Journal of Advances in Microbiology

3(2): 1-5, 2017; Article no.JAMB.33180
ISSN: 2456-7116

Antimicrobial Activity and Elemental Composition of *Sarcocephalus latifolius* Fruits: An Ethnopharmacological Based Evaluation

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AO and SA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MO, SY, YB, TE and AA managed the analyses of the study. Author AO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2017/33180
Editor(s):
(1) En Tao Wang, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México.
Reviewers:
(1) Eliton da Silva Vasconcelos, Federal University of São Carlos – UFSCar, Brazil.
(2) Birsa Mihail Lucian, Alexandru Ioan Cuza University of Iasi, Romania.
Complete Peer review History: http://www.sciencedomain.org/review-history/19177

Original Research Article

Received 1st April 2017
Accepted 2nd May 2017
Published 29th May 2017

ABSTRACT

Sarcocephalus latifolius have been part of Sudanese ethnomedicine since long time. The current study was conducted to evaluate the antimicrobial activity of *Sarcocephalus latifolius* fruits and to investigate its elemental composition. The antimicrobial activities of the ethanolic crude extract and solvents fractions (hexane chloroform, acetone and aqua's) were investigated by the disk diffusion method. These fractions were further screened for the presence of eight secondary metabolites using standard protocols. The XRF technique was used to evaluate the content of trace elements in the fruit sample ash. The chloroform fraction was the most active against both the targeted Gram positive and negative bacteria with (20, 19, 15 and 11 mm) against *Bacillus subtilis* (NCTC8236), *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853). It also found to be active against the yeast *Candida albicans* (ATCC7596) with 15 mm. *S. latifolius* fruits contained different elements with potassium as the most abundant metal (40.11 ppm). All these results support the medicinal and nutritional uses of this plant.

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Keywords: Sarcocephalus latifolius; antimicrobial; elemental composition; phytochemical screening.

1. INTRODUCTION

Recently the alarming of the antibiotic resistance by the pathogenic microorganisms has been increased. Infectious diseases caused by multidrug-resistant bacteria and fungi are major cause of hospitalization and drug failure which could lead to death in many cases [1].

Nowadays the emergence of multi-drug resistant phenotypes is increasingly responsible for community infections and all pathogenic microorganisms are concerned. Due to the presence of resistance nodulation cell division, Gram-negative bacteria can reduce the antibacterial component. This process makes bacteria capable to develop resistance to a wide range of antibiotics [2,3]. Gram-positive bacteria such as *Staphylococcus aureus* are also a major cause of hospitalization due to the resistance to methicillin. This situation has created a need to find more effective drugs. Due to the broad diversity and high sufficiency, secondary metabolites from medicinal plants represent the interesting sources of antibacterial agents. WHO estimates about eighty percent of people in developing countries, especially the African countries, are using medicinal plants for their health care [4]. Many trace elements play significant roles in various physiological and biochemical events, while the plants are important link in the transfer of trace elements from soil to humans [5]. Therefore, the fruits and vegetables are valuable sources of minerals [6].

Sarcocephalus latifolius (family: Rubiaceae), locally known as “Karmadoda”. It grows in humid areas across the tropical and austral regions of Africa. *S. latifolius* is a plant with many uses in traditional medicine in Africa for treating malaria, dysentery, fever, diabetes and AIDS [7,8,9]. In the current study we focused on the antimicrobial potential of *S. latifolius* fruits, due its wide use for this proposes in traditional medicine in Sudan.

2. MATERIALS AND METHODS

2.1 Fruit Sampling and Extraction

S. latifolius mature fruits were collected from South Kordofan state in January 2016, identified and authenticated by plant taxonomists at the herbarium of Medicinal and Aromatic Plants Research Institute, National Center for Research. Khartoum, Sudan. The fresh samples were dried in shades for 7 days, powdered then used for extraction by cold maceration methodology according to Osama et al. 2017 [10]. The crude extract was then fractionated using liquid- liquid extraction methodology, which were carried by dissolving the samples in dist. H_2O then partitioned between n-hexane, chloroform and acetone respectively using separating funnel apparatus.

2.2 Qualitative Phytochemical Evaluation

Phytochemical screening was conducted to determine the presence of natural products in the fractions using the method of Osama and Awdelkarim reported in 2015 [11].

2.3 Antimicrobial Evaluation

2.3.1 Preparation of bacterial suspensions

Aliquots (1 ml) of culture of the test organisms, *Bacillus subtil*is (NCTC8236), *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853), incubated at 37ºC for 24 h, were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial culture was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8-10^9 colony forming units (CFU) per /ml. The suspension was stored in a refrigerator at 4ºC till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique with the same medium supplied with 1.8% (w/v) of agar. Serial dilutions of the stock suspension were made in sterile normal saline solution aliquots of 0.02 ml (a drop) of the appropriate dilutions were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for drying the drops and then incubated at 37ºC for 24 hours. After incubation, CFU was counted, and CFU/ml was calculated for the stock suspension as the average CFU per drop (0.02 ml) × 50 × the dilution factor. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.3.2 Preparation of fungal suspension

The fungal culture *Candida albicans* (ATCC7596) was maintained on Sabouraud dextrose agar, incubated at 25ºC for 4 days. The cells were harvested and washed with sterile normal saline and finally suspension in 100 ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.3.3 *In vitro* testing of extracts for antimicrobial activity

3.3.3.1 Testing for antibacterial activity

The cup-plate agar diffusion method was adopted according to published method of Eltayeb et al. 2015 [12] with minor modifications to assess the antibacterial activity of the prepared extract against *B. subtilis* (NCTC8236), *S. aureus* (ATCC25923), *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC27853). One ml of the standardized bacterial stock suspension 10^8–10^9 CFU/ml was thoroughly mixed with 100 ml of sterile molten nutrient agar at 45ºC and then the nutrient agar was distributed into sterile Petri-dishes (20 ml per dish). After the medium was solidified 4 cups (10 mm in diameter) were cut in each plate using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic Microliter-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extracts against each of the test organisms. Simultaneously addition of extracts was carried out as controls. After incubation, the diameters of the resultants and growth inhibition zones were measured, and the mean values were tabulated.

3.3.3.2 Testing for antifungal activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used, which is considered to be more selective medium for fungi and yeasts. The *C. albicans* (ATCC7596) inoculated medium was incubated at 25ºC for three days.

2.4 Trace Elements Composition Analysis (X-ray Fluorescence)

The powdered plant material was analyzed for content of trace elements by energy dispersive x-ray fluorescence (XRF) spectroscopy. The elemental composition was determined by using XRF- 1800 (Shimadzu, Japan) with silicon drift detector SDD under resolution of 145 eV at 10000 pulses. The samples (0.1 g) were measured during 300 s at voltage of 25 kV and 50 kV, at current of 0.5 and 1.0 mA under helium atmosphere.

3. RESULTS AND DISCUSSION

In the present study, ethanol was able to solubilize about 32.75% of dry sample. When this crude extract was fractionated with different solvents the highest portion of mass was went to the water fraction with 51.9% followed with acetone, hexane and lastly chloroform (26.95, 16.33 and 4.8% respectively).

As shown in Table 1 different classes of secondary metabolites were detected in the extracts of *S. latifolius* fruit: phenols, tannins, flavonoids, quninous, alkaloid, triterpenes, steroids, saponins. All these types of compounds are known to have many medicinal proposes [13].

Table 1. Phytochemical screening of *S. latifolius* fruit

Type of compounds	Type of test	Inference			
		Hexane	$CHCl_3$	Acetone	H_2O
Phenols	FeCl3	-v	+v	+v	+v
Tannins	FeCl3	-v	-v	+v	+v
Flavonoids	KOH	+v	+v	-v	+v
	Alkiline test	+v	+v	-v	+v
Quninous	H2SO4	+v	+v	+v	+v
Alkaloid	Dragendorff's	+v	+v	+v	+v
	Wagner's	+v	+v	+v	+v
Triterpenes	Salkowski	+v	+v	+v	-v
Steroids	Salkowski	+v	+v	+v	-v
Saponins	Forth	+v	+v	+v	-v

+ve = positive result; -ve = negative result

Table 2. Zone of inhibition of *S. latifolius* fruit solvent fractions against pathogenic bacterial and fungal strains

Type of microorganism	Microorganism code	MDIZ (mm)				
		Extracts 100 mg/ml (0.1 ml/well)				
		Crude	n-hexane	$CHCl_3$	Acetone	H_2O
Gram –ve	*Escherichia coli*	-	11	15	-	-
	Pseudomonas aeruginosa	12	11.5	11	15	-
Gram +ve	*Staphylococcus aureus*	14.5	10	19	17	-
	Bacillus subtilis	9.5	12.5	20	17.5	-
Fugal	*Candida albicans*	15.5	10	15	8	-

MDIZ = mean diameter of growth inhibition zone = average of two replicates in millimeter.

Fractions of *S. latifolius* showed variable degrees of bactericidal activity as shown in Table 2 above. The highest antimicrobial activity was detected in the chloroform fraction, which was able to inhibit all the tested microbes with remarkable potency. The highest inhibition zone with 20 mm in diameter was observed against *B. subtilis*. The hexane and acetone fractions were also showed variable activities conversely to the aqua's fraction which was found to be inactive against any of the tested microorganisms. The antimicrobial activity of these solvent fractions could be due the presence of phenols, tannins and flavonoids.

Table 3. Elemental composition of *S. latifolius* fruits

Standard code	Concentration (%)	Concentration (ppm)
MgO	1.937	19.3
Al_2O_3	0.248	24.3
SiO_2	0.815	8.5
V_2O_5	1.408	1.6
SO_3	0.920	9.2
K_2O	4.011	40.11
CaO	1.282	12.82
TiO_2	0.008	0.08
Fe_2O_3	0.071	0.71
MnO	0.005	0.05
Cl	0.055	0.55
CuO	0.002	0.02
ZnO	0.122	1.22
Br	0.004	0.04
Rb_2O	0.002	0.02
SrO	0.010	0.1

XRF is one of the most sensitive, rapid and simple analytical technique to study the essential element content of medicinal plants [14,15]. In the current study, the ash was about 10.8% of dry sample weigh. By using XRF spectroscopy various concentrations of macro elements and microelements were detected in the samples (Table 3), including Mg, Al, Si, V, SO_3, K, Ca, Ti, Fe, Mn, Cl, Cu, Zn, Br, Rb and Sr in various quantities.

The results indicate that metal oxide content was 10.9% of ash. However, XRF is practically hard to detect the elements with mass lower than that of sodium, therefore it is more likely that the remaining percentage of ash is for other light elements. All detected elements play important roles in health and disease states of human and domestic animals [16].

4. CONCLUSION

The present study demonstrates that the *S. latifolius* fruits contain phytochemicals with valuable antibacterial activities and the presence of different amounts of trace elements. Further studies for isolation of the active compounds are recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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