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SYNERGISTIC ANTIMICROBIAL ACTIVITIES OF PHYTOESTROGENS IN CRUDE EXTRACTS OF TWO SESAME SPECIES AGAINST SOME COMMON PATHOGENIC MICROORGANISMS.

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Abstract

Intensive studies on extracts and biologically active compounds isolated from medicinal plants have doubled in the last decade worldwide. However, as a result of paucity of knowledge and folkloric claim on the effectiveness of sesame leaves in infectious disease treatments, we aimed to determine the synergistic antimicrobial activity of essential oils and lignans present in the crude leaves extracts of *Sesame radiatum* and *Sesame indicum.* Ethanolic, methanolic and aqueous extracts of both leaves were studied for their in-vitro synergistic antimicrobial activity against both Gram positive and Gram negative micro-organisms, and Yeast using Agar diffusion method. The GC-MS phytochemical screening of methanolic extract showed that the major compounds in essential oils are of carboxylic acids and phenolic groups especially, the most potent antioxidants known to man like sesamol, sesamolin and sesamin among others. Methanolic and ethanolic extracts have broad spectrum antimicrobial effect against all the tested pathogenic micro-organisms except *Streptococcus pneumoniae* and *Staphylococcus aureus* respectively, while the aqueous extract exhibited inhibitory activity on *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans*. The result confirmed the folkloric claims of the antimicrobial effectiveness of locally consumed sesame leaves extracts especially against bacterial and common skin infection in many areas of Nigeria .

Key words: Pathogenic micro-organisms, Gram-positive, Gram-negative,Yeast, Anti-microbial, Sesame leaves, GC-MS, MIC

Introduction

Concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the newer or modern antibiotics that have been produced in the last three decades worldwide (Cohen, 1992; Nascimento et al, 2000). Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine (Nascimento et al, 2000; Rios and Recio, 2005). For over thousands of years, natural plants have been seen as a

valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to synthetic drug agents (Iwe et al, 1999). However, the problem posed by the high cost, adulteration and increasing side effects of synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized (Shariff, 2001).

Sesame belongs to the family- Pedaliaceae and genus-Sesamum (Purseglove, 1974). The genus consists of about 36 species and 19 of which are indigenous to Africa (Weiss 1983; Uzo 1998). Sesame plant is believed to have originated from Africa (Ram et al. 1990). 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 inflamed membranes of the mouth. 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).

In Nigeria, three species, *S. alatum* (Thonn), *S. indicum* L. and *S. radiatum* Schum & Thonn, are widely cultivated for different purposes (Dabir 2000). However, in Tiv and Idoma areas of Nigeria's Benue state, two breeds of Sesame, *Sesame radiatum* and *Sesame indicum* are usually cultivated mainly for their seeds and leaves (Agboola, 1979). Sesame seeds could be consumed either through its oil, roasted or as animal feeds (Johnson et al., 1979). Sesame seed oil, known to man since the dawn of civilization has been used as a healing oil and is locally consumed as a staple food in Nigeria, especially in South-West and Middle Belt areas, where it is richly cultivated by local subsistence farmers (Akpan-Iwo et al, 2006). Thus, the use of sesame leaves and oil as food sources may account for the high fecundity among the adult male population in these areas (Shittu, 2006) .

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 the synergistic activity of both *Sesame radiatum* and *Sesame indicum*. This study is also to prove the folkloric claims of the antimicrobial activity of sesame leaves.

Materials and Methods

Collection of Plant materials

Two species of Sesame plants (*Sesame radiatum*, Schum and Thonn; *Sesamum indicum*-L - Pedaliacaea family) were bought from a vendor in Agege market Lagos, after being identified by Dr Shittu in May 2005 . The *Sesamum radiatum* plant was authenticated by the herbarium section of Forestry Institute of Research (FRIN) with FHI # 107513 on the 5th of August, 2005 while the *S .indicum* was already confirmed.(Shittu, 2006) .Voucher specimens were deposited in Botany Department of University of Ibadan and Lagos State University, respectively.

Preparation of Extracts

The leaves of the plants were air dried for 2 weeks and powdered. Modified Okogun (2000) method of extraction was adopted in the process. Such that the diluents (solvents) used were absolute ethanol, methanol and sterile distilled water. To 10 ml of each diluent (solvent) in a 20 ml screw cap bottle was added 0.5 g each of the raw air-dried and grinded leaves of both Sesame species. Extraction was allowed to proceed for 5 days (120 h) in the Refrigerator at 4^{o}C for stability of the essential oils, unlike in Okogun (2000) method. The combined extracts solution obtained were regarded as the full concentration.

Phytochemical screening using Gas Chromatography-Mass Spectroscopy

The crude methanolic extracts of sesame leaves were analyzed by GC/MS. GC analysis were 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) 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.The carrier gas Helium was at a flow rate of 22 cm/sec. One millilitre of extract mixed with methanol (80%), at a split ratio of 1:30 was injected. (Shimoda et al, 1996). 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 75K Library Software database. The capillary column and GC conditions were as described above. Mass spectra were recorded at 70 eV /200°C. The scanning rate of 1 scan/sec and the run time was 90minutes. 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 compounds.

Micro-organisms

Staphylococcus aureus (clinical), *Streptococcus pneumonia* (clinical) and *Candida albicans* (clinical) were the microorganisms 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 *Staphylococcus aureus* (ATCC 29213) of oxoid Culti-loop (Oxoid Ltd., Hampshire, England) was also used.

Preparation of 24 hours pure culture

A loop full of each microorganisms was suspended in about 10ml 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 hours except for *Candida albicans* which was incubated at 25ºC for 24-48 hours.

Standardization of micro-organisms

Each of the 24 hours old pure culture was suspended in a Roux bottle containing 5 ml of physio-logical saline. Each suspension of microorganisms was standardized to 25% transmittance at 560 nm using a Ultraviolet (UV)- visible spectrophotometer.

Antimicrobial screening

The modified Collin et al (1995) agar-well diffusion method was employed to determine the antimicrobial activities for ethanolic, methanolic and aqueous extracts. Various concentrations of each of the extracts was made by diluting 1 ml of each reconstituted extract in 2 ml, 4 ml, 6 ml and 8 ml of sterile distilled water respectively. The Mean Inhibitory Concentration (MIC) of the extracts against the tested microorganisms were obtained.

Agar-well diffusion method

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 hours 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 minutes. In the case of *Candida albicans*, Sabouraud Dextrose Agar (SDA) seeded with a 24 hours old *Candida 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 of each dilution of the extracts was dispensed into the wells and the plates were incubated at 37ºC for 24 hours except for the plates seeded with *Candida albicans* which were incubated at 25ºC for 24-48 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

Measurement of zone of inhibition

The zones of inhibition of the tested microorganisms by the extracts were measured by using the Fisher-Lilly antibiotic zone reader model 290 (USA) with serial number 003N007. The diameter sizes in mm of the zone of inhibition measured are shown in the respective tables.

Minimum Inhibitory Concentration (MIC) of extract

The MIC for each microorganism used was determined using microdilution method by Eloff (1998) as the last concentration/dilution (lowest) of the extract that inhibited the growth of the tested pathogenic micro-organisms.

Results

The results obtained in Table 1 showed that ethanolic extract of the two combined sesame spp. had a very strong and mild antimicrobial effect against S*treptococcus pneumoniae* and *Candida albicans* respectively at full concentrations and no activity against S*taphylococcus aureus.*The methanolic extract of two combined spp.

Table 1: Sensitivity of 3 microorganisms to ethanolic extracts of two combined Sesame species leaves

MICROORGANISMS	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 = 70.0µg/ml.
While for *Candida albicans* was at 1:2 = 36.6µg/ml.

Table 2: Sensitivity of 3 microorganisms to methanolic extracts of two combined Sesame species leaves

MICROORGANISMS	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* was at 1:2 = 39.3µg/ml.
The MIC for *Candida albicans* was at 1: 6 = 28.2µg/ml .

Table 3: Sensitivity of 3 microorganisms to aqueous extracts of two combined Sesame species leaves

MICROORGANISMS	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* was at 1: 6 = 60.7µg/ml.
The MIC for the *Streptococcus pneumoniae* was at 1:6 = 47.6µg/ml.
The MIC for the *Candida albicans* was at 1:6 = 36.6 µg/ml.

exhibited a mild antimicrobial activity against *staphylococcus aureus* at both the full concentration and 1:2 dilution of the extract and also had inhibitory effect against C*andida albicans* at full concentration and all dilutions of the extracts used. However, there was no inhibitory effect on S*treptococcus pneumoniae* (Table 2). The two combined aqueous extracts had a very strong inhibitory effect against *Staphylococcus 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

streptococcus pneumoniae at full concentration and 1:2, 1:4 and 1:6 dilutions. However, the inhibitory effect on *Candida albicans* was mild at full concentrations and 1:2; 1:4 and 1:6 dilutions of the extracts (Table 3). MICs of the two combined crude extracts of Sesame species on all the tested microorganisms are shown in Table 4.

The GC-MS showed that the methanolic sesame radiatum leaves extracts contain aromatic phenolic compounds- sesamol, sesaminol, carboxylic acids and other classes of compounds including palmitic acids, arachidonic/arachidic acid, stearic acid, oleic acid, linoleic acids, thiazole, pyrroles, disulphide and aldehyde.

Table 4 The minimum inhibitory concentrations (MICs) of crude extract of two combined Sesame species leaves on tested microorganisms.

MICs (µg/ml) of the crude extracts on tested microorganisms

Microorganisms	Crude Extract	Full	1: 2	1: 4	1: 6	1: 8
S. aureus	Methanolic		39.3			
	Ethanolic					
	Aqueous				60.7	
S.pneumoniae	Methanolic					
	Ethanolic	70.0				
	Aqueous				47.6	
C. albicans	Methanolic		28.2			
	Ethanolic		36.6			
	Aqueous				36.6	

Discussion

The GC-MS of the methanolic sesame radiatum leaves extract 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 Sesame spp 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. (Aquaculture Research, 2001).

In this study, the methanolic extracts have antibacterial effect against all the tested micro-organisms except the growth of *Streptococcus pneumoniae*. The ethanolic extract had no inhibitory effects against the *Staphylococcus aureus* but had both antibacterial and antifungal activities against both *Streptococcus aureus* and *Candida albicans* respectively. The aqueous extract of the same concentration showed antimicrobial effects on all the tested microorganisms. 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 processes of the Sesame leaves (Rios and Recio, 2005). The antimicrobial effect of *Sesamum radiatum* leaves observed in this study is supplementary to our earlier reports (Shittu et al, 2006). Furthermore, there was more significant antimicrobial effect of *Sesamum radiatum* when combined with *Sesamum indicum*. This confirms the use of Sesame leaves extracts as antimicrobial agent in folk medicine.

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, 1987). Similar finding is observed in this study where the ethanolic extract with a higher pH (less acidic) was more effective against the tested microorganisms than the methanolic extract. Also aqueous extract had an antifungal activity at a higher pH but of less potency as reflected in the various MICs .

These findings also underscored the importance of traditional ways of preservation of leaves extracts using local gins in which case, the ethanolic form was better effective in conservation of the active ingredients than the methanolic extractive procedure. The MIC against C*andida albicans* was the same in both ethanolic and aqueous extracts that is, 36.6 µg/ml. This showed that the popularly used local gins will be as effective as water. Both solvents are beneficial as they will better enhance the extraction of the oily and water soluble active ingredients

which have been proven to have anti-microbial properties especially against yeasts. No doubt, the cooling effect and longer duration of extraction using different solvents did contribute to conservation and availability of the active ingredients maximally.

Conclusion

This finding confirmed the folkloric claims of the antimicrobial effectiveness of locally consumed Sesame leaves extracts in Nigeria. However, it is very effective against bacterial and other common skin infection including yeast. Further study is on to elicit the antimicrobial effect of Sesame indicum, and to isolate the active ingredients that have been characterized in Sesame leaves in order to check their effects on the tested microorganisms.

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References

1. Agboola, S. A. (1979). An Agricultural Atlas of Nigeria Oxford University press, London, pp. 131-135.
2. Akpan-Iwo, G., Idowu, A. A. and Misari, S. M. (2006). Collection and evaluation of sesame (*Sesamum* spp.) germplasm in Nigeria. IGPR/FAO, **142:** 59-62.
3. Alade, P. I. and Irobi, O. N. (1993). Antimicrobial activities of crude leaf extract of Acalypha wilkensiana. J. Ethanopharmacol., **39:1**71-174.
4. Alma, M. H., Mavi, A., Yildirim, A., Digrak, M. and Hirata, T. (2003). Screening chemical composition and *in vitro* antioxidants and antimicrobial activities of the essential oils from the Origanum syriacum L, growing in Tuckey. Biol. Pharmaceu. Bull. **26:** 1725-1729.
5. Cohen, M .L. (1992). Epidemiology of drug resistance: implications for a post antimicrobial era. Science 257: 1050-1055.
6. Collins, C. H., Lynes, P. M. and Grange, J. M. (1995). Microbiological Methods. (7thEdn.) Butterwort-Heinemann Ltd., Britain, pp.175-190.
7. Dabir, J. D.. (2000). Cytogenotical studies of interspecific hybrids between *Sesamum indicum* and wild relatives. MSc thesis, Botany Department, University of Jos, Plateau State, Nigeria.
8. Dapkevicus, A., Venskutonis, R., Van Beek, T. A. and Linssen, J. P. H. (1998). Antioxidant activity of extract obtained by difgerent isolation procedures from some aromatic herbs grown in Lithuania. J. Sci Food Agric **77:** 140-6.
9. Davis, P. H. (1982). Flora of Turkey and East Eagean Island, Edinburg, Edinburg University Press. **7:**947 .
10. Ebana, R. U. B., Madunagu, B. E., Ekpe, E. D. and Otung, I. N**.** (1991). Microbiological exploitation of cardiac glycoside and alkaloids from *Garcinia kola, Borreria ocymoides, Kola nitida* and *Citrus aurantifolia.* J. Applied Biotechnol., **71:** 398 – 401.
11. Fabricant, D. S. and Farnsworth, N. R. (2001). The value of plant used in traditional medicine for drug discovery. Environmental Health Perspectives Supplements, **109:** 69-75.
12. Gills, L. S. (1992). Ethnomedical uses of plants in Nigeria, Uniben Press, Edo State Nigeria. 212 .
13. Janssen, A. M., Scheffer, J. .J. C. and Baerheim Svendsen, A. (1976). Antimicrobial activity of essential oils: a 1976–1986 literature review. Aspect of the test methods. Planta Medica **53:** 395–398.
14. Johnson, L. A., Suleiman, T. M. and Lucas, E. M. (1979). Sesame protein: A review andprospectus. J. Am. Oil Chem. Soc., **56:** 463-468.
15. Lis-Balchin, M. and Deans, S. G. (1997). Bioactivity of selected plant essential oils against Listeria monocytogenes. J. Applied Bacteriol., **82 :** 759-762.
16. Nascimento Gislene, G. F., Juliana Locatelli, Paulo C Freitas, and Giuliana L. Silva. (2000). .Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian J. Microbiol. **31:**247-256
17. Purseglove, J. W. (1974). Tropical Crops: Dicotyledons. Longman Group, London,UK. 1974; pp: 430–435.
18. Ram, R., D. Catlin, J. Romero, and Cowley, C. (1990). Sesame: New approaches for crop improvement.. In: J. Janick and J.E. Simon (eds.), Advances in new crops. Timber Press, Portland pp. 225–228
19. Reynolds, J. E. F. (1996). Martindale-the extra pharmacopia. (31st ed), Royal Pharmaceutical Society of Great Britain, London 1996.

20. R´ıos, J. L and. Recio. M. C. (2005). Medicinal plants and antimicrobial activity J. Ethnopharmacol. **100 :**80–84
21. Santos, P. R. V.; Oliveira, A.C.X., and Tomassini, T. C. B. (1995). Controle microbiógico de produtos fitoterápicos. Rev. Farm. Bioquím. **31:** 35-38.
22. Sesame (2000). Aquaculture Research August, 32: 623. In: Shittu Lukeman .A. Joseph. (2006). The effect of the aqueous crude leave extract of *Sesamum radiatum* using Mestorolone as a standard on the adult male Sprague Dawley rat Testis and Epididymis, MSc Dissertation, Lagos State University College of Medicine, Ikeja, Lagos, 2006.
23. Shariff, Z. U. (2001). Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series (Volume 1), Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, 2001 ; pp. 9 – 84.
24. Shimoda, M., Shiratsuchi, H., Nakada, Y., Yin Wu and Osajinwa, Y. (1996). Identification and sensory Characterization of volatile flavor compound in Sesame seed oil. J.Agric Food Chem. **44:** 3909-3912.
25. Shittu Lukeman .A. Joseph. (2006). The effect of the aqueous crude leave extract of Sesamum radiatum using Mestorolone as a standard on the adult male Sprague Dawley rat Testis and Epididymis, MSc Dissertation, Lagos State University College of Medicine, Ikeja, Lagos, 2006.
26. Shittu L. A. .J, Bankole, M. A., Ahmed, T., Akinsanya, M. A., Bankole, M. N., Shittu, R. K, Ashiru O. A. (2006). Differential antimicrobial activity of the various crude leaves extracts of *Sesame radiatum* against some common pathogenic micro-organisms. Scientific Res. Essay, 2006; **1 :**108-111.
27. Wang, M., Li, J., Rangarajan, M., Shao, Y., LaVoie, E. J., Huang, T. C. and Ho, C. T. (1998). Antioxidative phenolic compounds from sage (*Salvia officinalis*).J. Agric. Food Chem., **46:**4869-4873.