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Original Article

Antibacterial Activity of the Eerial Axtracts from *Xanthium brasilicum*

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Abstract

Antibacterial activity of the aerial extracts from *Xanthium brasilicum* prepared in methanol, diethyl ether, petroleum benzene and an equal mixture of the three solvents were studied against bacterial laboratory standards and clinical isolates using the disk diffusion method. The best antibacterial results were obtained when methanol or the solvent mixture was used. The crude extract with the highest antibacterial activity was fractionated by silica gel chromatography and the biologically active fractions were subjected to thin layer chromatography. All bands were separated and tested for antibacterial activity and the compounds of the active (TLC) bands were identified by ^{1}HNMR spectroscopy. The results showed the presence of two substances, a xanthanolide and a flavonoid.

Keywords: *Xanthium brasilicum*; Antibacterial activity.

Introduction

Xanthium brasilicum Velloso (Asteraceae) is one of the four species of *Xanthium* growing in Iran. The plant is generally found in Asia particularly in Iran, Afghanistan, India, Japan and parts of the Mediterrania (1, 2). *Xanthium brasilicum* is an annual weed with arrow shaped dentated leaves. The fruit is oblong and 1-1.5 cm long. Studies have shown that *X. strumarium* leaves and fruits have medicinal uses including antimicrobial activity and contain linoleic acid, vitamin C and a glucoside, xanthostrumarin (3, 4). However, no such information exists on *Xanthium brasilicum*.

We studied the antibacterial activity of *X. brasilicum* aerial crude extracts prepared in methanol, diethyl ether, petroleum benzene and a mixture of the three solvents. The biologically active extract was then partially purified using silica gel chromatography and fractions showing antibacterial activity were further fractionated by thin layer chromatography. The individual bands with antibacterial activity were then subjected to ^{1}HNMR spectroscopy to identify the active compounds.

Experimental

Plant material

The aerial parts of the plant were collected from Chitgar Park area in Tehran in June 2003. The plants were dried in the shade and identified at the Herbarium of the Biology Department, Shaheed Beheshti University (Tehran, Iran).

Extract preparation from the aerial parts of Xanthium brasilicum

The air dried aerial parts of *Xanthium*

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brasilicum were soaked in methanol, diethyl ether, petroleum benzene or an equal mixture of the three, (MEP) at room temperature (10% w/v). The mixtures were filtered after 24 h and the filtrates were concentrated using a rotavapor (Eyela) and placed at -15°C to remove heavy hydrocarbons and lipids. The extracts were then diluted with methanol, filtered and evaporated to complete dryness. Crude extracts were reconstituted in methanol (10% w/v) before use. The aqueous extracts were prepared by adding boiling water to the dried plant (10% w/v), placing the mixture in a boiling water bath for 1 h followed by filtration and drying over water bath. The dried pellets were reconstituted to 10% (w/v) in sterile distilled water before use.

Fractionation of the crude exracts

The pellet from the crude extract in MEP (100 ml) was dissolved in chloroform and mixed with silica gel powder (70-230 mesh ASTM, Merck) until well adsorbed. The mixture was fractionated on a silica gel column (6 x 40 cm) previously equilibrated with petroleum benzene. Gradient elution was carried out using 150 ml of petroleum benzene, petroleum benzene and diethyl ether at different ratios, diethyl ether, diethyl ether and methanol at different ratios and finally methanol alone. Twenty six fractions (80 ml each) were collected and each fraction was tested for antibacterial activity by the disk diffusion method. The fractions with good antibacterial activity were subjected to thin layer chromatography (TLC, 20 x 20 cm containing silica gel 60 F_{254}, Merck). Several bands were observed under UV lamp (254 and 365 nm) which were cut out, eluted using methanol and further concentrated before testing for antibacterial activity. One band with biological activity common to all active fractions was subjected to ^{1}HNMR spectroscopy to identify the active components.

Bacterial strains

Twelve bacterial strains were used which included; *Bacillus subtilis* (ATCC 465), two *Enterococcus faecail*is (ATCC 29737 and a clinical isolate), three *Staphylococcus aureus* (ATCC 25923, ATCC 29737 and a clinical isolate), three *Escherichia coli* (ATCC 25922, ATCC 10536 and a clinical isolate) and three *Pseudomonas aeruginosa* (ATCC 85327, ATCC 9027 and a clinical isolate) strains. The organisms were cultured overnight on Mueller Hinton Agar (MHA) plates from frozen stocks before each experiment.

Antimicrobial screening by the disk diffusion method

The antibacterial activity of the extracts and fractions were determined by the disk diffusion method (5). Concentrations of 2.5 mg/disk were used for assaying crude extracts against all test bacteria. In addition, a range of concentrations (7, 3.5, 2.5, 1.75 and 0.875 mg/disk) were also tested against the ATCC laboratory standards. Four to six colonies from overnight grown MHA plates were resuspended in tubes containing 5 ml of Mueller Hinton Broth (MHB) and placed at 37°C for 4 h before adjusting the turbidity to MacFarland standard 0.5. MHA plates were seeded using sterile cotton tip swabs in 3 planes before placing 6 mm sterile disks containing the appropriate amount of each extract. The plates were incubated at 37°C for 18-24 h at which time the zones of inhibition were measured and reported in mm. Triplicate tests were carried out for each extract.

Minimum inhibitory concentrations (MICs)

MICs of the crude extracts were determined against *E. coli* and *S. aureus* strains using a tube doubling dilution assay. Serial two fold dilutions of MEP or methanol extracts were made in MHB within the range of 2000-3.75 µg/ml. Fresh bacterial suspensions were prepared in the same medium and 10^6 bacteria were added/ml before incubating the tubes at

37°C for 18-24 h. The first dilution with no bacterial growth was recorded as MIC.

Results and Discussion

The disk sensitivity results for crude extracts showed that methanol, MEP and water extracts contained antibacterial activity. Extracts prepared in other solvents showed poor or no antibacterial activity and the results are not included. Table 1 shows the results of the antibacterial activity of the extracts prepared in methanol, MEP or water

Table 1. Antibacterial activity of *Xanthium brasilicum* extracts.

Microorganism	Inhibition zone (mm)[a]								
	Crude Extracts			Silica Gel Fraction No.			TLC	Antibiotics	
	MEP[b]	Methanol	H_2O	24	25	26	26(1)	G10	E10
B. subtilis ATCC 465	22	20	12	NT	NT	22	22	NT	24
E. faecalis ATCC 29737	10	6	6	6	6	6	6	NT	18
Clinical								NT	20
S. aureus ATCC 25923	26	22	15	20	20	28	28	23	24
ATCC 10536	30	25	15	20	22	22	22	20	24
Clinical	32	30	20	20	25	26	26	25	30
E. coli ATCC 25922	12	10	16	8	8	10	10	18	NT
ATCC 10536	12	10	12	10	10	10	10	18	NT
Clinical	14	10	16	8	10	12	10	20	NT
P. aeruginosa ATCC 85327	18	12	12	10	15	15	15	16	NT
ATCC 9027	10	8	8	8	8	10	10	18	NT
Clinical	18	12	12	10	13	15	15	18	NT

[a] Including disk diameter (6mm).
[b] Methanol:Diethylether:Petroleum Benzene (1:1:1).
[c] G10, Gentamycin (10µg), E10, Erythromycin (10µg). NT, not tested.

(2.5 mg/disk), silica gel fractions and the TLC band with antibacterial activity. Gentamycin and erythromycin were used as antibiotic controls (Table 1). As shown, the most susceptible organisms were *S. aureus, B. subtilis* followed by *P. aeruginosa* and *E. col*. *E. faecalis* was resistant to the extracts at the concentration used. Table 2 shows the activity of different concentrations of the MEP extract tested against 4 ATCC strains. As shown, at concentrations of 7 and 3.5 mg/disk, all test bacteria were susceptible including *P. aeruginosa* and *E. faecalis.* These results are significant and show that both Gram negative and Gram positive bacteria were susceptible if the appropriate concentrations are used. *Staphylococuus aureus* was the most sensitive organism and large inhibition zones were obtained even with 0.875 mg of extract/disk. MIC results agreed with the disk sensitivity profile and were 125 µg/ml for MEP and 15.5 µg/ml for methanol extracts against *S. aureus* isolates. As expected, *E. coli* strains were more resistant and the MIC for both extracts against all 3 isolates was 500 µg/ml. However, these values are significant since only a fraction of the crude extracts has been responsible for antibacterial activity. Unfortunately, because of the large volumes required for measuring MICs, purified fractions were not used.

Fractionation of the crude MEP extract on silica gel column resulted in the elution of the biologically active material by methanol. Only the final four fractions showed good inhibition zones against the test bacteria and the best result was obtained with fraction 26 (Table 1). Fraction 26 was further fractionated by TLC and

Table 2. Antibacterial activity of different concentrations of *Xanthium brasilicum* crude MEP[a] extract.

Microorganism		Inhibition zone (mm)[b]				
		7 mg	3.5 mg	2.5 mg	1.75 mg	0.875 mg
E. faecalis	ATCC 29212	21	13	10	9	8
S. aureus	ATCC 25923	40	27	26	23	22
E. coli	ATCC 25922	22	16	14	12	11
P. aeruginosa	ATCC 85327	20.5	18	18	11	9

[a]Methanol:Diethylether:Petroleum Benzene (1:1:1).
[b]Including disk diameter (6mm).

from several bands observed under UV, only one band showed strong antibacterial activity (26/1, Table 1). Finally, when band 26/1 from the TLC plate was subjected to ^{1}HNMR spectroscopy two compounds were identified, a xanthanolide and a flavonoid.

Flavonoids have been shown to have antibacterial and antiviral activities by several investigators (6-9). There are also reports on the antibacterial activity of xanthanolides isolated mostly from *Xanthium srtumarium* (3, 10-14) and *Xanthium spinosum* (15). This is the first report on the antibacterial activity of *Xanthium brasilicum*. Further work is needed to determine the ethnomedicinal potential uses of this plant.

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