

Antimicrobial Activity and Phytochemical Screening of *Ximenia americana* L Bark and Leaves

Zeinab M. M. Ali¹ Ahmed. E. M Saeed² and Hassan. S. Khalid³

¹Department of Chemistry, College of Science, Bakht Alruda University, Sudan.

²Department of Chemistry, College of Science, Sudan University for Science and Technology, P.O. Box 407. Khartoum - Sudan.

³Department of Pharmacognosy, Faculty of Pharmacy University of Khartoum

Abstract

Ximenia americana bark and leaves, which belongs to family Olacaceae traditionally used for treatment of different human ailments. The bark powder was successively extracted by petroleum ether and methanol using cold extraction method (maceration). The highest degree of activity was observed in methanol extracted of the bark was for *B. subtilis* (inhibition zone: 35-30mm). Followed by *S. aureus* (inhibition zone: 17-16mm). *P. aeruginosa* (inhibition zone: 16-17mm), *E. coli* (inhibition zone: 15-14mm).

The methanolic extract was fractionated by chloroform, ethylacetate and water. Anti- microbial activity was carried out for all extracts and fractions. The ethyl acetate fraction showed high activity against both bacteria and fungi with inhibition zone ranged from 20-23mm.). In all, the various extracts were found to have broad spectrum effect against standard organisms. In all the various extracts were found to have broad spectrum effect against standard organisms, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* and supports the traditional usage of this plant as remedy in treatment of microbial infections. Phytochemical screening was carried out for extract and fractions. Both extracts and fractions showed the presence of saponins, few alkaloids, tannins, flavonoids, terpenes, triterpenes sterols and coumarins in all extracts and fractions.

Key words: *Ximenia americana*, antimicrobial activity, phytochemical, Bark and Leaves.

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Introduction

Ximenia americana L. (Olacaceae), also known as 'wild plum', 'yellow plum' or sea Lemon is a semi-decandent shrub or small tree with small elliptic leaves and whitish to yellowish-green flowers borne in small cymes. *X.americana* is currently widespread throughout tropical and subtropical countries in Central and Southern America, Africa, India and Southeast Asia to Australia, New Zealand, and Pacific islands (Alpern, 2008). This specie is widely used in folk medicine of different countries to treat several human ailments. Leaves, barks, peeling and roots are used in different African countries for treating toothaches, mumps and conjunctivitis in frontal applications (Okigbo *et al.*, 2009). The presence of tannins and flavonoids (Ogunleye and Ibitoye, 2003) might support this action, due to their anti-inflammatory properties. Direct application of minced leaves or an infusion of bark and leaves are also used as an antidote in the case of snake and scorpion bites in different tropical countries (Teo, 1997; Feiberger and Vanderjagt, 1998). This use could be explained with the presence of chemical compounds such as alkaloids (Maikai, *et al.*, 2009), glycosides, phenols, tannins, saponins (Ogunleye; Ibitoye, 2003) and volatile oils (Mevy *et al.*, 2006).

Materials and Methods

Materials

Plant Materials

X. americana bark and leaves was collected from forest around Babanousa city in West Kordofan (western Sudan) on July – August 2010. The plant material was authenticated by Dr. Wail Elsadig & Dr. Haidar Abd-Algadir and herbarium specimens were deposited at the Herberium of Medicinal and Aromatic Plants Research Institute (MAPRI) National Centre for Research (NCR).

Test microorganisms**Bacterial microorganisms**

Bacillus subtilis NCTC 8236 (Gram + ve bacteria)

Staphylococcus aureus ATCC 25923(Gram +ve Bacteria)

Escherichia coli ATCC 25922(Gram -ve bacteria)

Pseudomonas aeruginosa ATCC 27853 (Gram -ve bacteria)

Fungal microorganisms

Candida albicans ATCC7596

Methods**Preparation of the plant**

Collected bark was cleaned and dried under shade. The dried bark was grounded to fine powder using grinder and stored in tightly container.

Preparation of extracts

The extraction was carried out according to method described by Sukhdev, *et al.*, (2008). Hundred g of each were successively extracted with petroleum ether and 80 % methanol using shaker extractor apparatus. Extraction carried out for about three days with daily filtration and evaporation the solvent for petroleum ether and five days for methanol. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in Petri dishes till complete dryness and the yield percentages were calculated as followed:

Weight of extract obtained /Weight of plant sample X100

Fractionation of the methanolic extract

Thirty g of the methanolic extract was dissolved in 2500 ml distilled water and shaken, three times with 100 ml of chloroform in each time using separatory funnel. Chloroform layers were combined together and evaporated under reduced pressure using rotary evaporator. Aqueous layer was re-shaken, three times with 100 ml ethyl acetate in each time using separatory funnel. Ethyl acetate layers were combined together and evaporated under reduced pressure using rotary evaporator apparatus. Aqueous layer was lyophilized using freeze-drier machine till dryness and the yield percentage of each fraction were calculated.

Phytochemical screening

Phytochemical Screening for the active constituents was carried out using the methods described by (Martinez & Valencia (1999) Sofowora (1993), Harborne (1984) and Wall et al., (1952) with many few modifications.

Anti-microbial activity test

The paper disc diffusion method was used to screen the antibacterial 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 (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to one hundred and eight cfu/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 (NCCLS, 1999).

Results

Table (1): The yield percentage

No.	Extract or Fraction	Leaves	Bark
		Yield %	Yield %
1	Petroleum ether extract	10.1%	0.820 %
2	Methanol extract	3.9%	17.819 %
3	Chloroform fraction	2.1%	0.502 %
4	Ethyl acetate fraction	1.98%	0.809 %
5	Aqueous fraction	12.1%	12.56 5 %

Table (2): Phytochemical screening of bark and leaves extracts of *X.americana*

Phytochemical screening	Bark			Leaves		
	Methanol	Chloroform	Ethylacetate	Methanol	Chloroform	Ethylacetate
Tannin	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
Flavonoid	(++)	(++)	(++)	(++)	(++)	(+)
Saponin	(+++)	(+++)	(+++)	(+)	(++)	(++)
Alkaloid	(-)	(-)	(-)	(+)	(-)	(-)
Sterol	(+)	(++)	(+)	(+)	(++)	(+)
Terpene	(+)	(+++)	(+)	(+)	(++)	(++)
Triterpenes	(+)	(+++)	(++)	(+)	(+)	(+)
Coumarin	(+)	(+)	(+)	(+)	(+)	(+)

+++ = highly presence, ++ = moderately present, += faintly present, - = absent

The phytochemical screening was performed for the methanol extract and chloroform, ethyleacetate fractions of *X. americana L.* bark and leaves, solvents. The results in table (2) showed highly present of tannin and saponin (+++) for the methanol extract and fractions of *X. americana L.* bark and leaves which is more comparable with ethanolic extract of *X. Americana L.* bark by (Abd alfatah, *et al.*, 2013), tannin and saponin were highly present (++), Flavonoid (++) showed similarity comparable with ethanolic and butanolic extract of *X. americana L.* bark by (Abd alfatah, *et al.*, 2013), alkaloids is moderately present (+) in methanolic extract which showed similarity comparable with ethanolic and butanolic extract of *X.americana L.* bark by (Abd alfatah, *et al.*, 2013) and alkaloids is absent in methanol extract and chloroform and ethylacetate fractions which showed similarity comparable with ethyl acetate extract of *X. americana L* bark by (Abd alfatah,*et al.*, 2013). Sterol is highly present (++) in the methanol extract of *X. americana L.* Terpene is present which showed similarity comparable with but ethanolic extract of *X. americana L.* bark by (Abd alfatah, *et al.*, 2013) is highly present (++) .

Table (3) Anti-microbial activity results (Bark)

Extract	Zone of Inhibition in (mm)				
	<i>S. a</i>	<i>B. s</i>	<i>E. c</i>	<i>Ps. a</i>	<i>Ca. a</i>
Petroleum ether	14	22	15	15	-
Methanol	14	20	14	14	-
Chloroform	15	14	-	15	-
Ethyl acetate	22	23	22	20	20

*P.E: Petroleum ether, MeoH: methanol, CHCl₃: Chloroform, E.A: Ethyl acetate.*S.a: *Staphylococcus aureus*, B.s: *Bacillus subtilis*, E.c: *Escherichia coli*, Ps.a: *Pseudomonas aeruginosa*, Ca.a: *Candida albicans*.

Table (4): Anti-microbial activity results (leaves)

Extract	Zone of Inhibition in (mm)				
	<i>S. a</i>	<i>B. s</i>	<i>E. c</i>	<i>Ps. a</i>	<i>Ca. a</i>
Petroleum ether	14	22	15	15	-
Methanol	14	22	15	15	-
Chloroform	14	20	20	14	-
Ethyl acetate	20	23	20	20	-

Tables (3& 4) showed antimicrobial activity of the bark and leaves *X. americana* by using four common bacterial isolates and one fungi (*Bacillus subtilis*: B.a, *Staphylococcus aureus*: S.a, *Escherichia coli*., E.st, *Pseudomonas aeruginosa*: Ps.a, and *Candida albicans*: Ca.a). *X. americana* antimicrobial activities of (petroleum ether, methanol, chloroform and ethyl acetate) extracts of bark were evaluated against five common microbial isolates (*Pseudomonas areuginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Candiaa albicans*) and was active against all of them. The highest degree of activity in Methanol extracted of the bark was for *B.subtilis* (inhibition zone: 35-30 mm), followed by *S. aureus* (inhibition zone: 17-16 mm), *P. aeruginosa* (inhibition zone: 16-15 mm), *E.coli* (inhibition zone: 15-14 mm), and *C.albicans* (inhibition zone: 12-11 mm). The bark extracts of *X.americana* were tested against

five microbes *Staphylococcus aureus* and *Escherichia coli* *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans* growth were inhibited by all these extracts and fractions. Several other studies to determine the presence of antimicrobial activity in crude extracts of *Ximenia americana* were performed (Magassouba *et al.*, 2007; Maikai *et al.*, 2009). In all, the various extracts were found to have broad spectrum effect against standard organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Bacillus subtilis*, and supports the traditional usage of this plant as remedy in treatment of microbial infections.

Conclusion

The antimicrobial activity of extracts and fractions of the bark of *X. americana* plant showed presences of secondary metabolites.

In some experiments, was remarked that the plants which accumulate polyphenols, tannins and unsaturated sterols/terpenes showed to inhibit the growth of bacteria and fungi.

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