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# Antimicrobial activity of the aqueous and methanolic extracts of *Sesamum radiatum* (Schum and Thonn.)

AO Akanmu<sup>1</sup>\*, ST Balogun<sup>1</sup>, AD Tuksa<sup>2</sup>, OA Sodipo<sup>1</sup> & IA Gulani<sup>3</sup>

- <sup>1.</sup> Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Medical Sciences, University of Maiduguri, PMB 1069, Maiduguri, Nigeria
- <sup>2.</sup> Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmacy, University of Maiduguri, PMB 1069, Maiduguri, Nigeria
- <sup>3.</sup> Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Maiduguri, Nigeria

#### \*Correspondence: Tel.: +2348037276800; E-mail: aoakanmu@gmail.com

Abstract
Abstract Sesamum radiatum (Schum and Thonn.) is a leafy vegetable belonging to the family Pedialiaceae, it is used traditionally in the treatment of conditions such as diarrhoea, dysentery and fungal infections. This study investigated the phytochemical constituents and the antimicrobial activity of Sesamum radiatum (Schum and Thonn.). The pulverized plant material was subjected to cold marceration using distilled water and methanol for the aqueous and methanolic extracts, respectively. The plant extracts were further subjected to phytochemical screening using standard procedures and <i>in</i> <i>vitro</i> antibacterial sensitivity tests using the disc diffusion method. Zones of inhibition, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined. Flavonoids, terpenoids, cardiac glycosides and cardenolides were found in both the aqueous and methanolic extracts. The results of the <i>in vitro</i> antimicrobial susceptibility test showed that the aqueous extract inhibited the growth of Candida albicans at the highest concentration of 600 mg/ml with a zone of inhibition of 8.00±0.00 mm while the remaining microorganisms were resistant at all the concentrations. The methanolic extract inhibited the growth of Salmonella typhi at concentrations of 200 mg/ml, 400 mg/ml and 600 mg/ml and Pseudomonas
<i>aeruginosa</i> at concentrations of 400 mg/ml and 600 mg/ml. In conclusion, the aqueous extract of <i>Sesamum radiatum</i> showed antifungal activities which may justify its folkloric use and the methanolic extract inhibited the growth of <i>S. typhi</i> which also justifies its use traditionally, in the treatment of dysentery and diarrhea.

#### Keywords: Antimicrobial, Phytochemical constituents, Sesamum radiatum, MIC, MBC

#### Introduction

Infectious diseases are the leading cause of deaths in developing countries (Pamar & Rawat, 2012). The frequent use of antimicrobial agents led to the emergence of widespread resistant strains of pathogenic organisms. Increase in resistance of these pathogenic organisms (Cohen, 1992), high cost, adulteration and potential side effects of these common antimicrobial drugs coupled with their inadequacy in treating diseases further compound the challenges of multi-drug resistant strains of pathogenic organisms (Shariff, 2001). The World Health Organization (WHO) reported that 80 % of African populations use traditional medicine to meet their primary healthcare needs, most of which involve the use of plants (Quave, 2016). There has been an increasing research on medicinal plants to validate their folkloric uses (Nascimeto *et al.*, 2000; Rios & Reico, 2005). These plants have been a valuable source of medicinal agents with proven potential of treating infections and minimal side effects when used cautiously (Iwe *et al.*, 1998). Plants are the treasure houses of potential drugs that could be the source to obtain variety of future drugs (Thite *et al.*, 2013).

Sesamum radiatum (Schum and Thonn), commonly called Benniseed or Sesame seed (English), eweatura (Yoruba) and karkashi (Hausa) (Jimam et al., 2015), is one of such plants with significant medicinal values. It is a plant of African origin belonging to the family Pedialiaceae (Purseglove, 1974). It occurs wild in West and Central Africa and is also cultivated there on a small scale. It does not occur in East and South Africa (except in Northern Angola), but it is sometimes cultivated and found naturalized in Tropical Asia (Bedigian, 2003). The decoction of the leaves is used for the treatment of catarrh, eye pains, bruises and erupted skins (Bankole et al., 2007) and many forms of intestinal disorders especially diarrhea and dysentery (Gills, 1992). Its warm water leaves infusion is used as gargle to treat inflamed oral membranes (Gills, 1992). The decoction of both leaves and root has been found to be effective against chicken pox and measles and has a cosmetic use as a shampoo for Taenia capitis (Gills, 1992). Several literatures also indicate that S. radiatum is used by several communities because of its ability to improve fertility and ease childbirth (Ojekale et al., 2006; Konan et al., 2013). The hypotensive effects of aqueous extract of Sesamum radiatum was also reported by Konan et al. (2013). In studies carried out by Konan et al. (2013) and Hamzah et al. (2013), Sesamum radiatum extract was found to contain flavonoids, phenols, tannins and terpenoids. These phytochemical constituents have been linked with antibacterial activities (Mujeeb et al., 2014). Shittu et al. (2006), Bankole et al. (2007), Ahmed et al. (2009), Osibote et al. (2010) and Agbankpe et al. (2016) reported the antimicrobial activities of the leaf extracts whereas Seukep et al. (2013) reported the antimicrobial activity of the leaves and stem of the plant. However, the present study was aimed at investigating the antimicrobial activity of the aqueous and methanolic extracts of the whole plant of *S. radiatum* (Schum and Thonn.), with focus on antibacterial and antifungal activities.

#### **Materials and Methods**

#### Plant collection and identification

Plant materials used in this study were purchased from the market in Maiduguri, Nigeria. The plant was identified and authenticated at the Biological Sciences Department, Faculty of Science, University of Maiduguri. A voucher specimen (Voucher no: 016) was deposited at the Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics, University of Maiduguri.

#### Preparation of plant extracts

The whole plant *S. radiatum* was shade dried at room temperature and pulverized using a mortar and pestle. The powdered material was weighed and stored. Two hundred and fifty grams each of the powdered material was subjected to maceration using 2 L of each solvent (99 % methanol and distil water). The solution was allowed to stand for 24 hours with periodic shaking and then filtered. The filtrate was evaporated using a water bath at  $50^{\circ}$  C. The percentage yield was determined for each solvent (De & Ifeoma, 2002) using the formula:

% Percentage yield extract =  $\frac{CX}{CY}$  X 100 where; CX = final weight (g) after extraction process

CY= initial weight (g) taken for extraction Measures of 10 grams and 5 grams respectively of the extract were reconstituted in 10 ml and 20 ml of distilled water to obtain solution of different concentrations used for the antimicrobial screening.

#### Test microorganisms

A total of nine microorganisms were used in this study: four Gram negative bacteria (*Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), four Gram positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis and Corynebacterium spp.*) and one fungal species (*Candida albicans*). These organisms were clinical isolates obtained from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria.

# Phytochemical Screening

The extracts were subjected to phytochemical screening to determine the presence of alkaloids, carbohydrates, flavonoids, saponins, tanins, glycosides, (cardiac, steroidal),

terpenes/terpenoids, fatty acids, resins using procedures described by Brian & Turner (1975); Vishnoi (1979); Markham (1982); Silva *et al.* (1998); Sofowora (2008); Evans (2009) as follows:

# Test for carbohydrates

General test (Molisch's test): A few drops of Molisch's reagent were added to the extract which was dissolved in distilled water. This was followed by the addition of 1 ml of concentrated tetraoxosulphate (VI) acid ( $H_2SO_4$ ) by the side of the test tube, so that the acid formed a layer beneath the aqueous layer. The mixture was then allowed to stand for two minutes and then diluted with 5 mL of distilled water. Formation of a dull violet colour at the interface of the layers showed a positive test (Evans, 2009).

Test for reducing sugars (Fehling's test): The extract (0.2 g) was dissolved in distilled water and filtered. The filtrate was heated with 5 ml equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide ( $Cu_2O$ ) indicated the presence of reducing sugar (Evans, 2009).

Test for combined sugars: The extract (0.2 g) was hydrolyzed by boiling with 5 ml dilute hydrochloric acid and the resulting solution was neutralized with sodium hydroxide solution. A few drops of Fehling's solution were added to it and then heated on a water bath for 2 minutes. Formation of a reddish brown precipitate of cuprous oxide indicated the presence of combined reducing sugar (Evans, 2009).

Standard test for ketones (Salivanoff's test): A few drops of resorcinol and 2 ml of hydrochloric acid were added to a small quantity of the extract and the solution boiled for 5 minutes. A red colouration indicated the presence of Ketones (Vishnoi, 1979).

Test for pentose: To a small quantity of the extract, 1 ml of hydrochloric acid and a little quantity of phloroglucinol were added. The mixture was heated on a low flame and appearance of a red colour indicated the presence of pentose (Vishnoi, 1979).

Test for soluble starch: A small quantity of the extract was boiled with 1 ml of 5 % potassium hydroxide (KOH), cooled and acidified with  $H_2SO_4$ . A yellow coloration showed the presence of soluble starch (Vishnoi, 1979).

# *Test for cardiac glycosides* Salkowiski's test

The plant extract (0.5 g) under study was dissolved in 2 ml of chloroform. Tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish brown colour at the interphase indicated the presence of a steroidal ring (i.e., aglycone portion of cardiac glycoside) or methylated steroids (Silva *et al.*, 1998).

#### Liebermann- Burchard's test

Steroidal nucleus: To 0.5 g of the extract, 3 ml acetic anhydride was added. After it had been dissolved, it was well cooled in ice. Concentrated tetraoxosulphate (VI) acid was carefully added. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e., the aglycone portion of cardiac glycoside (Silva *et al.*, 1998).

Test for terpenoids: A little amount of the extract was dissolved in ethanol. To it, 1 ml of acetic anhydride was added, followed by the addition of conc.  $H_2SO_4$ . A colour change from pink to violet indicated the presence of terpenoids (Silva *et al.*, 1998)

# Test for flavonoids

Shinoda's test: The extract (0.5 g) to be tested was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by a few drops of conc. HCl. A pink colouration indicated the presence of flavonoids (Markham, 1982).

Ferric chloride test: The extract was boiled with distilled water and then filtered. To the 2 ml of the filtrate, a few drops of 10 % ferric chloride were then added. A green-blue colour indicated the presence of phenolic hydroxyl group (Evans, 2009).

#### Lead ethanoate test

A small quantity of the extract was dissolved in water and filtered. To 5 ml of the filtrate, 3 ml lead ethanoate solution was added. Appearance of a buff coloured precipitate indicated the presence of flavonoids (Brian & Turner, 1975).

#### Sodium hydroxide test

A small quantity of the extract was dissolved in water and filtered. 2 ml of 10% aqueous sodium hydroxide was added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid indicated the presence of flavonoids (Evans, 2009).

# Test for saponins

One gram of the extract was boiled with 5 ml of distilled water, filtered and the filtrate was divided into two portions.

To the first portion, 3 ml of distilled water was added and then shaken for about 5 minutes. Frothing which persisted on warming was an evidence of the presence of saponins (Sofowora, 2008).

To the second portion, 2.5 ml of a mixture of equal volumes of Fehling's solutions was added. A brick red precipitate indicated the presence of saponin glycosides (Vishnoi, 1979).

# Test for phlobatannins

A small amount of each extract was boiled with distilled water and filtered. The filtrate was further boiled with 1 % aqueous HCL. The appearance of a red precipitate showed the presence of phlobatannins (Evans, 2009).

# Test for tannins

The extract (0.5 g) to be tested was stirred with about 10 ml of distilled water. The filtrate was used for the following test; To 2 ml of the filtrate, a few drops of 1 % ferric chloride solution was added and the occurrence of a blue-black precipitate showed the presence of tannins. Two millilitre of 10% lead ethanoate was added to an equal volume of the filtrate. Formation of a white precipitate indicated the presence of tannins. The filtrate of the extract was boiled with 3 drops of 10 % HCl and 1 drop of methanol and a red precipitate indicated the presence of tannins (Sofowora, 2008; Evans, 2009).

# Test for alkaloids

Preliminary test for alkaloids: The extract (0.5 g) was stirred with 5 ml of 1 % aqueous HCl on water bath and then filtered. Of the filtrate, 3 ml was taken and divided equally into 2 portions in test tubes. To the first portion, a few drops of Draggendoff's reagent were added. The occurrence of an orange-red precipitate was taken as a positive.

To the second portion, 1 ml Mayer's reagent was added and the appearance of a buff-coloured precipitate indicated the presence of alkaloids and to the last 1 ml, a few drops of Wagner's reagent was added and a dark-brown precipitate indicated the presence of alkaloids (Brian & Turner, 1975).

# Test for cardenolides

Keller-Killiani's test: The plant extract (0.5 g) was dissolved in 2 ml glacial acetic acid containing a drop of ferric chloride solution, and 1 ml of concentrated tetraoxosulphate (VI) acid was added. The appearance of a brown ring at the interphase indicated the presence of digitoxose sugar characteristic of cardenolide. A violet ring would appear just below the brown ring, while in the acetic acid layer a greenish ring would form just above the brown ring and gradually spread throughout this layer (Evans, 2009).

# Antibacterial susceptibility test of the extracts

The antimicrobial susceptibility test was carried out using the agar plate disc diffusion technique as described by Usman & Osuji (2007). The tests were carried out using a stock concentration of 1000 mg/ml of the aqueous extract and 500 mg/ml of the methanolic extract by dissolving 10 g and 5 g respectively into 20 ml and 10 ml sterile distilled water. Working volumes were 0.5 mL each of the concentrations prepared and then dispensed into each of the 9 mm bored holes to afford respectively of 600 mg, 400 mg, 200 mg and 100 mg/ hole of both aqueous and methanolic extracts. After incubation at 37 °C for 24 hours, the average diameter of three readings of the clear zone around the hole was recorded as the measure of inhibitory level of the extract against the test bacteria and reported as mean±SEM. The dilution ratio for grampositive bacteria and gram-negative bacteria was 1:1000 and 1:5000 respectively using peptone water (Usman & Osuji, 2007). The plates were inoculated with the same standardized inoculum to check for the activities of standard drugs against the tested organisms using standard antimicrobial disc Ciprofloxacin (10  $\mu$ g) and Tetracycline (50  $\mu$ g).

# Disc diffusion antifungal selectivity test

In testing for antifungal activity of *S. radiatum* against *C. albicans*, Sabouraud Dextrose Agar (SDA) seeded with a 24 hours old *Candida albicans* was layered on the Muller-Hinton Agar (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 and standard drug (fluconazole) were dispensed into the wells and the plates were incubated at 28 °C for 72 hours and then checked for activities (Doss & Anad, 2013). At the end of the period, inhibition zones formed on the medium were evaluated in millimetres.

#### Data analysis

Data obtained was subjected to statistical analyses using suitable statistical software (GraphPad InStat version 5.01, 2007). The results are expressed as Mean  $\pm$  SEM, p<0.05 was taken as accepted level of significant difference.

#### Results

The aqueous extract was greenish brown with a flaky texture and a pungent odour, while the methanolic extract appeared dark green with a gummy texture and pungent odour. The percentage yield of the aqueous and methanolic extracts were 10.74 % and 7.44 % respectively.

The results of the phytochemical screening of the aqueous and methanolic extracts are presented in Table 1. Flavonoids, terpenoids, cardiac glycosides and cardenolides were observed to be present in both extracts. Saponin was observed to be present in only aqueous extract whereas carbohydrates

were observed to be present in methanolic extract. The results of antibacterial susceptibility test showed marked differences in the susceptibility of various bacterial isolates (Tables 2 and 3). The aqueous extract did not demonstrate antibacterial activity against any of the isolates at the concentration tested. However, the methanolic extract was found to be active against *S. typhi* and *P. aeruginosa*. The methanolic extract of *S. radiatum* inhibited the growth of *S. typhi* at concentration of 200 mg/ml, 400 mg/ml and 600 mg/l while *P. aeruginosa* was inhibited at 400 mg/ml and 600 mg/ml which were significant when compared with the standard drug tested.

The extracts inhibited the growth of *Candida albicans* at the highest concentration of 600 mg/ml with a zone of inhibition of  $8.00\pm0.00$  mm while the remaining microorganisms were resistant at all the concentrations.

Table 1: Phytochem	nistry of the aqueo	us and methanolic extracts	of Sesamum radiatum	(Schum and Thonn.)
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		Results
Plant Constituents/Test	Aqueous	Methanolic
Carbohydrates		
General test (Molisch's Test)	-	+
Test for monosaccharide (Barfoed's Test)	-	-
Test for free reducing sugars (Fehling's Test)	-	+
Test for combined reducing sugars	-	+
Test for Ketose	-	+
Test for Soluble Starch	-	-
Test for Cardiac glycosides		
Salkowski's test	+	+
Lieberman-Burchard's test	-	+
Test for Terpnoids	+	+
Test for Flavonoids		
Shinoda's test	+	-
Ferric Chloride	-	-
Lead Acetate	-	-
Sodium hydroxide	+	+
Test for Saponin Glycoside		
Frothing test	+	-
Test for Phlobatannins	-	-
Test for Tannins		
Ferric Chloride	-	-
Lead acetate	-	-
Test for Alkaloids		
Dragendoff's reagent	-	-
Mayer's reagent	-	-
Test for Cardenolites		
Keller-Killani's test	+	+
Kev: Absent		

Key:- = Absent

+ = Present

	Zones of Inhibition (mm)/Resistance					
Name of the strain	100 mg/ml	200 mg/ml	400 mg/ml	600	Ciprofloxacin	Tetracycline
				mg/ml		
E. coli	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	21.67 ± 0.33	9.67 ±0.33
S. typhi	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	20.00 ± 0.00	13.33 ± 0.33
K. pneumoniae	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	$10.00 \pm 0.00$	9.33 ± 0.33
P. aeruginosa	R <sup>*</sup>	R <sup>*</sup>	R <sup>*</sup>	$R^*$	22.00 ± 1.00	R
Staph. aureus	$R^*$	$R^*$	R <sup>*</sup>	$R^*$	26.67 ± 0.88	R
S. pyogene	$R^*$	$R^*$	R <sup>*</sup>	$R^*$	25.33 ± 0.33	R
B. subtitis	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	$18.00 \pm 0.00$	10.67 ± 0.33
C. spp	R <sup>*</sup>	R <sup>*</sup>	R <sup>*</sup>	$R^*$	31.67 ± 0.33	R

Table 2: The zone of inhibition produced by the aqueous extract of Sesamum radiatum (Schum and Thor	nn.
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The results are expressed as mean ± SEM, n=3 per group

Key: R= Resistant

Statistically different when compared with Standard drug Ciprofloxacin (P<0.05)

<sup>#</sup> Statistically different when compared with Standard drug Tetracycline (P<0.05)

Name of the			Zones of Inh	ibition (mm)/Resis	stance		
strain	100	200 mg/ml	400 mg/ml	600 mg/mg	Ciprofloxacin	Tetracycline	
	mg/ml						
E.coli	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	21.67 ± 0.33	9.67 ± 0.33	
S. typhi	$R^{*\#}$	$7.00 \pm 0.00^{*#}$	$9.00 \pm 00^{*#}$	$11.00 \pm 00^{*#}$	$20.00 \pm 0.00$	13.33 ± 0.33	
K. pneumonia	$R^{*\#}$	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	$10.00 \pm 0.00$	9.33 ± 0.33	
P. aeruginosa	$R^*$	R <sup>*</sup>	$7.67 \pm 0.33^{*}$	$9.67 \pm 0.33^{*}$	$22.00 \pm 1.00$	R	
Staph. aureus	$R^*$	R <sup>*</sup>	R <sup>*</sup>	R <sup>*</sup>	26.67 ± 0.88	R	
S. pyogene	$R^*$	R <sup>*</sup>	R <sup>*</sup>	$R^*$	25.33 ± 0.33	R	
B. subtitis	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	R <sup>*#</sup>	$18.00 \pm 0.00$	10.67 ± 0.33	
C. spp	R <sup>*</sup>	R <sup>*</sup>	R <sup>*</sup>	R <sup>*</sup>	31.67 ± 0.33	R	

The results are expressed as mean ± SEM, n= 3 per group

Key: R = Resistant

\* Statistically different when compared with Standard drug Ciprofloxacin (P<0.05)

<sup>#</sup> Statistically different when compared with Standard drug Tetracycline (P<0.05)

Table 4: Antifungal activity of the aqueous and methanolic extracts of Sesamum radiatum(Schum
and Thonn.) against <i>C. albicans</i>

		Zones of Inhibition (mm)/Resistance					
Extracts	100	200	400	600	Fluconazole		
	mg/ml	mg/ml	mg/ml	mg/ml			
Aqueous	R	R	R	8.00 ± 0.00	15.33 ± 0.33		
Methanolic	R	R	R	$8.00 \pm 0.00$	15.33 ± 0.33		

The results are expressed as mean ± SEM, n= 3 per group Key: R = Resistant

#### Discussion

Phytochemical constituents show therapeutic effects against different infectious diseases (Omojate *et al.*, 2014) and these are responsible for different physiological actions and antimicrobial activities. The phytochemical screening of the extracts showed that *S. radiatum* is rich in some bioactive components such as flavonoids,

carbohydrates, cardenolides, cardiac glycosides, saponins and terpenoids. However, previous studies carried out by Hamzah *et al.* (2013) reported that the methanolic extract of *Sesamum radiatum* contains alkaloids, tannins and saponins which is contrary to the findings of this study. Altitude, temperature, illumination and moisture have been reported as an important factor that influence the accumulation and metabolism of secondary metabolites and their differences in different locations have also contributed to the differences in active ingredient contents of medicinal plants (Liu *et al.*, 2016). Most of these phytochemical constituents have been previously reported to have medicinal activity (Yahaya *et al.*, 2012). Terpenoid has been reported to be useful in herbal medicines and showed some strong antimicrobial significance against some potential enteric pathogens (Yahaya *et al.*, 2012).

Results of antibiotic susceptibility showed that nearly all the selected Gram negative and Gram positive bacteria were resistant to the aqueous extract at the tested concentrations. This may occur as a result of number of phenolases and hydrolases that are released when plant materials are ground in water or plant cells are damaged. These enzymes have been reported to modulate the activity of active components in plant extract (De & Ifeoma, 2002) and this may contribute to low activity of the aqueous extract. This might also occur as a result of inability of the water to extract some phytochemical components in the plant (De and Ifeoma, 2002). However, the in vitro antifungal susceptibility test of the plant extract of S. radiatum showed activity against Candida albican at higher concentrations. The in vitro antibacterial activities of methanolic extract of S. radiatum were found to have activity against S. typhi and P. aeruginosa. This study is in agreement with work done by Shittu et al. (2006) who reported that the ethanolic extract showed a mild inhibitory effect on S. pneumoniae and C. albicans while the aqueous extract of the same concentration showed no inhibitory effects on the tested microorganisms. These observations may be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the different media used in the assay. In the present study, lack of antibacterial activity of the aqueous extract observed may be due to loss of some active compounds during extraction process or there may be lack of active compound or lack of solubility of active constituents in aqueous solution (Anjana et al., 2009). Alternatively, dose levels employed may contain low or small quantities of active compounds inadequate enough to produce activities.

In conclusion, aqueous extract of *S. radiatum* has antifungal activities and methanolic showed activities against *S. typhi* and *P. aeruginosa* which has justified its use in traditional practice. Further studies should be done on the plant to know the active constituents of the plant responsible for antimicrobial activity and best solvent for extraction of these active phytochemical constituents.

# **Conflicts of Interest**

The authors declare they have no conflict of interest.

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