Advancement in Medicinal Plant Research
Vol. 2(4), pp. 58-61, November 2014
ISSN: 2354-2152
Full Length Research Paper

Antimicrobial activitiy of the fruits essential oil of *Xylopia aethiopica* [Dun.] A. Rich

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Accepted 3 November, 2014

ABSTRACT

The antimicrobial activity of the fruits essential oil of *Xylopia aethiopica* (family: Annonaceae) 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 Agar plate diffusion method. The fruits essential oil of *Xylopia aethiopica* dissolved in methanol (1:10) showed high activity (21 mm) against the Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa*. It also showed moderate activity (16 mm) against the Gram positive *Bacillus subtilis* and against the Gram negative *E. coli* (15 mm), *K. pneumoniae* (17 mm) and *P. vulgaris* (16 mm). The essential oil also showed high activity (19 mm) against *A. niger* and (21 mm) against *C. albicans*. The fruits 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.

Keywords: *Xylopia aethiopica*, fruits, essential oil, antimicrobial activity.

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INTRODUCTION

Xylopia aethiopica (Dunal) A. Rich (Annonaceae), is a tree of 20 m high or more and it is valuable medicinal plant widely distributed in west African rainforest from Senegal and to Sudan in Eastern Africa and down to Angola in Southern Africa (Irvine, 1961; Burkhill, 1985). The aromatic plant *X. aethiopica* commonly known as Ethiopia or Negro pepper has been used in Europe, Asia and Africa as pepper substitute and spice in local cooking. Various parts of the plant have been traditionally employed in different therapeutic preparations (Fall et al., 2003; Ogunkunle et al., 2006). The mature fruits of green color take a brown-black coloration after drying and are used as spices. The fruits of this plant are used against cough, stomach-aches, dizziness, amenorrhea, bronchitis, lumbago and neuralgia. *X. aethiopica* possesses nutritional and medicinal values (Nadia et al., 2013); it is also used as calmative, purgative, repulsive to pain and in the treatment of boils and skin eruptions (Tairu et al., 1999). The biological properties of aromatic plants such as Annonaceae plants are partially attributed to their essential oils (Aminimoghadamfarouj et al., 2011). Aromatic plant such as *X. aethiopica* is used as spices (fruits) all over central and western Africa and as a component of herbal medicines for the treatment of skin infections, dysentery, female sterility and fever (Burkhill et al., 1985; Ghana Herbal Pharmacopoeia, 1992). The fruits and seeds are dried and sold whole or ground to be used in stews, soups, cakes and desserts in medicine, the bark is used in treatments of febrile pains, eye diseases and hemorrhoids (Weiss, 2003).

Traditionally in Sudan the fruits of *X. aethiopica* are used as flatulence, tonic, and appetizer, for the treatment

of chest infection, *H. pylori* and topical skin diseases. The main constituents are mono and sesquiterpenes hydrocarbons (Karioti et al., 2004). Chemicals components of *X. aethiopica* have been helpful in the avoidance and treatment of cancerous tumors (Del-Rio et al., 1997). *X. aethiopica* fruits contain alkaloids, flavonoids terpenoids, fixed oil and volatile aromatic oil. Key constituents are diterpenic and xylopic acids (Iwu, 1993; Shanmugam et al., 2008). *X. aethiopica* oil contains carbohydrates, glycosides saponins, tannins and phytosterols (Ezekwesili et al., 2010). Adewoyin et al. (2006) reported the mosquito repellent activity of fruits essential oil. Aqueous extract of *X. aethiopica* fruit is rich in phenolics and volatiles compounds, which act as strong natural antioxidants. The aqueous extract normalized the toxic effect of panadol-induced hepatic and renal toxicity (Nadia et al., 2013)

The purpose of the study was to evaluate the antimicrobial activity of *X. aethiopica* against standard bacteria and fungi.

MATERIALS AND METHODS

Plant materials

The fruits of *Xylopia aethiopica* 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, Khartoum, Sudan). The specimen was deposited in the Herbarium of the Institute.

Method of extraction

The oil of the tested *Xylopia aethiopica* fruits 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. The oil was dissolved in methanol (1:10) and was then tested against the standard organisms.

Test microorganisms

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

Sixty clinical isolates of *B. subtilis, S. aureus*, *E. coli, K. pneumoniae, P. vulgaris* and *P. aeruginosa* were collected from Khartoum and Soba Hospital.

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

In vitro testing the oil *Xylopia aethiopica* 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 to 10^9 CFU/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 fruits of *X. aethiopica* family (Annonaceae) were screened for antimicrobial activity against eight standard microorganisms. Two Gram positive bacteria (*B. subtilis*, *S. aureus*), four Gram negative bacteria (*E. coli*, *K. pneumoniae, P. vulgaris* and *P. aeruginosa*) and two fungi namely (*A. niger* and *C. albicans*) using the cup-plate agar diffusion method.

The oil of *X. aethiopica* dissolved in methanol (1:10) showed high activity (21 mm) against *S. aureus*, *P. aeruginosa* and 19 to 21 mm against *A. niger* and *C. albicans*. It also showed moderate activity (15 mm) against *E. coli*, 16 mm against *B. subtilis*, *P. vulgaris* and 17 mm against *K. pneumoniae.* Therefore this result showed that the oil tested inhibited the growth of all microorganisms though the sensitivities of microorganisms varied. This result was similar to that reported by Fleischer et al. (2008) except that in their study *E. coli* was not sensitive to the oil and that *C. albicans* showed the least sensitivity. Husanya et al. (2012) found similar result that the methanol extract was active against *B. subtilis, S. aureus,* and *P. aeruginosa.* On the contrary, the fruits oil showed no activity against *E. coli* and *K. pneumoniae*. Some authors (Konning et al., 2004, Tatsadjieu et al., 2003, Asekun and Adeniyi, 2004; Okigbo *et al*., 2005) have reported *in vitro* activities of the oil and methanol extract against other microorganisms including *E. coli*, *K. pneumoniae* similar to our findings in

Table 1. Minimum inhibitory concentrations of *X. aethiopica.*

Concentration (µg/ml)	*B.s*	*S.a*	*E.c*	*K.p*	*Pr,v*	*Ps.a*	*A.n*	*C.a*
100	16	21	15	17	16	21	19	21
50	15	20	14	16	15	20	16	19
25	14	19	13	15	14	18	15	17
12.5	13	14	12	14	13	16	14	15

B.s = Bacillus subtilis, S.a = Staphylococcus aureus, E.c = Escherichia coli, K.p = Klebsiella pnumoniae, Pr.v = Proteus vulgaris, Ps.a= Pseudomonas aeruginosa, A.n = Aspergillus niger and C.a = Candida albicans.

Table 2. Antimicrobial activity of *Xylopia aethiopica* essential oil.

Family/botanical/vernacular names	Part used	Solvent system	MIZD* (mm)							
			Bacteria*						Fungi**	
			B.s	*S.a*	*E.c*	*K.p*	*Pr.v*	*Ps.a*	*A.n*	*C.a*
Annonaceae/*Xylopia aethiopica*/ Kumba	Fruits	Methanol	16	21	15	17	16	21	19	21

*: Bacteria: *B.s = Bacillus subtilis, S.a = Staphylococcus aureus, E.c = Escherichia coli , K.p = Klebsiella pnumoniae, 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*: Mean of Inhibition Zone Diameter in mm.
Interpretation of results: MIZD*: 14 mm = Resistant; 18 mm = Sensitive; 14 to 18 mm = Moderate.

Table 3. Activity of *X.aethiopica* fruits oil against different clinical isolates.

Clinical isolates microorganisms	Number of isolates	No. of clinical isolate		
		Sensitive	Moderate	Resistant
*S.a**	10	7	3	-
B.s	10	-	10	-
E.c	10	6	4	-
Kl.p	10	4	6	-
Pr.v	10	5	5	-
Ps.a	10	-	3	**7**

*: Abbreviations code according to Table 1.

this study. Therefore, the oil of *X. aethiopica* will be useful in treatment of diseases caused by *S. aureus, E. coli*, *P. aeruginosa* and *C. albicans.* The antimicrobial activity of the oil can be attributed to the contents of active ingredients such as mono and sesquiterpenes hydrocarbon reported by Karioti et al. (2004).

The result of minimum inhibition concentration from Table 1 showed that 12.5 µg/ml was the lowest concentration to inhibit the growth of all organisms tested.

Comparison of observation given in Tables 2 and 3 showed that the fruits oil of *X. aethiopica* dissolved in methanol inhibited *B. subtilis* higher than 40 µg/ml Ampicillin and less than 5 µg/ml Gentamicin It also inhibited *S. aureus* higher than 20 µg/ml Ampicillin. It inhibited *E. coli* less than 10 µg/ml Gentamicin and inhibited *K. pneumoniae* less than 5 µg/ml Gentamicin. The oil inhibited *P. vulgaris* similar to 40 µg/ml Ampicillin and *P. aeruginosa* similar to 10 µg/ml Gentamicin. The oil of *X. aethiopica* inhibited *A.niger* similar to 10 µg/ml Clotrimazole and *C.albicans* more than 50 µg/ml Nystatin.

From Table 4, it was clearly seen that the fruits oil of *X. aethiopica* showed high activity against clinical isolates of *B. subtilis*, *S. aureus, K. pneumoniae* and *P. vulgaris* and low activity against *S. aureus,* and *P. aeruginosa.*

CONCLUSION

The essential oil of *X. aethiopica* fruits showed the various degree of inhibitory activity against the microorganisms tested. The obtained results may justify the use of the Sudanese fruits of *X. aethiopica* as antimicrobial therapy in traditional medicine in Sudan and the neighbouring countries.

Table 4. Antibacterial and antifungal activity of reference drugs against standard microorganisms.

Drugs	Concentrations (µg/ml)	Standard microorganisms used MDIZ* (mm)					
		Tested bacteria used (M.D.I.Z mm)					
		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					
		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				-	

*: Abbreviations code according Table 1.

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Citation: Hassan AM, Almagboul AZ, Abdelgadir H, 2014. Antimicrobial activitiy of the fruits essential oil of *Xylopia aethiopica* [Dun.] A. Rich. Adv Med Plant Res, 2(4): xx-xx.