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Full Length Research Paper

Antimicrobial activity of the rhizome essential oil of *Zingiber officinale* Roscoe

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ABSTRACT

The antimicrobial activity of the rhizome essential oil of *Zingiber officinale* (family: Zingiberaceae) was tested against six standard bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and against two standard fungi namely *Aspergillus niger* and *Candida albicans* using the cup plate agar diffusion method. The rhizome essential oil of *Z. officinale* dissolved in methanol (1:10), showed high activity against the Gram positive *S. aureus* (23 mm)*, B. subtilis* (20 mm), Gram negative *E. coli* (21 mm), *P. vulgaris* (20 mm) and *P. aeruginosa* (27 mm). It also showed moderate activity against the Gram negative *K. pneumoniae* (15 mm)*, C. albicans* (16 mm) and no activity against *Aspergillus niger*. The rhizome oil was also tested against sixty clinical isolates, collected randomly from Khartoum and Soba Hospitals. The minimum inhibitory concentrations (MICs) of the essential oil against standard bacteria were determined using the agar diffusion method. The antimicrobial activity of the reference drugs were determined against the standard organisms and compared with the antimicrobial activity of the tested oil. The obtained results showed variable sensitivity against the organisms tested and confirmed its folkloric uses.

Keywords: *Zingiber officinale* (Rhizome), essential oil, antimicrobial activity.

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INTRODUCTION

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a well-known spice used in the daily diet in many Asian countries (Demin and Yingying, 2010). It is a rhizomatous plant grown throughout Southeastern Asia, China in parts of Japan, Austria, Latin America, Jamaica and Africa. It has been used as a spice and medicine in India and China since ancient times. It was known in Germany and France in the ninth century and in England in 10th century for its medicinal properties (Sasidharan and Nirmala, 2010). Over three quarters of the world population still rely on plants and plant extracts for health care (Sasidharan and Nirmala, 2010). Ginger compounds are active against specific type of diarrhea which is leading to cause death in infant in developing countries. Moreover, it has been found that ginger is effective in treating nausea caused by sea sickness, morning sickness and chemotherapy, though it was found superior over a place for post operative nausea (Demin and Yingying, 2010; Sebiomo et al., 2011).

In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and anti-tumor (Demin and Yingying, 2010; Sasidharan and Nirmala, 2010; Sebiomo et al., 2011; Lee et al., 1986; Penna et al., 2003; Islam and Choi, 2008; Isa et al., 2008; Wang et al., 2009; Shim et al., 2011).

In the Tibb and Ayurvedic systems of medicine preventive and ameliorative effects of ginger have been described in the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Chrubasik et al., 2005; Ali et al., 2008). Extracts were reported to have effects as an anti-inflammatory (Chang et al., 1995). It is considered safe herbal medicine with only few and insignificant adverse side effects (Ali et al., 2008). The aim of this

study was to determine the antimicrobial activity of the essential oil of ginger against standard bacteria, fungi and to determine its minimum inhibitory concentrations.

MATERIALS AND METHODS

Plant materials

The rhizome of *Zingiber officinale* were purchased from Alyhya Mole in October 2013 and it was identified and authenticated by the taxonomist Dr. Haider Abd alGader, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

Method of extraction

The oil of the tested *Z. officinale* leaves was obtained by hydrodistillation technique using Clevenger's apparatus. Hundred grams from plant materials were placed in a two liters round bottom flask and distilled water was added and mixed thoroughly. The contents of the flask were boiled gently for four hours until the volatile oil has been distilled. The crude volatile oil of plant was transferred by means of a pipette into a separate brown glass bottle. Anhydrous sodium sulphate was added agitated gently to absorb the water and the clear oil was decanted into brown glass bottle and kept in the refrigerator until needed for analysis.

Test microorganisms

The oily solution of *Z. officinale* was tested against two Gram positive bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Four Gram negative organsims, *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae (*ATCC 53657), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853) and two standard fungi, *Asperugillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596). The tested organsims were obtained from the Department of Microbiology, MAP TMRI and National Health Labroatory, Khartoum, Sudan.

60 clinical isolates of *Bacillus subtilis, Staphylococcus*, *Escherichia coli, Klebieslla pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa* were collected from Khartoum and Soba Hospitals.

The bacterial cultures were maintained on nutrient agar and incubated at 37°Cfor 18 h and then used for tests.

In vitro testing the oil *Zingiber officinale* for antimicrobial activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8 to10^9 C.F.U/ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of the oil using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for the oil against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated. Positive control involving the addition of the solvent (methanol) instead of the oil was carried out.

Testing for antifungal activity

The same method as for bacteria was adopted. Instead of Nutrient agar, Sabouraud dextrose agar was used the inoculated medium was incubated at 25°C for two days for *Candida albicans* and three days for *Aspergillus niger*.

RESULTS AND DISCUSSION

The rhizome of ginger (*Z. officinale*) a member of the Zingiberaceae family, was screened for its *in vitro* antimicrobial activity against two standard Gram positive bacteria *Bacillus subtilis, Staphylococcus aureus,* four standard Gram negative bacteria *Escherichia coli, Klebsiella pnumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa* and two standard fungi *Aspergillus niger* and *Candida albicans* using a cup plate agar diffusion method. Table 1 and Figure 1 represents the results of antimicrobial activity of the methanolic solution prepared from the rhizome of ginger which showed high activity against *B. subtilis* (20 mm), *S. aureus* (23 mm), *E. coli* (21 mm), *P. vulgaris* (20 mm), and *P. aeruginosa* (27 mm) and moderate activity against *K. pneumoniae* (15 mm) and *C. albicans* (16 mm). It showed no activity against *A. niger.* Similarly Yin and Cheng (1988), Belik (2014) showed that ginger had no significant action on some fungi (*A. niger* and *A. flaveus*). Jagetia et al*.* (2003) and Badreldein et al. (2008) found similar to our result that the ginger extract exhibited antimicrobial activity against *P. aeruginosa*, *E. coli* and *C. albicans*. The methanol extract of ginger showed the highest activity against *P. aeruginosa*, followed by *S. aureus*, *E. coli*, *B. subtilis*, *P. vulgaris* and *K. pneumoniae* was the least. Therefore methanol extract was more effective against the Gram negative organism *P. aeruginosa* than the Gram positive bacteria. This results was different from that reported by Hasan et al. (2012); Kaushik and Goyal (2011) who found that the methanol extract was more effective against the Gram positive bacteria compared to Gram negative bacteria.

Furthermore the Gram positive bacteria *S. aureus* was found more susceptible than the Gram negative bacteria *E. coli*, *K. pneumoniae* and *P. vulgaris* which is in agreement with the study of Chen et al. (1985) and Belik (2014). This is probably due to the difference in chemical composition and structure of cell wall of both types of microorganisms.

Therefore ginger inhibits the growth of all Gram positive, Gram negative and fungus *C. albicans*. This result is similar to that reported by Nielsen and Rios (2000) and Belik (2014). Gupta and Sharma (2014) found similar to our results that ginger inhibited the growth of *E. coli*, *Pr. Species, S. species, Streptococci* and *Salmonalla*. Shahnaz and Mohammed, (2015) found

Table 1. *In vitro* antimicrobial activity of the essential oil of *Zingiber officinale* rhizome.

Family/plant name/vernacular name	Part used	Solvent system	Yield (%)	Tested organisms used*/ MIZD (mm)							
				B.s	S.a	E.c	K.p	Pr.v	Ps.a	A.n	C.a
Zingiberaceae (Zingiber officinale) Genzabil	Rhizome	MeOH	0.6%	20	23	21	15	20	27	-	16

Key: Bacteria*****: *B.s = Bacillus subtilis, S.a = Staphylococcus aureus, E.c = Escherichia coli, K.p = Klebsiella pneumoniae, Pr.v = Proteus vulgaris, Ps.a =Pseudomonas aeruginosa,* Fungi**: *A.n= Aspergillus niger* and *C.a = Candida albicans.* Concentration of oil dissolved in methanol (1:10) at 0.1 ml/cup. MIZD*(mm): Mean of Inhibition Zone Diameter in mm.
Interpretation of result: MIZD* (mm): >14 mm: Resistant.<18 mm: Sensitive. , 14 -18 mm: Moderate.

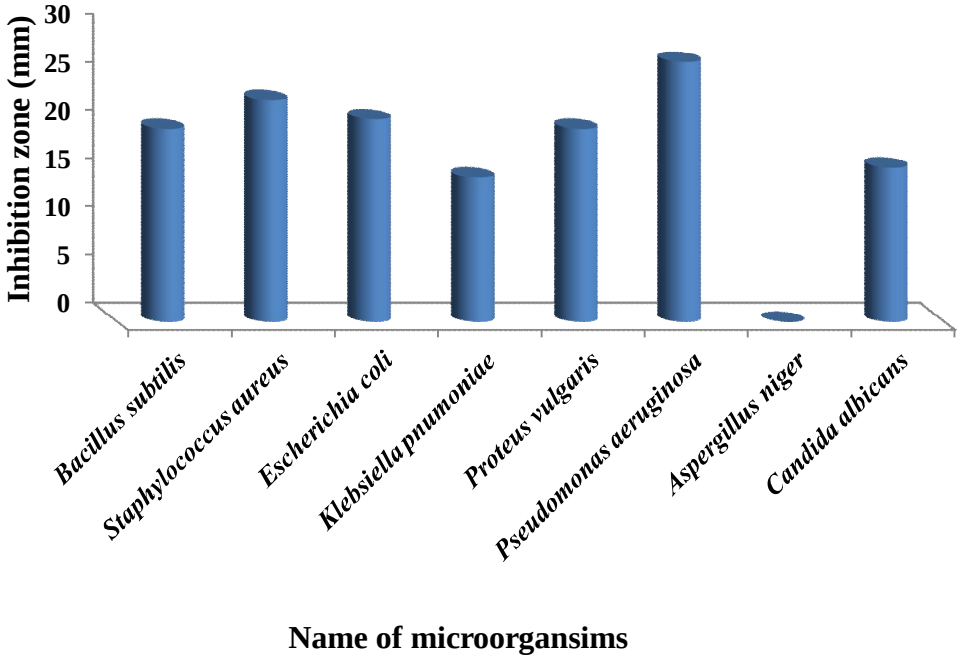

Figure 1. *In vitro* antimicrobial activity of the essential oil of *Zingiber officinale rhizome*.

Table 2. The activity of *Zingiber officinale* oil against different clinical isolates.

Microorganisms	No. of isolate	No. of clinical isolates		
		Sensitive	Moderate	Resistant
B. subtilis	10	7	3	-
S. aureus	10	10	-	-
E. coli	10	6	4	-
K. pneumoniae	10	-	9	-
P. vulgaris	10	3	7	-
P. aeruginosa	10	-	2	8

similar to our results that the oil showed significant antimicrobial activity against *E. coli*, *S. aureus*, *B. subtilis*, *C. albicans* but contrary to our result the oil was also active against *A. niger.* There are several reports on the inhibitory effect of ginger on the growth of *A. niger* (Singh et al., 2008; Sasidharan and Nirmala, 2010; Tagoe et al., 2011).

Comparison of observation given in Tables 1, 2 and 3, and Figure 1, 2 and 3 showed that the methanol extract of the rhizome of ginger inhibited *B. subtilis* and *P. aeruginosa* similar its 10 µg/ml Gentamicin, *S. aureus* more than 20 µg/ml and less than 40 µg/ml Ampicillin. It also inhibited *E. coli* more than 10 µg/ml Gentamicin, *K. pneumoniae* and *P. aeruginosa* less than 10 µg/ml

Table 3. Antibacterial and antifungal activity of reference drugs against standard microorganisms.

Drugs	Concentrations (µg/ml)	Standard microorganisms used MDIZ* (mm)					
		Tested bacteria used					
		B.s	***S.a***	***E.c***	***K.pn***	***Pr.v***	***Ps.a***
Ampicillin	40	15	25	-	35	16	16
	20	14	20	-	26	-	13
	10	13	18	-	25	-	12
	5	12	15	-	21	-	-
Gentamicin	40	29	35	32	26	25	23
	20	22	33	30	24	24	22
	10	20	30	17	21	23	21
	5	17	28	-	20	22	19
		Tested fungi used					
		A.n				***C.a***	
Clotrimazole	40	30				42	
	20	22				40	
	10	19				33	
	5	16				30	
Nystatin	50	28				17	
	25	26				14	
	12.5	23				-	

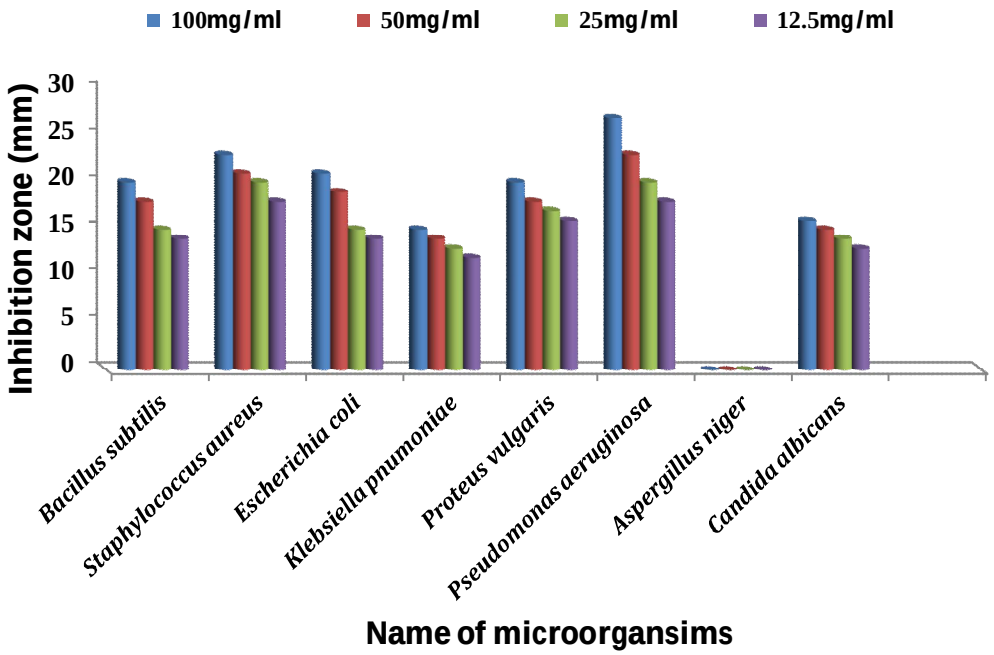

Figure 2. Minimum inhibitory concentrations of *Zingiber officinale* essential oil against standard microorganisms.

Gentamicin. It also inhibited *C. albicans* less than 50 µg/ml Nystatin. The methanolic extract of rhizome of ginger was subjected to MIC by the cup plate agar diffusion method against bacterial species (Table 4 and

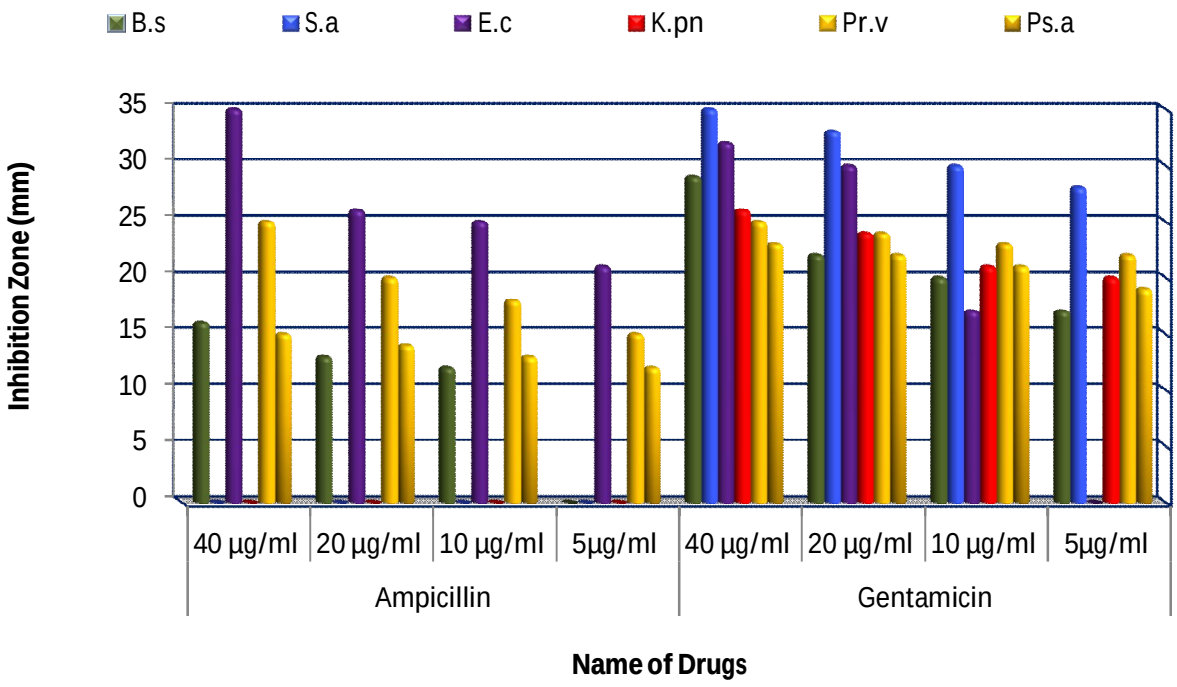

Figure 3. Antibacterial and antifungal activity of reference drugs against standard microorganisms.

Table 4. Minimum inhibitory concentrations of *Zingiber offinale* essential oil against standard microorganisms.

Concentration (mg/ml)	Bateria						Fungi	
	B.s	S.a	E.c	K.p	Pr.v	Ps.a	A.n	C.a
100	20	23	21	15	20	27	-	16
50	18	21	19	14	18	23	-	15
25	15	20	15	13	17	20	-	14
12.5	14	18	14	12	16	18	-	13

Figure 2). The results indicated that the methanol extract inhibited the organisms less than 12.5 µg/ml. Control experiments using methanol for extract preparation (that is, negative control) showed no inhibition of any bacteria indicating that ginger itself and not the solvent inhibited the growth of Gram positive, Gram negative. Ampicillin, Gentamicin, Clotrimozole and Nystatin (positive controls) showed variable inhibition diameters ranging from 12 to 35 mm against Gram positive Gram negative bacteria and from 14 to 42 mm against Clotrimozole and Nystatin.

The results of this study showed that *Z. officinale* possessed antimicrobial properties as antibiotics and antifungal and may be used to mange some infectious disease caused by the tested organisms. The high activity of ginger essential oil may be due to the presence of phenolic compounds as it is well known that ginger essential oil contains considerable amount of phenolic compounds (eugenol, shogaols, zingerone, gingerol etc (Pawar et al., 2011, Din et al., 2012).

CONCLUSION

The essential oil of *Z. officinale* showed various degree of inhibitory activity against the microorganisms tested. The obtained result may justify the use of the Sudanese *Z. officinale* as antimicrobial therapy in folkloric medicine in Sudan and the neighboring countries.

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