International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182 Print ISSN: 2349-5979, Impact Factor: RJIF 5.72
www.allsubjectjournal.com
Volume 3; Issue 5; May 2016; Page No. 206-211

In Vitro antimicrobial activity and cytotoxicity of fermented leaves and the seeds of cassia tora methanol extract

[1] Aisha B Ali, [2] Ahmed O shareef, [3] Aisha Z Almagboul

[1, 2] Department of Microbiology and parasitology, Medicinal and Aromatic plant and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404.National Center for Research, Khartoum, Sudan.
[3] Professor at Microbiology and Parasitology Department, Medicinal and Aromatic plant and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404.National Center for Research, Khartoum, Sudan.

Abstract
The methanol extract of the fermented leaves, seeds of *Cassia tora* belonging to the family Leguminosae were screened for their antimicrobial activity against four standard bacteria: two Gram positive (*Bacillus subtilis, Staphylococcus aureus),* two Gram negative (*Escherichia coli,* and *Pseudomonas aeruginosa*) and two fungi (*Aspergillus niger* and *Candida albicans*) using the cup plate agar diffusion method. The methanol extracts of two parts showed high activity against the bacteria and fungi tested. The minimum inhibitory concentrations of the methanol extracts of leaves and seeds were determine against standard bacteria and fungi using the agar plate dilution method. The minimum bactericidal concentrations and minimum fungicidal concentrations were determined using the macro- broth dilution method. The antibacterial activity of five reference drugs and the antifungal activity of two reference drugs were determined against four bacteria and two fungi and their activities were compared with the activity of the plant extracts.Two extracts were also evaluated for their cytotoxic activity using Brine shrimp lethality bioassay method.

Keywords: *Cassia tora* methanol extract, Antimicrobial activity, Cytoxicity

1. Introduction
Cassia tora is perennial herbs or under shrubs, up to 2 m. high. Habitat water chatchment, distribution Rashad and Al Abassiya. It is used as substitute of coffee and against ringworm the roots are used as diuretic and for wounds (El Ghazali *et al.,* 1987).
Cassia tora (*C. tora*) (sub-family: Caesalpinioideae; Family Leguminosae Fabaceae) is a small shrub which grows up in warm moist soil throughout the tropical parts of Asian and African countries (Ingle *et al*., 2012) [6].
The chloroform, methanol and aqueous extracts of leaves of *C. tora* L. observed to have antimicrobial property, as the extracts displayed activity against some bacteria and fungi which can cause skin infection and gastro-intestinal disorder (0-5000 µg mL). Methanol extracts also showed antifungal activity (0-64 mg mL1).As per *in-vitro* study five strains of *Shigella dysenteriae*, four strains of *Staphylococcus aureus* and three strains of *Escherichia coli*, have shown sensitivity against *in vitro* treatment of the methanol extracts (up to 2000µg mL1) (Das *et al*., 2010) [2]. The methanol extract of the leaves of *C. tora* showed good activity against carageenin, serotonin, histamine and dextran induced rat hind paw edema in a dose dependent manner (Maity *et al*., 1998; Jain and Patil, 2010) [11, 7]. Preliminary phytochemical analysis of different extracts of *C. tora* gave positive test for phenolics, flavonoids, tannins and anthraquinons (Jiun *et al.,* 2011) [8].

2. Materials and methods
Plant Material and Extraction
Fermented leaves and seeds of *Cassia tora* sample were collected from Khartoum state from local markets. The plant was authenticated by the researcher Dr. Haider Abdel Gadir, Medicinal an Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI).Voucher specimen was deposited at the herbarium of the institute

Preparation of crude extracts
Each of the coarsely powdered plant materials (fermented leaves and the seeds) (1oo g) was allowed to soak for 3 days in methanol with shaking, then it was filtered. The residue was then dried.

Test organisms
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 (*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 ^{0} C for 18 hrs before being used for the tests. All the microorganisms were obtained from the stock cultures of the institute.

In vitro testing of extracts for antimicrobial activity
Testing for antibacterial Activity
The cup-plate agar diffusion method (Kavanagh, 1972) [9] 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 ºC. 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 ºC 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.

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*.

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, 6,25, 3,125 mg / ml. MIC is the least concentration of antimicrobial agent that completely inhibits the growth. Results were reported as MIC.

Determination of Minimum Bactericidal Concentrations (MBCs)

The minimum bactericidal concentrations (MBCs) Were determined by the macro- broth dilution method. A set of tubes containing inoculated broth with the methanol extract *Cassia tora* extract were prepared in the series of decreasing concentrations in the following order 100, 50, 25, 12,5 and 6.125 mg /ml MBCs were determined by sub-culturing 10µg/ml from each negative tube and from the positive growth control. MBCs were defined as the lowest concentration yielding no growth or only one colony.

Brine shrimp lethality bioassay

The cytotoxic activity of the honey samples were evaluated using Brine shrimp lethality bioassay method where 3 graded doses (viz 1000 µg/mL, 100 µg/mL and 10 µg/mL) were used (Meyer *et al*, 1982) [12]. Brine shrimps (Artemia salina Leach) nauplii (Ocean 90, USA) were used as test organisms. For hatching, eggs were kept in brine with a constant oxygen supply for 48 h. The mature nauplii were then used in the experiment. Methanol was used as a solvent and also as a negative control. Vincristine sulfate was used as a reference standard in this case. The numbers of survivors were counted after 24 h. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation. The larvae did not receive food. To ensure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation; we compared the dead larvae in each treatment to the dead larvae in the control.

3. Results and discussion

3.1 Results

The average of the diameters of growth inhibition zones produced by *Cassia tora* fermented leaves and seeds methanol extracts against standard organisms are shown in Table (1). The minimum inhibitory concentrations of (MICs) was determined for methanol extract of *Cassia tora* against standard organisms Table (2). The minimum Bactericidal concentrations of (MBCs) was determined for methanol extract of *Cassia tora* against standard organisms Table (3). The antimicrobial activity of standard chemotherapeutic agents against the standard strains of certain bacterial and fungal species was shown in Tables (5, 6). Cytotoxic activity of methanol extract of the fermented leaves and seeds of *Cassia tora* was determined using Brine shrimp lethality bioassay method as shown in Table (6).

Screening for antimicrobial activity of Cassia tora extracts

From Table (1) the fermented leaves methanol extract exhibited high activity (20 mm) against *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were as *Bacillus subtilis* was (21mm) while it was more active against *A.niger* and *C. albicans* (25mm) and (24mm) respectively. The methanol extract of the seeds exhibited high activity (21mm) against *Escherichia coli* against followed by (20mm) *Bacillus subtilis*, *S. aureus* and *Pseudomonas aeruginosa*. The lowest (MICs) value was l2.5 mg/ml for the crude fermented leaves methanol extract against all microorganisms tested (Table 2).The lowest (MICs) value was less than 3.125 mg/ml for the crude seeds methanol extract.

Table1: Antimicrobial activity of *Cassia tora* methanol extract against the standard microorganisms

Family/Botonical/Vernacular names	Plant part	Solvent System	Concentration mg/ml	Micro-organism MDIZ					
				B.s	***E.c***	***Ps.a***	***S.a***	***A.n***	***C.a***
Cassia tora **Kawal**	**Fermented leaves**	**Methanol**	**100**	21	20	20	20	25	24
	Seeds	**Methanol**	**100**	20	21	20	20	22	17

Table2: The minimum inhibitory concentrations (MICs) of Cassia tora methanol extract

Family/Botonical/Vernacular names	Plant part	Solvent System	Concentration mg/ml	Micro-organism MDIZ					
				B.s	*E.c*	*Ps.a*	*S.a*	*A.n*	*C.a*
Cassia tora									
Kawal	**Fermented leaves**	**Methanol**	50	19	19	18	19	24	22
			25	17	18	17	19	20	20
			12.5	16	17	15	18	19	19
	Seeds	**Methanol**							
			25	18	19	18	17	20	20
			12.5	18	18	17	17	22	17
			6.25	16	17	15	15	19	15
			3.125	14	15	13	14	17	13

Key: B.*s* = *Bacillus subtilis, S.a= Staphylococcus aureus, E.c = Escherichia coli and Ps.a=Pseudomonas aeruginosa, As.n =Aspergillus niger, C.a = Candida albicans*
MDIZ= Mean diameter of growth inhibition zone in mm.
Interpretation of results : <14 mm =low , 14-15mm= moderate ,> 15mm = High
(−)= No inhibition

The fermented leaves methanol extract of *Cassia tora* showed (MBCs) of 50 mg/ml against all tested organisms 6.25 mg/ml against all tested organisms.

Table3: The minimum bactericidal concentrations (MBC) of fermented leaves and seeds of *Cassia tora*

Family/Botonical/Vernacular names	Plant part	Solvent System	Concentration mg/ml	Micro-organism MDIZ					
				B.s	*E.c*	*Ps.a*	*S.a*	*A.n*	*C.a*
Cassia tora									
Kawal	**Fermented leaves**	**Methanol**	50	-	-	-	-	-	-
			25	+	+	+	+	+	+
			12.5	+	+	+	+	+	+
			6.25	+	+	+	+	+	+
	Seeds	**Methanol**	50	-	-	-	-	-	-
			25	-	-	-	-	-	-
			12.5	-	-	-	-	-	-
			6.25	-	-	-	-	-	-

Key: B.*s* = *Bacillus subtilis, S.a= Staphylococcus aureus, E.c = Escherichia coli and Ps.a=Pseudomonas aeruginosa, As.n =Aspergillus niger, C.a = Candida albicans*
Interpretation of results : (+)Growth / (−)= No Growth

Ccomparison of the activities *Cassia tora* fermented leaves and seeds methanol extracts against standard microorganisms with reference drugs

The fermented leaves methanol extract of *Cassia tora* inhibited *B.subtilis* similar to 5 µg /ml Gentamicin and Tetracycline. It inhibited *S.aureus* similar to 20µg /ml Ampicillin. It inhibited *E.coli* similar to 40µg /ml Ampicillin. It inhibited *P. aeruginosa* similar to 5µg /ml Ampicillin. It inhibited *C. albicans* similar to 5µg /ml Clotrimazole. It inhibited *A.niger* similar to 20µg /ml Clotrimazole. The seeds methanol extract of *Cassia tora* inhibited *B.subtilis* similar to 10µg /ml Gentamicin. It inhibited *S.aureus* similar to 10µg /ml Ampicillin. It inhibited *E.coli* similar to 40µg /ml Ampicillin. It inhibited *P. aeruginosa* similar to 5µg /ml Gentamicin. It inhibited *C. albicans* similar to 50µg /ml Nystatin. It inhibited *A.niger* similar to 20µg /ml Clotrimazole and 12.5µg /ml Nystatin.

Table 4: Antibacterial activity of reference drugs against standard bacteria.

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

Table5: Antifungal activity of reference drugs against standard fungi

Antifungal drugs	Cocentrations µg/ml	Standard fungi used MDIZ (mm)	
		As.n	***C.a***
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.
(−)= No inhibition

The cytotoxic activity of the fermented leaves methanol extracts of *Cassia tora* was determined using brine shrimp where the ED 50 was 4652873 and seeds methanol extract was 86.15.

Table(6): Cytotoxic activity of *Cassia tora* amples on brine shrimp

Name of extract source	Solution (µg/ml)	Number of dead organisms	Number of survivors organisms	ED_{50} (µg/ml)	The degree of toxicity
Fermentwd Leaves	**1000**	11	19		
	100	12	18	4652873	Non - toxic
	10	10	20		
Seeds	**1000**	23	7		
	100	12	18	86.15	Highly toxic
	10	11	19		
Ctrl +ve	**1000**	30	0		
	100	27	3	0.423	Highly toxic
	10	25	5		

Key: ED50 < 249 µg/ml : High toxic ; 250 – 499 µg/ml : Median toxicity ; 500 – 1000 µg/ml Light toxicity ;> 1000 µg/ml Non-toxic .

3.2 Discussion

In this study the methanol extract *Cassia tora* (Caesalpinaceae) was tested *in vitro* for their antimicrobial activity against six standard organisims.Two Gram positive (*Bacillus subtilis, Staphylococcus aureus),* four Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa)* and two standard fungi (*Aspergillus niger and Candida albicans*) using cup agar diffusion method.

Fermented leaves methanol extract of the *Cassia tora* was active against all organisms tested. This was similar to EL-Kamali (2010) who reported that EtOH extracts of *C. occidentalis, C. tora, C. senna, R. minima* var. *memnonia, A. marítima* and *C. nervatus* showed equal or nearly equal antibacterial activity against both Gram-positive and Gram-negative bacteria. Chavan (2011) [1] found that ethanolic and aqueous extracts from the leaves of *Cassia tora* were investigated for their antibacterial activity. Both the extracts exhibited significant antibacterial activity.

The fermented leaves methanol extract showed activity against both Gram positive organisms and Gram negative organisms. The methanol extract showed higher activity (20mm) against *S.aureus* and *E.coli*.

The fermented leaves methanol extract show high activity against the two fungi tested (24-25mm) and this result is in agreement with Ashok (2011) who found that methanol extract of *Cassia tora* leaves demonstrated significant antifungal activity against *C. albicans* (24mm) contrary to Hamuel (2008) [5] reported that leaves methanol extract demonstrated the least activity against the clinical and laboratory isolates of both bacteria and fungi using the disc diffusion method (7 mm zone diameter of inhibition, MIC 400 g/mL and MBC 400 g/mL).

The *Cassia tora* seeds methanol extract showed activity against both Gram positive organisms and Gram negative organisms these results were in contrary to Roopashree *et al.*, (2008) [13] found that seeds methanol extract of *Cassia tora* showed no activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

It also showed high activity against *Aspegillus niger and Candida albicans* this was similar to Kim (2004) [10] reported that *Cassia tora* (Leguminosae) seeds showed an antifungal property against phytopathogenic fungi at 1 g/L, the chloroform fraction of *C. tora* showed a strong fungicidal activity.

These results of high activity of methanol extracts of fermented leaves and seeds of *Cassia tora* can provide promising information for the potential use of their crude extracts in the treatment of microbial infections.

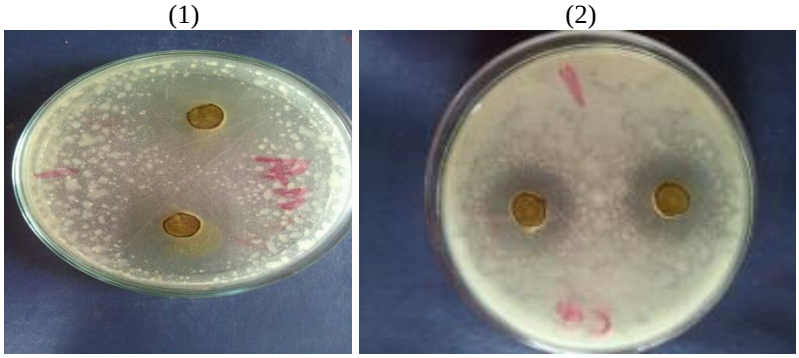

Fig 1: Antimicrobial activity of methanol crude extract of *Cassia tora fermented leaves.* Zone of inhibition of methanol extract against standard Fungi (1) *Angier (2) C.albicans.*

4. Conclusions

1. The fermented leaves and seeds methanol extract of *Cassia tora* exhibited high antibacterial activity against Gram positive and Gram negative organisms.
2. The two extracts exhibited a very high activity against the two standard tested fungi.
3. The fermented leaves and seeds methanol extract of *Cassia tora* showed no toxicity while the seeds showed a high toxicity.

5. References

1. Chavan R, Deshmukh VL, Kadam AS. Antibacterial activity of Cassia tora leaves. Recent Research in Science and Technology. 2011; 3(5):12-14.
2. Das G, Ojha D, Bhattacharya BM, Samanta M, Ghosh S. Evaluation of antimicrobial potentialities of leaves extract of the plant Cassia tora Linn. (Leguminosae/Caesalpinioideae). J Phytol. 2010; (2):64-72.
3. EL-Kamali H, EL-amir M. Antibacterial Activity and Phytochemical Screening of Ethanolic Extracts Obtained from Selected Sudanese Medicinal Plants. Current Research Journal of Biological Sciences. 2010; 2(2):143-146.
4. Gamal E, Ekhlas A, Ahmed K, Kariem M. Medicinal plant of the Eastern Nuba Mountains. NCR Khartoum University press. P.o. Box 321 khartoum, 1987. Part II.
5. Hamuel J. Antimicrobial activity of leaf extracts of Senna obtusifolia (L)more. African Journal of Pharmacy and Pharmacology. 2008; 2(1):7-13.
6. Ingle A, Ranaware P, Ladke A, Damle M. Cassia Tora Phytochemical and pharmacological activity. Int. Imperial J. Pharmacognosy Natural Products. 2012; (2):14-23.
7. Jain S, Patil U. Phytochemical and pharmacological profile of Cassia tora- an overview. Ind. J Natural Products Resour. 2010; (1):430-437.
8. Jinu Archana J, Pradeep Mehta M. Antimicrobial and phytochemical analysis of Cassia tora leaf extracts. Journal of Basic and Applied Biology. 2011; 5(1):125-131.
9. Kavanagh F. Analytical Microbiology, Academic press, (Pub) New York and London, 1972; II:11.
10. Kim Y, Lee C, Kim H. Anthraquinones Isolated from Cassia tora (Leguminosae) Seed Show an Antifungal

Property against Phytopathogenic Fungi. J Agric Food Chem. 2004; 52(20):6096-6100.
11. Maity T, Mandal S, Mukharjee P, Saha K, Das J. Studies on anti-inflammatory effect of Cassia tora leaf extract (fam. Leguminosae). Phytother. Res. 1998; (12):221-223.
12. Mayer BN, Ferrigin NR, Putnam JE, Jacobesn LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient bioassy for active plant constituents. Planta Medica, 1982.
13. Roopashree T, Dang R, Rani S, Narendra C. Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. International Journal of Applied Research in Natural Products. 2008; 1(3):20-28.