

Antibacterial and Antifungal Activities of Essential Oils of Crude Extracts of *Sesame Radiatum* against Some Common Pathogenic Micro-Organisms

SHITTU LAJ, BANKOLE M.A., AHMED T., BANKOLE M.N., SHITTU R.K., SAALU C.L., ASHIRU O.A.

For author affiliations, see end of text.

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ABSTRACT

Concern about the rising prevalence of antibiotics-resistant strains of pathogenic microorganisms 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. As a result of paucity of knowledge and folkloric claim on the leaves effectiveness in infectious disease treatments, we aimed to determine the antimicrobial activity of essential oils and lignans present in the crude *Sesame radiatum* leaves extracts. Ethanolic, Methanolic and Aqueous extracts of Sesame radiatum leaves were studied for their in-vitro 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 the presence of carboxylic acids and phenolic groups in essential oils especially some of the most potent antioxidants like Sesamol, Sesamolin and Sesamin. Both the methanolic and ethanolic extracts have broad spectrum antimicrobial effect against all the tested micro-organisms except *Streptococcus pneumoniae*, *Candida albicans* and *Staphylococcus aureus* respectively, while the aqueous extract exhibited no inhibitory effect on *Staphylococcus aureus* and *Streptococcus pneumoniae* except on *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 the Country (Nigeria).

Keywords: Pathogenic micro-organisms, Anti-microbial, Sesame leaves, GC-MS

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. [1].

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 [2, 3, 4, 5].

More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal [6] and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies [7, 8, 5].

Santo et al, [9] remarked that the World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic

drugs. No doubt, some studies have identified and isolated the main active ingredients in the plants responsible for this antimicrobial activity [10, 11]. However, the study on medicinal plants will allow for the demonstration of their physiological activity and also catalyze many pharmacological studies that will lead to the development of more potent drugs with no or minimal toxicity and high sensitivity especially towards the emerging microbial agents [12, 13].

Sesame belongs to the family Pedaliaceae and genus *Sesamum* [14]. The genus consists of about 36 species of which 19 species are indigenous to Africa [15, 16].

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 has been found to be

Table 1. Sensitivity of 3 micro-organisms to ethanolic extracts of *Sesamum radiatum* leaves

Micro-Organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
<i>Staphylococcus aureus</i>	-	-	-	-	-
<i>Streptococcus pneumoniae</i>	+++	-	-	-	-
<i>Candida albicans</i>	++	+	-	-	-

(+) susceptibility (inhibition zone ≥ 10 mm)

(-) absence of susceptibility

The MIC of *Streptococcus pneumoniae* was at full concentration = 76.2 μ g

While for *Candida albicans* was at 1:2 = 28.2 μ g/ml.

effective against chicken pox and measles (anti-viral) and used as hair shampoo for *Taenia capitis* (antifungal properties) [17].

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 *S. alatum* (Thonn), *S. indicum* L. and *S. radiatum* Schum & Thonn, are widely cultivated for different purposes [18]. However, in Tiv and Idoma areas of Nigeria's Benue state, two breeds of Sesame -the *Sesame radiatum* and *Sesame indicum* are usually cultivated mainly for their seeds and leaves [19]. 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 [20] and this may account for the high fecundity of the people especially among the adult male population [21]. The seeds could be consumed either through its oil, roasted or as animal feeds [22].

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 *Sesamum radiatum*, hence the basis for this study in order to prove the folkloric claims

MATERIALS AND METHODS

Collection of Plant materials

Sesame plants (*Sesamum radiatum*, Schum and Thonn - Pedaliaceae family) were bought from a vendor in Agege market, Lagos after being identified by the author 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 [21]. Voucher specimens were deposited at the Botany departments of University of Ibadan and Lagos State University respectively.

Preparation of Extracts

The leaves having been separated from the rest of the plants were air dried for 2 weeks and later grounded into a powdery form using a grinder.

Aqueous, Ethanolic and Methanolic Extraction of Sesame leaves

Modified Okogun [23] method of extraction was adopted in the process. Such that the diluents used were absolute ethanol, methanol and sterile distilled water. To 10ml of each diluent in a 20ml screw cap bottle was added 1g of the raw air-dried and grinded Sesame leaves.

The modification was in the extraction time which was for 5 days (120 hours) and the storage of the solution took place in the refrigerator at 4°C. The extracts obtained were regarded as the full concentration.

Phytochemical screening using Gas Chromatography-Mass Spectral

Crude methanolic extracts of Sesame leaves were analyzed by GC/MS.

GC analyses 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 (μ 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 22cm/sec. One millilitre of extract mixed with methanol (80%), at a split ratio of 1:30 was injected. [24].

GC/MS analyses were carried out on a Agilent Technologies Network mass spectrometer (model 5973) coupled to H.P. gas chromatograph (model 6890)

Table 2. Sensitivity of 3 Micro-organisms to Aqueous extracts of *Sesamum radiatum* leaves

Micro-Organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
<i>Staphylococcus aureus</i>	-	-	-	-	-
<i>Streptococcus pneumoniae</i>	-	-	-	-	-
<i>Candida albicans</i>	+	+	+	+	-

(+) susceptibility (inhibition zone ≥ 10 mm)

(-) absence of susceptibility

The MIC for *Candida albicans* was at 1: 6 = 31.0 μ g/ml

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

Micro-Organisms	Sensitivity				
	Full	1:2	1:4	1:6	1:8
<i>Staphylococcus aureus</i>	+	+	-	-	-
<i>Streptococcus pneumoniae</i>	-	-	-	-	-
<i>Candida albicans</i>	-	-	-	-	-

(+) susceptibility (inhibition zone ≥ 10 mm)

(-) absence of susceptibility.

The MIC for the staphylococcus aureus was at 1: 2 = 39.3µg/ml.

equipped with NBS 75K Library Software data. The capillary column and GC conditions were as described above. Helium was the carrier gas, with a flow rate of 22cm/s. Mass spectra were recorded at 70 eV /200°C. The scanning rate of 1scan/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 pneumoniae* (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 micro-organisms 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 of the 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 37°C for 24 hours except for *Candida albicans* which was incubated at 25°C for 24-48 hours.

Standardization of micro-organisms

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

Standard antibiotics

The primary standard of Cloxacillin was obtained from Sigma-Aldrich (St Louis, MO, USA) was used. However, the secondary standards with antibacterial and antifungal activity used for comparison with the extract were from local manufacturers in the Nigeria.

Antimicrobial screening

The modified Collin et al [25] 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 1ml of each reconstituted extract in 2ml, 4ml, 6ml and 8ml 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 [25], approximately 10ml of sterile Muller-Hinton Agar

(MHA) was poured into sterile culture plates and allowed to set. About 10ml of the antibiotic medium No 2 seeded with 0.5ml of 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 8mm in diameter were punched on the plates. About 0.5ml of each dilution of the extracts was dispensed into the wells and the plates were incubated at 37°C for 24 hours except for the plates seeded with *Candida albicans* which were incubated at 25°C for 24-48 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

Table 4. The minimum inhibitory concentrations (MICs) of crude extract of *Sesamum radiatum* leaves on tested microorganisms.

Microorganisms	Crude Extract	Full	1: 2	1: 4	1: 6	1: 8
<i>S. aureus</i>	Methanolic		39.3			
	Ethanolic					
	Aqueous					
<i>S.pneumoniae</i>	Methanolic					
	Ethanolic	76.2				
	Aqueous					
<i>C. albicans</i>	Methanolic					
	Ethanolic		28.2			
	Aqueous				31.0	

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

Table 5. Antimicrobial effect of primary (1o) standard (Cloxacillin) and secondary (2o) standard antibiotics (Amoxycillin tablet, Ampiclox tablet, 500mg) and antifungal (Clotrimazole vaginal tablet 200mg and Fluconazole tablet, 500mg) on tested pathogenic microorganisms.

Name of antibiotic/ antifungal	Zone diameter of Microorganisms			Concentration Used ($\mu\text{g/ml}$)
	Staph. aureus	Strept pneumoniae	Candida albicans	
Fluconazole tablet 500mg (2o standard)	—	—	40mm	100
Clotrimazole Vaginal tablet (2o standard)	—	—	35.5mm	100
Ampiclox tablet 500mg (2o standard)	30.5mm	—	—	100
Amoxycillin (2o standard)	15.5mm	11.5mm	—	100
Cloxacillin tablet (1o standard)	30 mm	—	—	100

Measurement of zone of inhibition

The zones of inhibition of the tested microorganisms by the extracts were measured using a Fisher-Lilly antibiotic zone reader model 290 (U.S.A). The diameter sizes in mm of the zone of inhibition are shown in the respective tables.

Minimum Inhibitory Concentration (MIC) of extract

The MIC for each microorganism used was determined using microdilution method described by Eloff [26] as the last dilution of the extract that inhibited the growth of the tested pathogenic micro-organisms. The various MIC are shown in the respective tables.

RESULTS

The results obtained in table 1 showed that ethanolic extract had a very strong antimicrobial effect against *Streptococcus pneumoniae* at full concentration while a strong and mild antimicrobial effect on *Candida albicans* at full and 1:2 dilution of the extracts respectively. Ethanolic extract of Sesame leaves had no inhibitory effects on *Staphylococcus aureus*.

Table 2 reveals that the methanolic extract exhibited a mild antimicrobial activity against *Staphylococcus aureus* at both the full concentration and 1:2 dilution of the extract and no inhibitory effect on either the *Streptococcus pneumoniae* or *Candida albicans*.

Table 3 showed that *Candida albicans* was mildly inhibited 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 microorganisms.

Table 4. Displays the minimum inhibitory concentrations (MICs) of different crude extracts of *Sesamum radiatum* leaves on tested microorganisms.

Table 5. Displays the obtained inhibition zones of the different types of standard antibiotics and antifungal agents against the selected tested microorganisms. This

also includes the minimum concentrations of the standards used as well.

The GC-MS showed that the methanolic extract of sesame radiatum leaves contained mainly essential oils such as aromatic phenolic compounds- sesamol, sesaminol, sesamin, carboxylic acids and other classes of compounds including fatty acids like palmitic acids, arachidonic/arachidic acid, stearic acid, myristic acid, oleic acid, linoleic acids, thiazole, pyrroles, disulphide and aldehyde.

DISCUSSION

To our knowledge this study appears to be the first that actually looked at the antimicrobial effect of *sesamum radiatum* leave extracts. However, there has been extensive study on sesame seeds and oil respectively in the past.

Muller-Hinton Agar diffusion (MHA) method was 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 [27].

Several studies conducted in the past three decades had focused on the antimicrobial properties of herbs, spices and their derivatives such as essential oils, extracts and decoctions [28, 29, 30, 31]. Some researchers had also reported that there was a relationship between the chemical structures of the most abundant compounds in the tested extracts or essential oils and the antimicrobial activity [32, 33].

The seed oil of Sesame spp was found to contain certain natural antibacterial agents that were effective against common skin pathogens, such as *Staphylococcus* and *Streptococcus* bacteria, as well as common skin fungi including the athlete's foot fungus [34].

The GC-MS of the methanolic *Sesame radiatum* leaves extract did show the presence of mainly essential oils such as aromatic phenolic compounds, which were found to possess antimicrobial properties [31] for example, Sesamol that is one of the most potent antioxidants known to man discovered in the leaves was reported for the first time by us [21].

In this study, the methanolic extracts showed antibacterial effect against all the tested micro-organisms except *Streptococcus pneumoniae* and *Candida albicans*. The ethanolic extract had no inhibitory effects against *Staphylococcus aureus* but had both antibacterial and antifungal activity [5].

The pH of compounds in dilutions had 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 deodorawas* was most active at pH 9.0 [35].

Ethanolic extracts (less acidic) was more effective against *Candida albicans* than the methanolic extract which had no inhibitory effect. In addition, aqueous extract had antifungal activity at a higher pH but with less potency compared to the ethanolic extracts as reflected by the different MICs obtained.

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 extracted effectively during extraction processes of the Sesame leaves [36].

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 than the methanolic extractive procedure in preservation of the oily and water soluble active ingredients with proven anti-microbial properties especially against yeasts.

The MIC against *Candida albicans* was lower in the ethanolic than in the aqueous extracts that is, 28.2 µg/ml compared to 31.9 µg/ml. No doubt, the cooling effect of the refrigerator (4°C) and the longer duration of extraction about 5 days compared to the room temperature and 2-3 days in the standard procedure using different solvents respectively did contribute to adequate preservation and extraction of the active ingredients from the sesame plants.

The modified procedure of Okogun [23] used in this study also made room for more effective potency of the extracts to be appreciated compared to the standard way of extraction using lysophilization and fractional distillation/rotatory evaporator with standard procedure [21] which have often times destroyed some of the active ingredients in the plants [5]. However, the antimicrobial findings of *Sesamum radiatum* leaves observed in this study were supplementary to our earlier reports [37].

The antimicrobial effectiveness of the different sesame leaves extracts was similar to that of standard antibiotics and antifungals used as seen in the tables 4 and 5 respectively. More so, the zone of inhibition obtained especially against the *staphylococcus aureus* of the methanolic extract of sesame leaves (39.3 mm) was found to be higher than that of the primary standard antibiotic-cloxacillin (30.0mm) used in this study. This implied a relative effectiveness of the extract over the standard primary antibiotic.

CONCLUSION

This finding confirmed the folkloric claims of the antimicrobial effectiveness of locally consumed Sesame leaves extracts in many areas of the Country (Nigeria). However, it is very effective against bacterial and other common skin infection including yeast.

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CURRENT AUTHOR ADDRESSES

Shittu LAJ, Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria. E-mial: drlukemanjoseph@yahoo.com (Corresponding author)

Bankole M.A., Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos/Lagos University Teaching Hospital, Idi-araba, Lagos, Nigeria.

Ahmed T., Department of Microbiology, Drug Quality Control Laboratory/Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria.

Bankole M.N., Diarrhea Unit, Microbiology Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.

Shittu R.K., Medical Microbiology Unit, Bolomedics Laboratories, Egbeda, Lagos, Nigeria.

Saalu C.L., Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.

Ashiru O.A., Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.