration and 1:2, 1:4 and 1:6 dilutions. However, the inhibitory effect on *C. albicans* was mild at full concentrations and 1:2; 1:4 and 1:6 dilutions of the extracts. Table 7 showed the zones of inhibition of standard antibiotic and antifungal against tested microorganisms as well as minimum concentrations of the standards used. Table 8 showed that the mean inhibitory effect of the combined *Sesame* leaves extracts irrespective of the diluents/solvents used was relatively higher than that of *S. radiatum.* The GC-MS of the methanolic *S. radiatum* leaves extracts showed the presence of mainly essential oils such as aromatic phenolic compounds- sesamol, sesaminol and carboxylic acids. Also rich in palmitic acids, arachidonic/arachidic acid, stearic acid, oleic acid, linoleic acids

Table 7. Antimicrobial effect of secondary standard antibiotics (Ofloxacin tablet 200 mg) and antifungal (Clotrimazole vaginal tablet 200 mg) on tested pathogenic microorganisms.

Name of antibiotic/ antifungal	Zone diameter of microorganisms			Concentration(µg/ml)
	Staph. aureus	*S. pneumoniae*	*C. albicans*	
Ofloxacin	28 mm	31.5 mm	–	100
Clotrimazole	–	–	35.5 mm	100

Table 8. Minimum inhibitory concentrations (MICs) of crude extract of two combined *Sesame* species leaves compared to S. *radiatum* on tested microorganisms.

Microorganisms	Crude extract	MICs (µg/ml) of the crude extracts on tested microorganisms							
		S. radiatum				Combined *Sesame* spp.			
		Full	1:2	1:4	1:6	Full	1:2	1:4	1:6
C. albicans	Ethanolic		28.2				36.2		
	Methanolic								28.2
	Aqueous				31.0				36.6
S. pneumoniae	Ethanolic	76.2				70.0			
	Methanolic								
	Aqueous								47.6
S. aureus	Ethanolic								
	Methanolic		39.3				28.2		
	Aqueous								60.7

and eicosanioc acids among others.

DISCUSSION

This appears to be the first study that actually looked at the comparative antimicrobial activities of *S. radiatum* leaves extracts and its combination with *S. indicum* leaves extracts in parallel with standard antibiotics to our knowledge. However, there has been extensive study on *Sesame* seeds and oil respectively in the past. Muller-Hinton Agar diffusion (MHA) methods are extensively used to investigate the antibacterial activity of natural antimicrobial substance and plant extracts. However, for solution/ extracts with a low antimicrobial activity, one will need a large concentration or volume made possible with holes or cylinders using MHA rather than the disk method with limited applications (Bastner et al., 1994).

Several studies conducted in the past three decades have been focused on the antimicrobial properties of herbs, spices and their derivatives such as essential oils, extracts and decoctions (Kivanc and Akgül, 1986; Dorman and Deans, 2000; Hsieh et al., 2001; Ozcan and Erkmen, 2001; Alma et al., 2003). Some researchers have also reported that there is a relationship between the chemical structures of the most abundant compounds in the tested extracts or essential oils and the antimicrobial activity (Farag et al., 1989; Deans and Svoboda, 1989). The GC-MS of the methanolic *S. radiatum* leaves extracts did show the presence of mainly essential oils such as aromatic phenolic compounds which have been found to possess antimicrobial properties (Alma et al., 2003) for example, Sesamol which is one of the most potent antioxidants was discovered in the leaves and reported for the first time by us (Shittu et al., in preparation). The seed oil of *Sesamum* sp. has been found to contain natural antibacterial agents that are effective against common skin pathogens, such as S*taphylococcus* and S*treptococcus* bacteria, as well as common skin fungi including the athlete's foot fungus (Annussek, 2001).

In this study, the methanolic extracts of *S. radiatum* had antimicrobial effect against all the tested micro-organisms except the growth of *S. pneumoniae* and *C. albicans*, while its combination with *Sesame indicum* had antimicrobial effects on *Staph. aureus* and *Candida albicans* (Tables 1and 4). The ethanolic extract of Sesame radiatum or its combination with *S. indicum* had no inhibitory effects against *Staph. aureus* with the exception of *S. pneumoniae* and *C. albicans (*Tables 2 and 5). However, the aqueous extract of *S. radiatum* had no inhibitory effects on *Staph. aureus* and *S. pneumoniae* except *C. albicans*, where as its combination with *S. indicum* leaves

extracts had synergistic inhibitory effects on all the tested pathogenic micro-organisms (Tables 3, 6 and 8). This may reflect the significance of the preservation of some of the active ingredients - sesame lignans such as sesaminol and its glucosides which are water soluble in nature and were extracted effectively during extraction processses of the *Sesame* leaves (Rios and Recio, 2005).

The pH of compounds in dilutions have also been found to modify the results outcome, as usually observed in the case of phenolic or carboxylic compounds present in plants extracts . However, not only do ionisable compounds change the activity; studies have shown that the different effects of neutral essential oil are pH dependents. Thus, for example, anise oil had higher antifungal activity at pH 4.8 than at 6.8, while the oil of *Cedrus deudora*was is most active at pH 9.0 (Janssen et al., 1976). Similar finding is observed in this study where the ethanolic extract of *S. radiatum* or its combination with *S. indicum* with a higher pH (less acidic) had greater inhibittory effective against all the tested pathogenic microorganisms than the methanolic extracts. Also aqueous extract has an antifungal activity at a higher pH but with less potency as reflected in the various MICs (Tables 1-6). These findings also underscored the importance of traditional ways of preservation of leave extracts using local gins in which case, the ethanolic form was better effective in conservation of the active ingredients than the methanolic extractive procedure. This finding confirmed the folkloric claims of the antimi-crobial effectiveness of locally consumed *Sesame* leave extracts in many areas of the Country (Nigeria). It is very effective against bacterial and other common skin infection including yeast.

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