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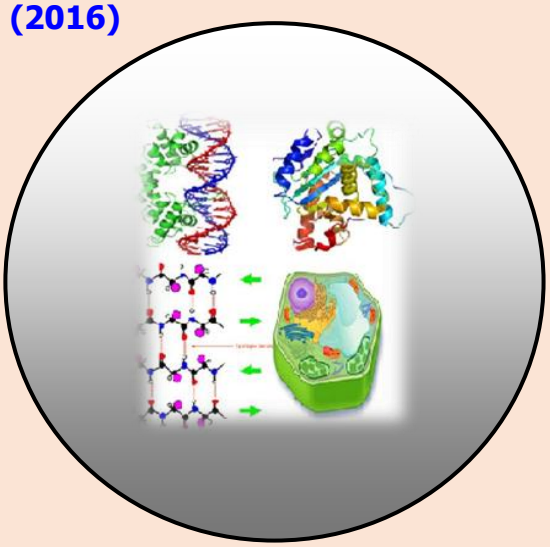

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Antimicrobial, Antioxidant, Cytotoxicity Activities and Phytochemical Screening of Fruits of *Abelmoschus esculentus* (*Malvaceae*) Grown in Kassala Town-Sudan

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ABSTRACT

Okra (Abelmoschus esculentus L.) is the only vegetable crop of significance in the Malvaceae family and is very popular. Its original home is Sudan, Ethiopia and North-eastern African countries. A. esculentus fruits were brought from Kassala town in Sudan, it has been extracted with methanol. Then the A. esculentus was fractionated by using different solvents (Petroleum ether, Chloroform, ethyl acetate, n-butanol and aqueous). For both extracts and fractions of A.esculentus, the antimicrobial activity studied by using disc diffusion and minimum inhibitory concentration (MIC) assays against four standard strains bacteria, two Gram-positive (Bacillus subtilis and Staphylococcus aureus), two Gram-negative bacterial strains (Escherichia coli and Pseudomonas aeruginosa) and two fungal strains (Apergillusniger and Candida albicans). Most of the extracts and fractions showed variable degree of activity against all microorganisms tested and all of them showed antioxidant activity when screened using DPPH method. The phytochemical screening and cytotoxicity via MTT-assay of A. esculentus fruits fractions were investigated, the cytotoxicity showed that A. esculentus was non-toxic. The aqueous fraction showed the highest values more than other fractions in all studied. These A. esculentus (Okra) is an important medicinal plant, its medicinal usage has been revealed that in the traditional systems of medicine such as Ayurveda, Siddha and Unani.

Keywords: Antioxidant, Antimicrobial, Phytochemical, Cytotoxicity and Abelmoschus esculentus.

INTRODUCTION

Okra (*Abelmoschus esculentus L.*) is an important vegetable which is widely distributed in Africa, Asia, southern European and America. It plays an important role in the human diet by supplying

carbohydrates, minerals, and vitamins (Arapitsas, 2008, Moyin-Jesu, 2007). Also its flower hasbeen consumed as health tea and herbal medicine for hundreds years. It is reported tohave many curative effects, such as antioxidant, anti-inflammatory and antitumor activities (Klimp et al., 2002,Zheng et al., 2014).Although some reviews are available describing medicinal properties okra (Kumar et al., 2010; Indah Mohd, 2011; Fong, Toh, Rajen, & Rao, 2011; Jain et al., 2012; Nwachukwu, Nulit & Rusea, 2014), but no specific review are present describing nutritional values, phytochemistry, preclinical pharmacological properties and the possible future application of the okra.

MATERIALS AND METHODS

Plant materials

Abelmoschus esculentus L. was collected from Kassala town in Sudan, it identified and authenticated at Botany Department – University of Al-Neelain. The specimens were deposited at the herbarium of Al-Neelain University.

Preparation of crude extract

Plant samples were extracted according to methods of (Sukhdev *et al.,* 2008). Each 500 grams of sample were extracted with 80% ethanol using shaker. After evaporating the solvent using rotary evaporator, dry extracts were stored in the refrigerator till used.

Antimicrobial Activity of Prepared Extracts

Disc diffusion method

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines. Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

The antimicrobial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while 13-18mm as active and >18mm as highly active).

Antioxidant activity of 80%ethanol extracts

In order to evaluate the antioxidant potentials, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and iron chelating techniques were used.

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of Shimada *et al*. (1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 µml. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multi plate reader Spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and Propyl Gallate (PG). All tests and analysis were run in triplicate.

Fractionation the most active extract/s

The most active extract/s was subjected to liquid-liquid fractionation by petroleum ether, chloroform, ethyl acetate, n-butanol and water using separating funnel.

Phytochemical screening of the ethanolic extracts and fractions of the four plants

Ethanolic extracts of sample and petroleum ether, chloroform, ethyl acetate, n- butanol and water fractions of stem and leaves, which showed the highest antimicrobial activity were subjected to qualitative chemical screening for the identification of various classes of Phyto-constituents using methods described by (Martinez and Valencia (1999); Sofowora (1993); and Harborne (1984).

RESULTS

Table 1. Yields percent of *Abelmoschus esculentus*(Okra).

Solvent	Weight of sample (g)	Weight of extract (g)	Yield (%)
Methanol	500	60.820	12.164

Table 2. Antimicrobial activity against standard microorganisms.

Solvent	Standard tested organisms* /M.D.I.Z (mm)**					
	B. s	S. a	E. c	Ps. a	A. n	C. a
MeOH	22	25	22	21	20	19

*Standard organisms tested: *B.s.= Bacillus subtilis, S.a.=Staphylococcus aureus, E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa, A.n= Aspergillusniger, C.a= Candida albicans*** M.D.I.Z=: Mean diameter of growth inhibition zone in (mm).Concentration used =100mg/ml

Table 3. Minimum inhibitory concentrations of against standard microorganisms.

Concentrations (mg/ml)	B.s	S.a	E.c	Ps.a	A.n	C.a
100	22	25	22	21	20	19
50	15	16	18	17	16	16
25	11	13	11	12	15	13
12.5	9	10	8	11	9	10
6.5	-	-	-	-	-	-

Table 4. Antimicrobial activity of reference antibiotics against standard microorganisms.

No.	Drugs	Concentrations (µg/ml)	Standard microorganisms used MDIZ* (mm)			
			Tested bacteria used (M. D. I. Z mm)			
			B.s	*S. a*	*E. c*	*Ps. a*
1	Ampicillin	100	15	25	11	16
		50	14	20	9	13
		12.5	13	18	-	12
		6.5	12	15	-	-
2	Gentamicin	40	29	35	32	23
		20	22	33	30	22
		10	20	30	17	21
		5	17	28	-	19
			Tested fungi used (M. D. I. Z mm)			
			A. n		*C. a*	
3	Clotrimazole	40	30		42	
		20	22		40	
		10	19		33	
		5	16		30	
4	Nystatin	50	28		17	
		25	26		14	
		12.5	23		-	

Key: *Standard organisms tested: *B.s. = Bacillus subtilis, S.a. =Staphylococcus aureus, E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa, A.n= Aspergillusniger, C.a= Candida albicans*

Table 5. Antioxidant results of *Abelmoschus esculentus* (Okra).

Sample	%RSA ± SD (DPPH)
Methanol extract	93 ± 0.01
PG (STD)	92 ± 0.04

Key: PG= Propylgalate**RSA= Radicals scavenging activity**
DPPH= 2, 2, diphenyl -1- picrylhydrazyl**.**

Table 6. Methanolic extracts by Finney probity Analysis (model).

Solvent	LD50	Results
Methanolic extract	1056.735	Non Toxic

Key: ≤ 249: highly toxic; 250 – 499: Moderate; 500 - 1000: non-toxic.

Table 7. Yield percent of Fractions of Methanolic extracts of *Abelmoschus esculentus.*

Plants used	Petroleum ether		Chloroform		Ethyl acetate		n. Butanol		Aqueous	
	W (g)	Yield (%)	W(g)	Yield (%)	W (g)	Yield (%)	W (g)	Yield (%)	W (g)	Yield (%)
	6.250	51.120	0.075	0.440	1.645	13.454	5.035	19.764	6.250	51.120

Table 8. Antimicrobial activity of plant sample fractions of methanol extracts against Standard microorganisms.

Solvent	Standard tested microorganisms* /M.D.I.Z (mm)**					
	B. s	*S. a*	*E. c*	*Ps.a*	*A. n*	*C. a*
Petroleum ether	6	8	10	9	-	-
Chloroform	11	7	14	13	10	8
Ethyl acetate	15	18	20	22	15	12
n- butanol	13	14	13	9	-	-
Aqueous	22	30	25	30	20	21

Table 9. Results of Antioxidant activity of extract of fractions.

Samples	Solvent	%RSA ±SD (DPPH)
	Petroleum ether	50± 0.02
	Chloroform	65± 0.01
	Ethyl acetate	75± 0.01
	n-Butanol	80± 0.04
	Aqueous	95± 0.03
Standard		89 ± 0·01

Key: PG= Propylgalate **RSA=** Radicals scavenging activity
DPPH= 2, 2, diphenyl-1-picrylhydrazyl**.**

Table 10. Fraction of extracts by Finney probity Analysis (model).

Solvent	LD50	Results
Petroleum ether	15.653	High toxic
Chloroform	494.545	Moderate
Ethyl acetate	600.735	Non toxic
n-Butanol	100.084	High toxic
Aqueous	3048.203	Non toxic

Key: ≤ 249: highly toxic; 250 – 499: Moderate; 500 - 1000: non-toxic.

Table 11. Phytochemical screening of *Abelmoschus esculentus* (Okra).

Solvent used	Tested								
	Saponins	Coumarins	Tannins	Alkaloids	Sterol	Triterpenes	Flavonoids	Cyanogenic glycosides	Anthraquinoe glycosides
Petroleum ether	+	+	+	-	+	++	++	-	-
Chloroform	+	-	+	++	+	-	+	-	-
Ethyl acetate	+	+	+++	-	+	-	-	-	-
n- butanol	-	-	++	-	+	+	++	-	-
Aqueous	++	++	+++	++	+	++	+++	-	-

Key: (-): Absent (+): Low concentration (++): Moderate concentration (+++): High concentration

Table 12. Cytotoxicity activity of *Abelmoschus esculentus* (Okra)by using MTT-assay.

Name of Solvent	Concentration (µg/ml) Inhibition (%) ± SD			IC_{50} (µg/ml)	IC_{50}
	500	250	125		
Methanol	60.1 ± 0.04	45.4 ± 0.02	40.9 ± 0.03	270.6	> 100
Petroleum ether	65.3 ± 0.50	55.1 ± 0.03	40.1± 0.02	206.5	> 100
Chloroform	60.1 ± 0.04	45.4 ± 0.02	40.9 ± 0.03	270.6	> 100
Ethyl acetate	62.7 ± 0.08	53.0 ± 0.01	35.9 ± 0.05	243.4	> 100
n-butanol	64.1 ± 0.05	55.5± 0.03	44.1± 0.02	206.2	> 100
Aqueous	65.4 ± 0.06	50.3 ± 0.09	45.0 ± 0.01	199.6	> 100

Key: **IC_{50}**<30 µg/ml : High toxic .

*Control = Triton-x100 was used as the control positive at 0.2 µg/mL.
The maximum concentration used was 500 µg/mL. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.
IC_{50}< 30 µg/ml : High toxic.

Figure 1. Okra *Abelmoschus esculentus L.*

DISCUSSION

The present study was conducted to investigate the antimicrobial, antioxidant, Phytochemical screening of leaves and stem of (*Abelmoschus esculentus L.*).

Antimicrobial activity

The antimicrobial activities of (*Abelmoschus esculentus L.*) of ethanol extracts against the microorganisms were examined in the present study. The plant extract was screened for it antimicrobial activity against four standard bacteria; two Gram-positive bacterial strains, (*Bacillus subtilus* and *Staphylococcus aureus*) and two Gram-negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal strains (*Aspergillus niger* and *Candida albicans*) using the cup plate agar diffusion method.

In the present study, it is observed that gram-positive are more resistant than gram-negatives bacteria, this results showed differently with previously studies reported by(Brink *et al.*, 2012); Kumarasamy *et al*., 2010 and Kluytman *et al*., 2013).

Antioxidant activity

The DPPH assay is used to determine antioxidant potential, which is based on the reduction of stable radical DPPH to yellow colored diphenylpicryl hydrazine. Thus, the ability of the test samples to quench this radical is a measure of its antioxidative ability of (*Abelmoschus esculentus L.*). In a study shows that the antioxidant activities of *A. moschatus* as determined by the total phenol, flavonoids, total antioxidant methods were higher, the aqueous extract has shown significant radical scavenging activity as in 1, 1- Diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide, hydroxyl radical, superoxide and lipid peroxidation. This result is in agreement with previously reported results (Gul *et al*., 2011 and Vayssade *et al*., 2010).

Phytochemical screening

Phytochemical screening results of extraction of (*Abelmoschus esculentus L.*)by different solvents extraction (petroleum ether, chloroform, ethyl acetate, n-butane and aqueous) were illustrated the presences and absent of second metabolism compounds, all these secondary metabolites are associated with various biological activities, therefore, the antimicrobial activity of *A. esculentus*leaves must be due to the presence of alkaloids as they are ranked the most efficient therapeutically significant plant substance used as a basic medicinal agent for its bactericidal effect. The presence of coumarins can be accounted for anti-proliferative activity of *A. esculentus* leaves on the basis of its anti-inflammatory and antimicrobial activities, whereas the presence of carbohydrate, coumarins and glycosides are beneficial for the action of immune system by increasing the strength of body and hence these are valuable as dietary supplements. The other important bioactivities of *A. esculentus* leaves viz. antioxidant and anticancer are solely due to the presence of flavonoids, and this result is in agreement with previously reported results (Tiwari et al., 2016, Dibyajyoti et al., 2011 and Sathish et al., 2014).

Cytotoxicity activities

The cytotoxicity screening results of extraction of (*Abelmoschus esculentus L.*).The extracted of *Abelmoschus esculentus* is non toxic, this results showed similarity with previously studies reported by (Ravi*et al.*, 2009).

CONCLUSION

We are grateful to Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) National Center for Research. Khartoum, Sudan.

AKNOWELDEGMENTS

This study from the result it can be concluded that the identified compounds may have many applications like antimicrobial, anticancer and anti-inflammatory and treatment of bacterial and fungal in vitro several infections disease.

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