Scientific Research and Essay Vol. 1 (3), pp.108-111, December 2006
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2006 Academic Journals

Full Length Research Paper

Differential antimicrobial activity of the various crude leaves extracts of *Sesame radiatum* against some common pathogenic micro-organisms

Shittu, L. A. J.[1]*, Bankole, M. A.[2], Ahmed, T.[3], Aile, K.[4], Akinsanya, M. A.[5], Bankole, M. N.[6], Shittu, R. K.[7] and Ashiru, O. A.[1]

[1]Department of Anatomy, Lagos State University College of Medicine, Ikeja, Nigeria.
[2]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos/Lagos University Teaching Hospital, Idi-araba, Lagos, Nigeria.
[3]Department of Microbiology, Drug Quality Control Laboratory/Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria.
[4]Department of Medical Microbiology, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria.
[5]Department of Medical Biochemistry, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.
[6]Diarrhea Unit, Microbiology Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.
[7]Medical Microbiology Unit, Bolomedics Laboratories, Egbeda, Nigeria.

Accepted 12 December, 2006

Concern about the rising prevalence of antibiotics resistant strains pathogenic micro-organisms has been expressed in the last three decades. However, intensive studies on extracts and biologically active compounds isolated from medicinal plants have also doubled in the last decade. Ethanolic and aqueous extracts of *Sesame radiatum* leaves were studied for *in-vitro* antimicrobial activity using agar diffusion method. The gas chromatography-mass spectrometry (GC-MS) phytochemical screening showed the presence of essential oils mainly the phenolic and carboxylic acids groups. The ethanolic extract mildly inhibited the growth of *Streptococcus pneumoniae* and *Candida albicans*, while there was no inhibitory effect on *Staphylococcus aureus*, *Pseudomonas aurogenosa* and *Escherichia coli*. However, aqueous extract exhibited no inhibitory effect on all the five tested micro-organisms.

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

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 (Cohen, 1992; Nascimento et al., 2000). Also, the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized (Shariff, 2001). 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 now, 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 the synthetic drug agents (Iwe et al., 1999).

Sesame belongs to the family- Pedaliaceae and genus-Sesamum (Purseglove, 1974). It is believed to have originated from Africa (Ram et al., 1990). It 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. Also, similar to palm oil, it is known to be effective against many forms of intestinal disorders especially diarrhea and dysentery (Gills, 1992; Ekpa, 1996). 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

*Corresponding Author's E-Mail: drlukemanjoseph@yahoo.com.

measles (anti-viral) and used as hair shampoo for *Taenia capitis* (antifungal properties) (Gills, 1992). Sesame seed oil has been used as healing oil for thousands of years and also enjoyed by humans since the dawn of civilization.

Sesame is also a staple food consumed locally in Nigeria especially in south-west and middle belt areas where it is richly cultivated by local subsistence farmers (Akpan-Iwo, 2002). In Tiv and Idoma areas of Nigeria's Benue state, two breeds of sesame seeds are usually cultivated the *Sesame radiatum* and *Sesame indicum* 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 (Akpan-Iwo, 2002) and this may account for the high fecundity of the people especially among the adult male population (Shittu, 2006) .

The seeds could be consumed either through its oil, roasted or as animal feeds (Johnson et al., 1979).

Extensive study has been carried out on the seed and oils. However, there is paucity of knowledge on the antimicrobial action of the leaves especially on the *S. radiatum* species. More so to confirm the folkloric claims of anti-microbial effect of sesame plants.

This appears to be the first study that looked at the antimicrobial effect of the *S. radiatum* leaves.

MATERIALS AND METHODS

Collection of Plant materials

Sesame plants (*Sesame radiatum*, Schum and Thonn - Pedaliacaea family) were bought from a vendor in Agege market, Lagos after being identified by me 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.

Preparation of extracts

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.

Aqueous extraction of sesame leaves

100 g of the powdered leaves were weighed using a BA210S electronic balance scale and transferred into a 1000 ml size beaker. Distilled water was later added in the ratio of 1:10 to the leaves, and the mixture was heated to boiling temperature and was intermittently stirred on the hotplate for 3 h. This was then filtered using a white sieve cloth into another 1litre beaker. The concentrated filtrate was later dried in a desiccator for 5 days and lyophilized to produce a black shinning crystal powder form with a yield of 83% w/w of the extract. The crude extract was kept in the fridge before being reconstituted and later used for the *in-vitro* study.

Ethanolic and ether-extracts preparation of sesame leaves

The plant parts were air-dried. Each dry powdered plant material (100 g) was extracted with 500 ml of 40% diethyl ether and 98% ethanol for 72 h with Soxhlet equipment using the 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 using gas chromatography-mass spectrometry

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/sec. l µ of extract mixed with diethyl ether (40%), at a split ratio of 1:30 was injected (Shimoda et al., 1996). MS analyses were carried out on a Agilent Technologies Network mass spectrometer (model 5973) coupled to HP 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 1scan/sec 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

Micro-organisms

Staphylococcus aureus, *Pseudomonas aurogenosa*, *Streptococcus pneumoniae*, *Candida albicans* and *Escherichia coli* were obtained from the Microbiology Laboratory of the Lagos State University Teaching Hospital (LASUTH). These micro-organisms were identified and confirmed at the Microbiology department of the Drug Quality Control Laboratory, LASUTH, Ikeja, Lagos. Standard strain of *S. aureus* (ATCC 29213) of oxoid Culti-loop (Oxoid Ltd., Hampshire, England) was also used.

Using modified Collins et al. (1995) method, 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 an ultraviolet (UV) - visible spectrophotometer.

Antimicrobial screening

The modified Collins et al. (1995) agar-well diffusion method was employed to determine the antimicrobial activities for both ethanolic and aqueous extracts. One millilitre (1 ml) of reconstituted and ten folds dilution of aqueous and ethanolic extracts of sesame leave extracts were used against the test microorganisms. Approximately 10 ml of sterile Muller-Hinton Agar (MHA) was poured into sterile

Table 1. Sensitivity of different microorganisms on full concentration and 10 folds dilution of ethanol extract of *Sesame radiatum*.

Micro-organisms	Sensitivity at full concentration	Sensitivity at 10- folds dilution
Streptococcus pneumoniae	++	+
Candida albicans	++	+
Staphylococcus aureus	-	-
Escherichia coli	-	-
Pseudomonas aerugenosa	-	-

Table 2. Sensitivity of different microorganisms to full concentration and 10 folds dilution of aqueous extract of *Sesame radiatum*.

Micro-organisms	Sensitivity at full concentration	Sensitivity at 10- folds dilution
Streptococcus pneumoniae	-	-
Candida albicans	-	-
Staphylococcus aureus	-	
Escherichia coli	-	-
Pseudomonas aerugenosa	-	

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 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.

RESULT AND DISCUSSION

The results obtained showed that full concentration and 10 folds dilution of ethanolic extracts of the leaves of *S. radiatum* plant had inhibitory effects on two of the five tested microorganisms as represented in Tables 1 and 2 respectively. Table 1 shows that the full concentration of the ethanolic extracts had mildly active inhibitory effect on *St. pneumoniae* and *C. albicans*, while Table 2 shows that 10 folds dilution of the ethanolic extract had a mild inhibitory effects on *St. pneumoniae* and *C. albicans*. At both concentrations the ethanolic extracts had no inhibitory effects on *S. aureus, E. coli* and *P. aerugenosa*. Aqueous extracts at both full concentration and 10 folds dilution had no inhibitory effects on all the five tested microorganisms. The mean zone of inhibition was 5 mm for *St. pneumoniae* and that of *C. albicans* was 3 mm.

This appears to be the first study that actually investigated at the antimicrobial effect of *S. radiatum* leaves. However, there has been extensive study on sesame seeds and oil. The seed oil has been found to contain natural antibacterial agents that are effective against common skin pathogens, such as staphylococcus and streptococcus bacteria, as well as common skin fungi including the athlete's foot fungus (Annussek, 2001).

However, in this study, the ethanolic extract had mild inhibitory effects on the *St. pneumoniae* and *C. albicans* while the aqueous extract of the same concentration showed no inhibitory effects on the tested microorganisms. This may be due to loss of some of the active sesame lignans such as sesaminol and its glucosides in view of their water solubility nature during extraction processes of the sesame leaves.

No doubt, several studies have been conducted in the past three decades that focused on the antimicrobial properties of herbs, spices and their derivates 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 researches report 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, 1990). The GC-MS of the methanolic *S. radiatum* leaves extract showed the presence of mainly essential oils such as aromatic phenolic compounds which have been found to have antimicrobial properties (Alma et al., 2003)

However, the pH of compounds in dilutions also may have modified the results, as we observed that when phenolic or carboxylic compounds are present in any extract which is the case in this study. It has been reported that the different effects of neutral essential oil depend on the pH. Thus, for example, anise oil had higher antifungal activity at pH 4.8 than at 6.8, while the oil of *Cedrus deudora* is most active at pH 9.0 (Janssen et al., 1987)

In conclusion, this finding confirmed the folkloric claims of the antimicrobial effectiveness of locally consumed

sesame leaf extracts in many areas of Nigeria. Further study is on to elucidate the active ingredient which is responsible for the antimicrobial properties.

ACKNOWLEDGEMENT

The authors wish to appreciate the technical assistance of the staff of the drug Quality Control Laboratory of LASUTH. The authors provided the financial support for this study.

REFERENCES

Agboola SA (1979). .An Agricultural Atlas of Nigeria Oxford University press, London. pp. 131-135.

Alma MH, Mavi A, Yildirim A, Digrak M, 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. Pharm. Bull. 26: 1725-1729.

Annussek, G. 2001. Sesame oil. In: Gale encyclopedia of alternative medicine.GaleGroupandLooksmart.http://www.hort.purdue.edu/newcrop/ncnu.html accessed in July, 2006.

Cohen ML (1992). Epidemiology of drug resistance: implications for a post antimicrobial era. Science 257: 1050-1055.

Collins CH, Lynes, PM, Grange JM (1995). Microbiological Methods. (7thEdn.) Butterwort-Heinemann Ltd., Britain, pp. 175-190.

Deans SG, Svoboda KP (1989). Antimicrobial activity of summer savory (*Satureja hortensis* L.) essential oil and its constituents. J. Hortic. Sci. 64: 205-210.

Dorman HJD, Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88: 308-316.

Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA (1989). Antimicrobial activity of some Egyptian spice essential oils. J. Food Protect. 52: 665-667.

Gills LS (1992). Ethnomedical uses of plants in Nigeria, Uniben Press, Edo State Nigeria. p. 212.

Hsieh PC, Mau, JL, Huang, SH (2001). Antimicrobial effect of various combinations of plant extracts. Food Microbiol. 18: 35-43.

Janssen AM, Scheffer JJC, Baerheim Svendsen A (1976). Antimicrobial activity of essential oils: a 1976–1986 literature review. Aspect of the test methods. Planta Medica 53: 395–398.

Johnson LA, Suleiman TM, Lucas EM (1979). Sesame protein: A review and prospectus. J. Am. Oil Chem. Soc. 56: 463-468.

Kivanc M, Akgul A (1986). Antibacterial activities of essential oils from Turkish spices and Citrus. Flavour and Fragrance 1: 175-179.

Nascimento Gislene GF, Juliana Locatelli, Paulo C Freitas, Giuliana L Silva (2000). Antibacterial activity of plant extracts and phytochemcals on antibiotic resistant bacteria. Braz. J. Microbiol. 31: 247-256

Purseglove JW(1974) Tropical Crops: Dicotyledons. Longman Group, London, UK. pp. 430–435.

Ram, RD Catlin J, Romero, Cowley C (1990). Sesame: New approaches for crop improvement. p: 225–228. In: J. Janick, JE Simon (eds.), Advances in new crops. Timber Press, Portland

R´ıos JL, Recio MC (2005). Medicinal plants and antimicrobial activity J. Ethnopharmacol. 100: 80–84.

Shariff ZU (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. pp. 9 – 84.

Shittu LAJ (2006). The effect of the aqueous crude leaves extract of Sesamum radiatum compared to mesterolone (proviron) on the adult male sprague -dawley rats testis and epididymis. MSc. dissertation.