

Antimicrobial activity of fruits, leaves, seeds and stems extracts of *Ziziphus spina -Christi*

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Abstract: Petroleum ether , chloroform , methanol and aqueous extracts of the fruits, leaves, seeds and stems of *Ziziphus spina -Christi* belonging to the family Rhamnaceae were screened for their antimicrobial activity against six standard bacteria: two Gram positive (*Bacillus subtilis* ,*Staphylococcus aureus*),four Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and two fungi (*Aspergillus niger* and *Candida albicans*) using the cup plate agar diffusion method. The methanol extracts of all parts showed the highest activity against the bacteria tested followed by chloroform then petroleum ether. The aqueous extracts of all parts were inactive against all bacterial organisms. All four parts of the plant extracts showed no antifungal activity against the two fungi tested. The minimum inhibitory concentrations of the most active methanol extracts of fruits, leaves and stems were determine against standard bacteria using the agar plate dilution method. The antibacterial activity of five reference drugs and the antifungal activity of two reference drugs were determined against six bacteria and two fungi and their activities were compared with the activity of the plant extracts.

Keywords: methanol extract, pathogens, *Ziziphus spina – Christi* L.

1. Introduction

Christ's Thorn Jujube (*Ziziphus spina-Christi*) name derives both its common and scientific from the belief by many that this tree provided the crown of thorns said to have been placed on Jesus' head before he was crucified. The plant belongs to the family Rhamnaceae, and it can tolerate high temperatures and grows in desert areas. In some areas, such as the Nile Valley in Egypt. The fruits of Christ's thorn Jujube are used as food especially by people in Western and Central Sudan and other Saharan regions. (Dafni *et al.*, 2005; Ward *et al.*, 2006; Saied *et al.*, 2008). There are many subspecies of the jujube tree; such as *Ziziphus lotus*, *Ziziphus vulgaris* and *Ziziphus jujube* and locally known as Seder (Arabic) (Hasan *et al.*, 2014). For a long time, *Z. spina-christi* has been used in alternative medicine for the treatment of fever, pain, dandruff, wounds and ulcers, inflammatory conditions, asthma and to cure eye diseases. *Z. spina-christi* has recently been shown to have antibacterial, antifungal, antioxidant and anti-hyperglycemic. Its extracts are important in drug development with pharmacological activities in the Middle East and South and East of Asia (Asgarpanah and Haghghat 2012). The phytochemicals identified were cardiac glycosides, polyphenols, saponins and tannins (Abalaka *et al.*, 2010). (Chems-Eddoha *et al.*, 2014) in his paper evaluated the anti-ulcer properties of Moroccan *Z. lotus* (fruits) methanol extract (ZLM) as well as its anti-*Helicobacter pylori* and anti-scavenging properties, (ZLM) showed a significant reduction of gastric juice secretion and total acidity also it inhibited three *H. pylori* clinical strains at 128 µg/ml.

2. Materials and methods:

2.1. Plant Material and Extraction

Fruit, leaves, seeds and stems of *Ziziphus spina-Christi* sample were collected from Khartoum state. The plant was authenticated by the researcher Dr. Haider Abdel Gadir, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). Voucher specimen was deposited at the herbarium of the institute with herbarium number (MAPTMRI-H- /Aisha / 013).

2.2. Preparation of crude extracts

Each of the coarsely powdered plant material (50 g) was exhaustively extracted with petroleum ether in Soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rota- Vap. The extracted plant material was then air-dried,

repacked in the Soxhlet and exhaustively extracted with chloroform for 20 hours. The chloroform extract was filtered and evaporated under reduced pressure using Rota-vap. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with methanol. The methanol extract was filtered and evaporated under reduced pressure again using Rota-vap. Each residue was weighed and the yield percentage was determined. The petroleum ether residue was dissolved in petroleum ether. The chloroform residue was dissolved or suspended in a mixture containing methanol: petroleum ether (2:1) to a final volume 10ml (con. 100 mg/ml). The methanol residue was dissolved in methanol 10 ml (con. 100mg/ml), and kept in refrigerator until used. Simultaneously the aqueous extract of the dried ground plant (10 g) was prepared by infusion using boiled distilled water. It was allowed to soak for 2 hours, then it was filtered. The residue was then dried, weighed and the yield percent was obtained. The final volume of the residue was adjusted to 10 ml distilled water and used immediately.

2.3. Test organisms

Petroleum ether, chloroform, methanol and aqueous extracts of the fruits, leaves, seeds and stems of *Ziziphus spina -Christi* were screened for their antimicrobial activity against six standard bacteria: Two Gram positive *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* ATCC 25923), two Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and two fungi (*Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596). The bacterial cultures were maintained on nutrient agar slopes they were grown on nutrient agar plate and incubated at 37 °C for 18 hrs before being used for the tests. All the microorganisms were obtained from the stock cultures of the institute.

2.4. In vitro testing of extracts for antimicrobial activity

2.4.1. Testing for antibacterial Activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension $10^8 - 10^9$ C.F.U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 °C. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile corborer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts 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 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated. In addition, Ampicillin, Gentamicin, Tetracyclin , Clotrimazole and Nystatin were used as standard antimicrobial controls to compare their activity with the activity of the extracts.

2.4.2. Testing for antifungal activity

The same method as for antibacterial activity was used. Sabouraud dextrose agar was used instead of nutrient agar. The inoculated medium was incubated at 25°C for three days for the *Aspergillus niger* and two days for *Candida albicans*.

2.4.3. Determination of Minimum Inhibitory Concentrations (MICs) by agar well diffusion method

Ziziphus spina -Christi extracts were prepared in the series of decreasing concentrations in the following order 50, 25 and 12.5 mg / ml. MIC is the least concentration of antimicrobial agent that completely inhibits the growth. Results were reported as MIC.

3. Results and discussion

3.1. Results

The average of the diameters of growth inhibition zones produced by *Ziziphus spina –Christi* extracts against standard organisms are shown in Table 1 and MICs in Table 2 on the other hand, Tables 3 , 4 showed the antimicrobial activity of standard chemotherapeutic agents against the standard strains of certain bacterial and fungal species.

3.1.1. Screening for antimicrobial activity of *Ziziphus spina Christi* extracts

From table (1) the petroleum ether, chloroform and aqueous of the fruits extracts showed no activity against all organisms tested. Its methanol extract exhibited high activity (20 mm) against *Proteus vulgaris* followed by *S. aureus* (18mm) then *Bacillus subtilis* (17mm), *Escherichia coli* and *Pseudomonas aeruginosa* (16mm).

The petroleum ether extract of leaves exhibited high activity (19 mm) against *B.subtilis* ,(18mm) against *Proteus vulgaris*, moderate activity (15-14mm) against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and inactive against *S.aureus* . Its chloroform extract showed high activity (16mm) against *B.subtilis*, *E.coli* and *Proteus vulgaris* and (17mm) against *Klebsiella pneumoniae* and inactive against *Pseudomonas aeruginosa* and *S.aureus* . Its methanol extract possessed pronounced activity(24-22mm) against *Proteus*

vulgaris and *E.coli* and very high activity (20 mm) against *B.subtilis* and *S.aureus* and high activity (16mm) against *Pseudomonas aeruginosa* and inactive against *Klebsiella pneumoniae*. The aqueous extract was inactive against all bacterial organism.

Table 1 : Antimicrobial activity of *Ziziphus spina- Christi* extracts against standard microorganisms.

Family/Botanical/Vernacular names	Plant parts	Yield %	Solvent System	Microorganism MDIZ							
				<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>KL.p</i>	<i>Pr.v</i>	<i>Ps.a</i>	<i>As.n</i>	<i>C.a</i>
Rhamnaceae	Fruits	3.273	Petroleum ether	-	-	11	-	-	-	-	-
<i>Ziziphus spina -Christi</i>		0.673	Chloroform	-	-	-	-	-	-	-	-
Sidr		22.3	Methanol	17	18	16	-	20	16	-	-
				Water	-	-	-	-	-	-	-
	Leaves	1.24	Petroleum ether	19	-	15	15	18	14	-	-
		2.64	Chloroform	16	-	16	17	16	-	-	-
		19.312	methanol	20	20	22	-	24	16	-	-
				Water	-	-	-	-	-	-	-
	Seeds	22.7084	Petroleum ether	-	-	-	-	-	-	-	-
		0.978	Chloroform	-	-	-	-	-	-	-	-
		6.77	Methanol	-	-	16	-	-	-	-	-
				Water	-	-	-	-	-	-	-
	Stems	0.72	Petroleum ether	15	-	15	-	15	16	-	-
		0.366	Chloroform	20	-	16	12	19	-	-	-
		5.071	Methanol	17	17	15	12	21	17	-	-
				Water	-	-	-	-	-	-	-

Key: *B.s* = *Bacillus subtilis*, *S.a* = *Staphylococcus aureus*, *E.c* = *Escherichia coli*, *KL.P* = *Klebsiella pneumoniae*, *Pr.v* = *Proteus vulgaris*, *Ps.a* = *Pseudomonas aeruginosa*, *As.n* = *Aspergillus niger*, *C.a* = *Candida albicans*.

MDIZ= Mean diameter of growth inhibition zone in mm.

Interpretation of results: MDIZ >15 mm = High , 14-15mm= moderate , < 15mm = low

(-)= No inhibition

Concentration used = 100mg/ml of 0.1 ml/cup

Table 2: The minimum inhibitory concentrations (MICs) of fruits, leaves and stems of *Ziziphus spina-Christi* methanol extracts against the standard microorganisms .

Family/Botonical/Vernacular names	Plant part	Solvent System	MIC Concentration mg/ml	Micro-organism MDIZ					
				<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>KL.p</i>	<i>Pr.v</i>	<i>Ps.a</i>
Rhamnaceae	Fruits	Methanol	100	17	18	16	–	20	16
<i>Ziziphus spina-Christi</i>			50	15	15	15	–	17	15
Sidr			25	14	14	14	–	15	–
			12.5	13	13	13	–	13	–
	Leaves	Methanol	100	20	20	22	–	24	16
			50	18	18	20	–	20	13
			25	15	17	17	–	16	14
			12.5	14	16	15	–	15	15
	Stems	Methanol	100	17	17	15	12	21	17
			50	15	15	14	–	16	–
			25	14	15	13	–	13	–
			12.5	13	14	13	–	13	–

Key: *B.s* = *Bacillus subtilis*, *S.a*= *Staphylococcus aureus*, *E.c* = *Escherichia coli*, *KL.P*= *Klebsiella pneumoniae*, *Pr.v*= *Proteus vulgaris*, *Ps.a*=*Pseudomonas aeruginosa*.

MDIZ= Mean diameter of growth inhibition zone in mm.

(–)= No inhibition

The petroleum ether, chloroform and aqueous extracts of *Ziziphus spina-Christi* seeds were inactive against all bacterial organisms. The methanol extract showed only high activity (16mm) against *E.coli* while it was inactive against the rest of bacterial organisms tested .

The petroleum ether extract of the stems of *Z.spina-Christi* showed moderate activity (15mm) against *B.subtilis* , *E.coli*, *Pr.vulgaris* and high activity (16 mm) against *Ps. aeruginosa* while inactive against *Klebsiella pneumoniae* and *S.aureus* .Its chloroform extract showed very high activity (20- 19mm) against *B.subtilis* and *Pr.vulgaris* and high activity (16mm) against *E.coli*, low activity (12mm) against *Klebsiella pneumoniae* and no activity against *Ps. aeruginosa* and *S .aureus* .Its methanol extract possessed pronounced activity (21mm) against *Pr.vulgaris*, high activity (17mm) against *B.subtilis* and *S.aureus*, *Ps. aeruginosa*, moderate activity (15 mm) against *E.coli* and low activity (12mm) against *Klebsiella pneumoniae* and in active against *S.aureus* . The aqueous extract was completely devoid of any activity against all bacterial organisms .All parts extracts were inactive against both fungi.

3.1.2. The minimum inhibitory concentrations of (MICs) was determined for the most active *Ziziphus spin-Christi* extracts against standard organisms.

The lowest (MICs) values was 12.5mg/ml for the crude methanol extract of the fruit against *B.subtilis* ,*S.aureus*,*E.coli* and *Pr. vulgaris* .The leaves methnol extract of *Ziziphus spina-*

Christi showed MIC of 12.5 mg/ml against *B.subtilis*, *S.aureus*, *E.coli* , *Pr. vulgaris* and *Pseudomonas aeruginosa*.

The stems methanol extract showed MIC of 12.5mg/ml against *B.subtilis*, *S.aureus*, *E.coli* and *Pr.vulgaris*.

3.1.3. Comparison of the activities *Ziziphus spina-Christi* extracts against standard microorganisms with reference drugs

The fruit methanol extract of *Ziziphus spina- Christi* inhibited *B.subtilis* similar to 5 µg /ml Gentamicin and Tetracycline. It inhibited *S.aureus* similar to 10 µg /ml Ampicillin .It also inhibited *Proteus vulgaris* almost similar to 40µg/ml Gentamicin.

The leaves petroleum ether extract inhibited *Bacillus subtilis* similar to 10µg/ml Gentamicin and Tetracycline. It also inhibited *Klebsiella pneumoniae* almost similar to 5µg/ml Tetracycline and inhibited *Proteus vulgaris* similar to 40 µg/ml Tetracycline.

The chloroform extract of the same part inhibited *Bacillus subtilis* and *Klebsiella pneumoniae* similar to 5 µg/ml Gentamicin and inhibited *Proteus vulgaris* similar to 10 µg/ml Gentamicin 20 µg/ml Ampicillin and Tetracycline. The leaves methanol extract inhibited *Bacillus subtilis* similar to 10 µg/ml Gentamicin. It also inhibited *S.aureus* similar to 20 µg/ml Ampicillin and inhibited *Proteus vulgaris* more than 40mg/ml Ampicillin, Gentamicin and Tetracycline.

The stems petroleum ether extracts of *Ziziphus spina- Christi* inhibited *Bacillus subtilis* to 40 µg/ml Ampicillin and inhibited *Proteus vulgaris* similar to 5 µg /ml Gentamicin and 10 µg /ml Tetracycline. The chloroform extract of *Ziziphus spina- Christi* stems inhibited *Bacillus subtilis* similar to 10 µg/ml Gentamicin and inhibited *Proteus vulgaris* similar to 40µg/ml Gentamicin. Its methanol extract inhibited *Bacillus subtilis* similar to 5 µg/ml Gentamicin and inhibited *Proteus vulgaris* more than 40µg/ml Ampicillin, Gentamicin and Tetracycline. It inhibited *Pseudomonas aeruginosa* less than 5µg/ml Gentamicin (Table 3).

Table 3: Antibacterial activity of reference drugs against standard bacteria.

Antibiotics	Cocentrations	Standard bacteria used					
	µg/ml	MDIZ (mm)					
		<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>Kl.pn</i>	<i>Pr.v</i>	<i>Ps.a</i>
Ampicillin	40	15	25	-	35	17	-
	20	14	20	-	26	16	-
	10	13	18	-	21	-	-
	5	12	15	-	29	-	-
Gentamicin	40	29	35	-	26	19	29
	20	22	33	-	24	17	21
	10	20	30	-	21	16	20
	5	17	28.5	-	20	15	19
Tetracycline	40	25	25	-	24	18	-
	20	23	-	-	22	16	-
	10	19	-	-	18	15	-
	5	18	-	-	14	14	-

Key: *B.s* = *Bacillus subtilis*, *S.a* = *Staphylococcus aureus*, *E.c* = *Escherichia coli*,

KL.P = *Klebsiella pneumoniae*, *Pr.v* = *Proteus vulgaris*, *Ps.a* = *Pseudomonas aeruginosa*,

MDIZ (mm) = Mean diameter of growth inhibition zone in mm.

(-) = No inhibition

3.2. Discussion

The petroleum ether, chloroform, methanol and aqueous of the fruits, leaves, seeds and stems of *Ziziphus spina-Christi* (Rhamnaceae) were tested *in vitro* for their antimicrobial activity against eight standard organisms. Two gram positive (*Bacillus subtilis*, *Staphylococcus aureus*), four Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) and two standard fungi (*Aspergillus niger* and *Candida albicans*) using cup plate agar diffusion method.

Petroleum ether, chloroform and aqueous extracts of the fruit of *Ziziphus spina-Christi* were inactive against all organisms tested. Al Samiry (2007) found that the aqueous extract of the fruit was not active against *S.aureus*.

The methanol extract showed activity against both Gram positive organisms and Gram negative organisms and inactive against *K. pneumoniae*. Therefore the methanol extract was more active than the other three extracts. The methanol extract showed higher activity against the two Gram positive bacteria (*B.subtilis*, *S.aureus*) than the two Gram negative (*E.coli*, *Ps.aeruginosa*) and *Pr.vulgaris* was the most susceptible bacteria. [Motamedi et al. \(2009\)](#) showed that the lowest activity was demonstrated by the methanol extract against *K. pneumoniae*.

The petroleum ether extract of *Ziziphus spina-Christi* leaves showed variable activities against the organisms tested. The chloroform extract showed high activity against *B.subtilis*, *E.coli*, *K.pneumoniae* and *Pr.vulgaris* and inactive against the rest of organisms tested. Contrary to [Gergiri \(2009\)](#) who found that the chloroform extract of leaves of *Ziziphus spina-Christi* showed moderate activity against *B.subtilis* and low activity against the other organisms

The methanol extract showed high activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, this result was similar to [Khalid et al. \(2011\)](#) who found that the methanol extract of the leaves has inhibitory effects against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Contrary to [Gergeir \(2009\)](#) found that the leaves methanol extract of *Ziziphus spina-Christi* showed only high activity against *B. subtilis*, moderate activity against *Pseudomonas aeruginosa* and low activity against the rest organisms tested. Therefore the methanol extract of the leaves showed higher activity against the organisms tested than the other extracts and the high activity could be due to presence of secondary metabolites (alkaloids, tannins, saponins, glycosides, steroids, flavonoids and terpenoids) which were detected by [Abalaka et al. \(2010\)](#) and [Dangoggo et al. \(2012\)](#).

Also it has been reported by [Asgarpanah and Haghgha \(2012\)](#) that flavonoids, alkaloids and saponins are the main phytochemicals that are reported from *Ziziphus spina-Christi*.

It also showed no activity against two fungi tested and this result is in agreement with [Alabaka et al. \(2010\)](#) who found that the ethanolic leaves extract of *Ziziphus spina-Christi* showed no activity against the fungal isolates *A. niger* and *C. albicans*. [Gergeir \(2009\)](#) found that the methanol extract showed low activity against *A.niger* and *C.albicans*

The aqueous extract of the leaves was inactive against all bacterial and fungal organisms tested. Unlike [Khalid et al., \(2011\)](#) who found that the aqueous extract of *Ziziphus spina-*

Christi. has inhibitory effects at various concentrations against five bacterial species among them *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *E.coli* and also unlike [Dangoggo et al. \(2012\)](#) who reported that the leaves aqueous extract of *Ziziphus spina Christi* showed significant activity against *S. aureus*. Also [Gergeir \(2009\)](#) found that the aqueous leaves extract of *Ziziphus spina –Christi* showed low activity against both *A.niger* and *C.albicans* also [Korji \(2012\)](#) who found that the leaves aqueous extract showed low activity against *K. pneumoniae* and *E.coli*. On the other hand our results are similar to [Coopoosamy et al ., \(2011\)](#) who reported that water extracts of leaves roots and stem bark of other species *Ziziphus mucronata* (Willd) showed no activity against all tested gram negative and gram positive bacteria .

Seeds extracts showed no activity on all tested bacteria except the methanol extract showed high activity against *E.coli* .This was not different from [Khalid et al.\(2011\)](#) who reported that seeds aqueous extract and methanol extract of *Ziziphus spina Christi* has inhibitory effects against *S. aureus*, *Ps.aeruginosa* and *E.coli* and that the leaves aqueous and methanol extracts had better antimicrobial activities than seeds aqueous and methanol extracts.

The petroleum ether extract of stems showed moderate activity against *B.subtilis* ,*E.coli*, *Pr.vulgaris* and high activity against *Ps.aruginosa* .

Its chloroform extract showed high activity against *B.subtilis*, *E.coli* and *Pr.vulgaris* . Low activity against *K.pneumoniae* and in active against *Ps. aeruginosa* and *S. aureus*.

Its methanol extract exhibited high activity against *Pr.vulgaris* , *B.subtilis* ,*S.aureus* and *Ps.aeruginosa* low activity against *Klebsiella pneumoniae* and moderate activity against *E.coli*.

The aqueous extract of the stems was inactive against all bacterial organisms. Unlike [Korji \(2012\)](#) who found that *Ziziphus spina- Christi* aqueous stem extract showed activity against all organisms among them *Proteus spp*, *Klebsiella spp* ,*Ps.aeruginosa* and *E.coli*. [El-Kamali and Mahjoub \(2009\)](#) reported that aqueous extract of *Ziziphus spina- Christi* stem bark was the most active of all aqueous extracts tested. Also it was inactive against both fungi tested and this result was similar to that reported by [Mohammed et al.\(2012\)](#) the stem barks (aqueous) extract showed no inhibition of *C. albicans* at low dose but was susceptible above 600mg/ml suggesting that the extract possess antifungal activity.

The leaves methanol extract of *Ziziphus spin -Christi* was the most active, followed by the fruits then the stems and the seeds was the least active. Although all extracts of all parts of

Ziziphus spin -Christi showed no activity against both fungi tested but Moroccan *Ziziphus Lotus* chloroform extract showed anti fungal activity at lowest concentrations because it countenance on terpenic compounds as reported by [Lahlou et al.,\(2002\)](#) .

The high activity of the methanol extracts of all parts could be due to the presence of secondary metabolites. These results of high activity of methanol extracts of different parts of *Ziziphus spina- Christi* can provide promising information for the potential use of their crude extract or purified active ingredients in the treatment of bacterial infections.

Table 4: Antifungal activity of reference drugs against standard fungi

Antifungal drugs	Cocentrations µg/ml	Standard fungi used	
		MDIZ (mm)	
		<i>As.n</i>	<i>C.a</i>
Clotrimazole	20	24	43
	10	19	33
	5	16	30
Nystatin	50	28	17
	25	28	14
	12.5	23	-

Key: *A.n* = *Aspergillus niger*, *C.a* = *Candida albicans* .

MDIZ (mm) =Mean diameter of growth inhibition zone in mm.

Average of two replicates .

(-)= No inhibition

4. Conclusions

1. The fruits, leaves and stems methanol extracts of *Ziziphus spina –Christi* exhibited higher antibacterial activity against Gram positive and Gram negative organisms than the seeds methanol extracts.
2. All extracts showed no activity against the two standard tested fungi.
3. The aqueous extracts exhibited no activity against all tested bacteria and fungi.
4. The leaves methanol extract of *Ziziphus spina- Christi* was the most active followed by the fruits then the stems and the seeds were the least active .The high activity of the methanol extracts of all parts could be due to the presence of secondary metabolites.

Recommendations

1. Pharmacological, toxicological and clinical studies should be carried out on the active plant extract to assess its safety, therapeutic efficacy and potential for commercial utilization.
2. Formulation of the active extracts in suitable dosage forms.
3. Isolation and characterization of the active ingredients responsible for the antimicrobial activity.

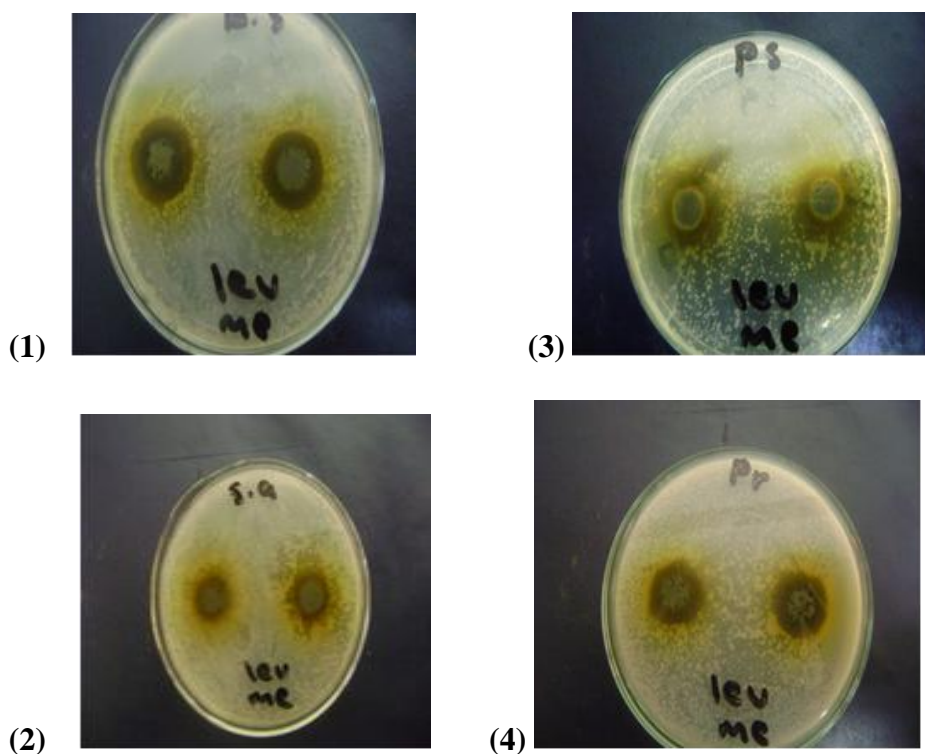


Fig.1 Antimicrobial activity of methanol extract of *Ziziphus spina-christi*. Zones of inhibition of leaves methanol extract against standard bacteria (1) *Bacillus subtilis* (2) *Staphylococcus aureus* (3) *Escherichia coli* (4), *Ps.aeruginosa* (5) *Proteus vulgaris*.

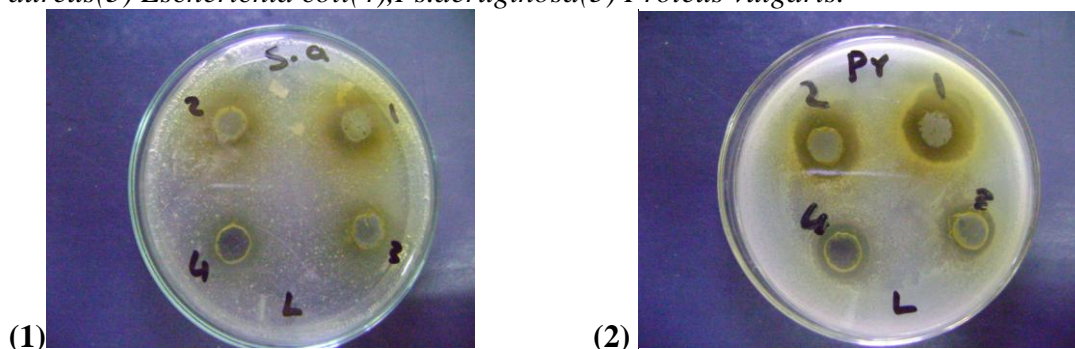


Fig.2 MICs of methanol extract of *Ziziphus spina-christi*. Zones of inhibition (100, 50, 25, 12.5 mg/ml) of leaves methanol extract against standard bacteria (1) *Staphylococcus aureus* (2) *Proteus vulgaris*.

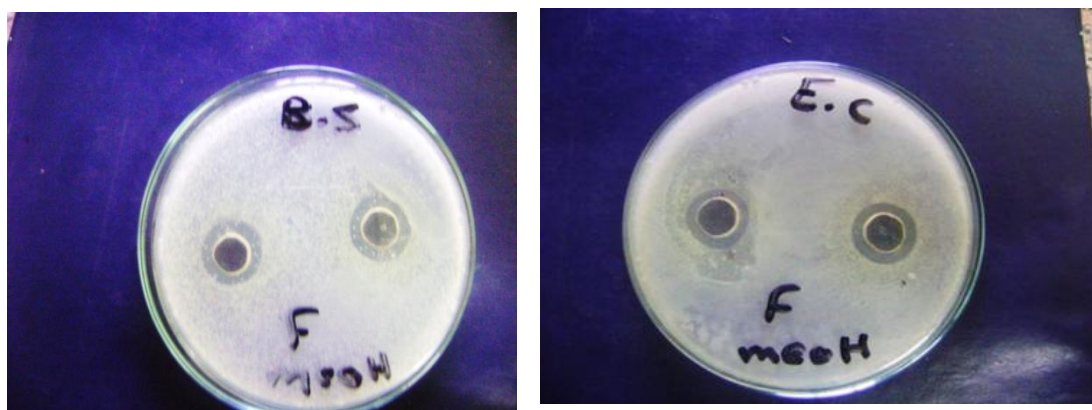


Fig.3 Antimicrobial activity of methanol extract of *Ziziphus spina-christi*. Zones of inhibition of fruit methanol extract against standard bacteria (1) *Bacillus subtilis* (2) *Escherichia coli*.

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