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In vitro antimicrobial activity of aerial parts of *Artemisia herba alba* and cytotoxicity methanol extract

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Abstract
The methanol extract of the aerial parts of *Artemisia herba alba* belonging to the family Asteraceae 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 Disc 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 aerial parts 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. The extracts was also evaluated for its cytotoxic activity using Brine shrimp lethality bioassay method.

Keywords: *artemisia herba alba*, methanol extract, antimicrobial activity, cytoxicity

Introduction
Mohamed *et al.*, (2010) [7] *Artemisia herba-alba* is classified into Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: *Magnoliophyta*, Class: Magnoliopsida, Subclass: Asteridae, Order: Asterales, Family: Asteraceae, Subfamily: Asteroideae, Tribe: Anthemideae, Subtribe: Artemisiinae, Genus: Artemisia L., subgenus: Seriphidium and species: *Artemisia herba-alba* Asso. The genus *A. herba-alba* is a medicinal and aromatic dwarf shrub that grows wild in arid areas of the Mediterranean basin, extending into northwestern Himalayas. This plant is abundant in the Iberian Peninsula and reaches highest population in the centre of Spain spreading over the eastern, southeastern and southern Spain. This taxon grows wild on nitrofilous and gypsum-rich substrata.

Various secondary metabolites have been isolated from *A. herba-alba*, perhaps the most important being the sesquiterpene lactones that occur with great structural diversity within the genus *Artemisia*. Additional studies have focused on flavonoids and essential oils.

The antibacterial activity of *Artemisia herba-alba* collected near Only the essential oil was found to be active against some Gram-positive bacteria (*Streptococcus hemolyticus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Shigella sonnei* and *Salmonella typhosa*,). The essential oil was fractionated by column chromatography, and these fractions were tested for antibacterial activity. The principal component of the most active fraction was santolina alcohol.

(Djeridane *et al.*, 2006) [6] study on *A. herba-alba*. Showed stronger antioxidant activity and content in phenolics than the common nutritional plants. It has been also noted in this study that these Algerian plants are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses

Various secondary metabolites have been isolated from A. herba-alba, perhaps the most important being esquiterpene lactones that occur with great structural diversity within the genus Artemisia (Mohamed *et al.*, 2010) [7].

Materials and methods
Plant Material and Extraction
Aerial parts of *Artemisia herba alba* sample were collected from Khartoum state. 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
The coarsely powdered plant material (100 g) was allowed to soak for 3 days in methanol with shaking, then it was filtered. The residue was then dried.

Test organisms
The methanol extract of *Artemisia herba alba* was screened for its 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 disc-diffusion method **(**Kirby-Bauer) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

The sterile solid surface of nutrient agar media were thoroughly inoculated (swap) with standardized bacterial stock suspension 108 –109 C.F.U/ ml.

Discs were filled with 0.02 ml sample of each extracts using automatic micro titer 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.

In addition, Ampicillin, Gentamicin, Tetracyclin, Clotrimazole and Nystatin were used as standard antimicrobial controls to compare their activity with the activity of the extracts.

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 Disc diffusion method

Artemisis herba alba extract was 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, 6.125, 0.781 and 0.039 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). 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.

Results and discussion

Results

The average of the diameters of growth inhibition zones produced by *Artemisia herba alba* (AHA) methanol extract against standard organisms are shown in Table (1). The minimum inhibitory concentrations of (MICs) and (MBCs) were determined for methanol extract of (AHA) against standard organisms Table (2) and (3) respectively. 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 Artemisia herba alba extract

From table (1) the aerial parts methanol extract exhibited the highest activity (20 mm) against *Escherichia coli* followed by *S. aureus* (19mm), *Pseudomonas aeruginosa* (18mm) then *Bacillus subtilis* (17mm). It also exhibits high activity (21mm) against *C.albicans* and (19mm) *A.niger*.

Table 1: The antimicrobial activity of *Artemisia herba alba* methanol extract against the standard microorganisms

Family/Botanical/Vernacular Names	Plant part	Solvent System	Concentration mg/ml	MIC					
				Micro-organisim MDIZ					
				B.s	***S.a***	***E.c***	***Ps.a***	***A.n***	***C.a***
Asteraceae *Artemisia herba alba* **Shieh**	**Arial parts**	**Methanol**	**100**	17	19	20	18	19	21

Key: *B.s = Bacillus subtilis, S.a= Staphylococcus aureus, E.c = Escherichia coli and Ps.a=Pseudomonas aeruginosa, As.n =*

MDIZ= Mean diameter of growth inhibition zone in mm.

Interpretation of results : <12mm= Resistant, 12-15mm= Intermediate, >15mm= Susceptible. (−)= No inhibition

The lowest (MICs) value was 0.781 mg/ml for the methanol extract against all microorganisms tested except *Pseudomonas aeruginosa* (Table 2).

Table 2: The minimum bacteriocidal concentrations (MIC) *Artemisia herba alba* methanol extract against the standard microorganisms

Family/Botanical/Vernacular Names	Plant part	Solvent System	MIC Concentration mg/ml	Micro-organism MDIZ *B.s*	*S.a*	*E.c*	*Ps.a*	*A.n*	*C.a*
Asteraceae	**Arial parts**	**Methanol**	50	16	18	19	17	18	20
Artemisia herba alba			25	16	14	17	16	18	18
Shieh			12.5	16	14	16	15	17	16
			6.25	15	14	15	15	16	15
			3.125	14	14	14	14	15	14
			1.56	13	14	12	12	14	14
			0.781	12	13	11	10	13	13
			0.039	10	13	10	-	11	12

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 : <12mm = Resistant, 12-15mm= Intermediat, >15mm = Susceptible. (−)= No inhibition

The methanol extract of *Artemisia herba alba* showed (MBCs) of 1.56 mg/ml against all tested organisms except *E.coli* and *Pseudomonas aeruginosa* Table (3).

Table 3: The minimum bacteriocidal concentrations (MBCs) *Artemisia herba alba* methanol extracts against the standard micro-organisms

Family/Botanical/Vernacular Name s	Plant part	Solvent System	MBCs Concentration mg/ml	Micro-organism MDIZ *B.s*	*S.a*	*E.c*	*Ps.a*	*A.n*	*C.a*
Asteraceae	**Aerial parts**	**Methanol**	50	–	–	–	–	–	–
Artemisia herba alba			25	–	–	–	–	–	–
Shieh			12.5	–	–	–	–	–	–
			6.25	–	–	–	–	–	–
			3.125	–	–	–	–	–	–
			1.56	–	–	–	–	–	–
			0.781	–	–	–	–	–	–
			0.039	+	+	+	+	+	+

Key : (−)= No growth
(+)= growth

Comparison of the *Artemisia herba alba* activities methanol extracts against standard microorganisms with reference drugs

The fermented leaves methanol extract of *Artemisia herba alba* at 100 mg/ml inhibited *B.subtilis* similar to 40 µg /ml Ampicillin. It also inhibited *S.aureus* similar to 40 µg /ml Ampicillin. It inhibited *E.coli* similar to 40µg /ml Ampicillin. It inhibited *P. aeruginosa* similar to 5µg /ml Gentamicin. On the other hand it inhibited *C. albicans* similar to 50µg /ml Nystatin. While inhibition of *A.niger* was similar to 10µg /ml Clotrimazole.

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*	*Ps.a*
Ampicillin	40	15	25	-	-
	20	14	20	-	-
	10	13	18	-	-
	5	12	15	-	-
Gentamicin	40	29	35	-	29
	20	22	33	-	21
	10	20	30	-	20
	5	17	28.5	-	19
Tetracycline	40	25	25	-	-
	20	23	-	-	-
	10	19	-	-	-
	5	18	-	-	14

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

Table 5: Antifungal activity of reference drugs against standard fungi

Antifungal drugs	Concentrations 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	–

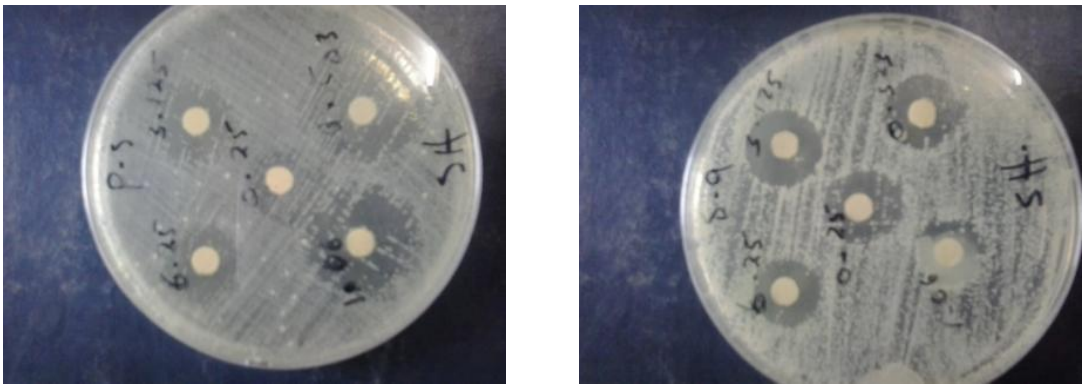
Key: *A.n = Aspergillus niger, C.a = Candida albicans.*
MDIZ (mm) =Mean diameter of growth inhibition zone in mm. (−) = No inhibition

The cytotoxic activity of the methanol extract of *Artemisia herba alba* was determined using brine shrimp where the ED 50 was 141.52 Table (6).

Table 6: Cytotoxic activity of *Artemisia herba alba samples* on brine shrimp

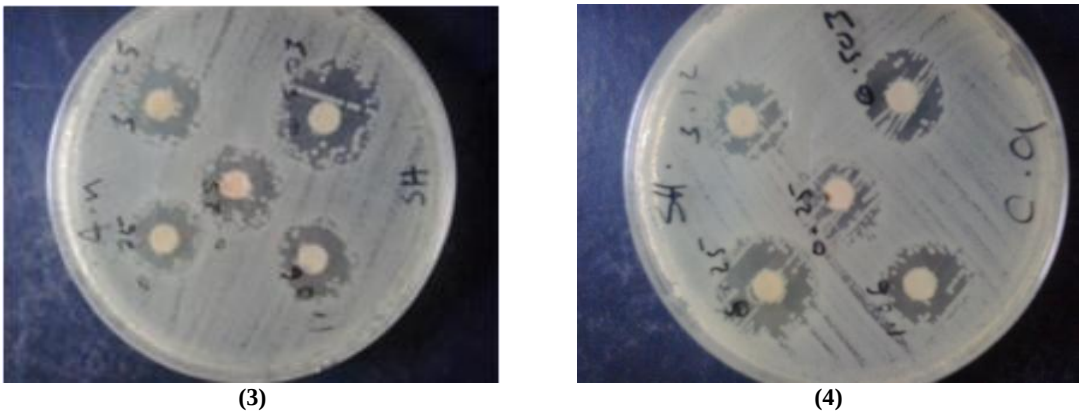
Extract source	Solution (µg/ml)	Number of dead organisms	Number of survivors organisms	ED50 (µg/ml)	The degree of toxicity
Artemisa herba alba	**1000**	30	0		
	100	11	19	141.52	**Highly toxic toxic**
	10	0	30		
Ctrl +ve	**1000**	30	0		
	100	27	3	0.423	**Highly toxic**
	10	25	5		

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



(1) **(2)**

Fig 1: MICs of methanol crude extract of *Artemisia herba alba aerial parts.* Zone of inhibition of methanol extract against standard Fungi (1) *S. aureus* (2) *Pseudomonas aeruginosa*



(3) **(4)**

Fig 2: MICs of methanol crude extract of *Artemisia herba alba aerial parts.* Zone of inhibition of methanol extract against standard Fungi (1) *C.albicans* (2) *A.niger*

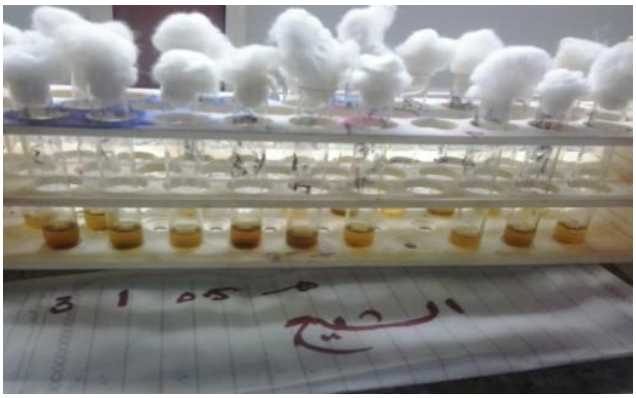


Fig 3: MBCs of methanol crude extract of *Artemisia herba alba aerial parts*

Discussion

In this study the methanol extract *Atremisia herba alba* (Asteraceae) was tested *in vitro* for their antimicrobial activity against eight standard organisims. Two Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*), four Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa)* and two standard fungi (*Aspegillus niger and Candida albicans*) using disc diffusion method. Our study indicated that methanol extract of the *Artemisia herba alba* was active against all organisms tested organisms at different concentrations. This was similar to (Guangrong *et al.*, 2008) [3] the methanol extract from *A. anomala* had great antibacterial activity against all five tested organisms. The methanol extract showed activity against both Gram positive organisms and Gram negative. In this study the methanol extract showed higher activity against *S.aureus*, *E.coli* and *Pseudomonas aeruginosa*. This agreed with (Aljebouri *et al.*, 2005) [1] who reported that methanol extract of *Artemisia herba alba* were more effective, than others and it inhibited the growth of Gram negative bacteria like *E.coli*, *Pseudomonas aeruginosa* and inhibit Gram positive bacteria like *Staphylococcus aureus*, but on the contrary Mohammed (2013) [9] who found that *Artemisia herba-alba*, *Allium sativum and Withania somnifera* extracted by methanol and ethanol showed little activity against *S. aureus. Also in contrary to* (*Ahmadizadeh et al., 2014*) [2] in his study on methanolic extract of (*Artemisia vulgaris*) reported that antibacterial effect of essence showed that the concentration of 1000mg/ml don't has activity against *P. aeruginosa*. On the other side our results showed that (*Artemisia herba alba*) methanol extract had MIC (0.781mg/ml) and MBC (1.56mg/ml) values were less than those of (*Artemisia annua*) tested by (Owuna et al., 2013) [10] were MIC was 125 mg/ml while MBC was 250mg/ml.

The aerial parts of methanol extract showed high activity against the two fungi tested and this result was similar to (Suresh, 2010) [11] who reported that the *Artemisia abrotanum* and *Artemisia pallens* extract showed the maximum antifungal activity against *Trichosporon* beigelii (17mm). Similarly *Artemisia abrotanum* was effective against *Saccharomyces cerevisiae* (17mm) indicating that the ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* shown both antibacterial and antifungal activity.

In this study the aerial parts extract reveiled a high toxicity unlike (Moufid and Eddouks 2012) [8] who reported that the aerial parts are characterized by very low degree of toxicity.

Conclusions

1. The crude methanol extract of *Artemisia herba alba aerial parts* showed high antibacterial activity against Gram positive and Gram negative organisms and Tested fungi.
2. The two extracts exhibited a very high activity against the two standard tested fungi.
3. The methanol extract of *Artemisia herba alba aerial parts* showed a high toxicity.

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