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Antimicrobial, Antimalarial and Antileishmanial Activities of *Abutilon hirtum*

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Abstract

The total ethanolic extract, different fractions and crude polysaccharides of leaves of *Abutilon hirtum* (Lam.) Sweet, were evaluated for their antimicrobial and antiparasitic activities. The antimicrobial activity was determined using the disc diffusion method against *Staphylococcus aureus* (Gram-positive, Facultative anaerobic bacteria), *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* (Gram-negative, Facultative anaerobic bacteria), *Candida albicans*, *C. krusei* (Diploid fungi) and *C. glabrata* (haploid fungus). The antimalarial activity was examined on chloroquine-sensitive (D6, Sierra Leone) strain of *Plasmodium falciparum* protozoan and the antileishmanial activity was tested against *Leishmania donovani*. The results of the present study showed that the total extract exhibited the lowest MIC (11.8 µg/ml) against *P. aeruginosa*. While, the chloroform fraction showed low MIC (59.03, 181.72 and 364.03 µg/ml) against *S. aureus*, *K. pneumonia*e and *P. aeruginosa*, respectively. The petroleum ether fraction exhibited the lowest MIC value (3.00 µg/ml) against *C. albicans*, while the total extract showed the lowest MIC (33.11 µg/ml) against *C. glabrata*. *A. hirtum* leaves extract and fractions showed a weak antimalarial activity. On the other hand, the chloroform fraction of *A. hirtum* leaves exhibited a potent antileishmanial activity with the highest percentage of inhibition (78%) against *Leishmania donovani* AMASTTHP_Pinh.

Key words
Malvaceae, *Abutilon hirtum*, antimicrobial activity, antimalarial activity, antileishmanial activity.

1. Introduction

Infectious and parasitic diseases are one of the serious problems for human beings causing numerous diseases by lot of microorganisms and parasites. They remain primary causes of pediatric mortality in developing countries. Medicinal plants are natural resources, yielding valuable chemical substances such as terpenes, antioxidant phenolics, flavonoids and other biologically active compounds, which are often used in the treatment of many diseases [1-4].

Abutilon hirtum (Lam.) Sweet is a perennial herb or shrub, commonly known as Florida Keys Indian Mallow. It is a pantropical species having an ornamental value, growing along the road sides and on waste lands. The folk uses in Thailand revealed that the roots are used for cough and toothache and as antipyretic, while the leaves or flowers are applied to abscesses. In Kenya, the fruits are eaten raw, while the leaves are browsed by goats and camels. The water extract of the bark is given to ease childbirth in Kenya and Uganda [5-7]. Phytochemically, only flavonoids and phenolic acids have been isolated from this plant [8]. The present study deals with evaluation of antibacterial, antifungal, antimalarial and antileishmanial activities of leaves of *A. hirtum*.

2.Materials and Methods

2.1. Chemicals and Reagents

Nutrient agar (Lab M Limited, UK). Sabouraud dextrose agar (Lab M Limited, UK). Ampicillin (Ampicillin®, Chemical Industries Development (CID), Egypt), gentamicin (Gentamicin®, Alexandria pharmaceutical, Egypt), sulbactam/ampicillin (Unictam®, Medical Union Pharmaceuticals, Egypt) and ketoconazole powder (Amriya, Egypt) were used for antimicrobial activity. Solvents used in this work, e.g. light petroleum ether (b.p. 60-80 °C), chloroform, ethyl acetate and ethanol were purchased from El-Nasr Company for Pharmaceuticals and Chemicals, Egypt. Autoclave (Raypa, Spain), laminar flow (Bioair, Italy) and incubator (GallenKamp, UK) were used for antimicrobial activity.

2.2.Plant material

The leaves of *A. hirtum* were collected in November 2012 from El-Zohria botanical garden, Cairo, Egypt. It was kindly identified by Prof. Dr. Mahmoud Abdelhady Hassan Professor of Horticulture, Faculty of Agriculture, Minia University. A voucher sample (Mn-ph-Cog-016) was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

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2.3.Preparation of the extract and fractions

The air dried powdered leaves (5 Kg) of *A. hirtum* were extracted with 95% ethanol at room temperature with occasional agitation and concentrated under reduced pressure. The concentrated ethanolic extract (550 g) was suspended in the least amount of distilled water, transferred to a separating funnel and partitioned successively by liquid/liquid extraction with petroleum ether, chloroform and finally with ethyl acetate. The fractions were concentrated under reduced pressure to afford petroleum ether (150 g), chloroform (8 g) and ethyl acetate fractions (18 g). The remaining mother liquor was concentrated to give the aqueous fraction (280 g).

2.4.Preparation of crude polysaccharides

The concentrated aqueous fraction (280 g) was dissolved in the least amount of distilled water, transferred to a conical flask and polysaccharides allowed to settle by drop wise addition to 1 liter of methanol followed by vigorous shaking, then filtrated using glass Büchner funnel and vacuum pump. The residue (crude polysaccharides) was collected and dried using vacuum drying oven and then kept for further investigation.

2.5.Microorganisms

Staphylococcus aureus (Gram-positive, Facultative anaerobic bacteria), *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* (Gram-negative, Facultative anaerobic bacteria), *Candida albicans, C. krusei* (Diploid fungi) and *C. glabrata* (haploid fungus). The investigated bacterial and fungal strains used were obtained from Microbiology Department, Faculty of Pharmacy, Minia University.

2.6. Agar diffusion disk method

The antimicrobial activities of the isolated compounds were evaluated using agar well diffusion technique [9]. Fifteen millimeters of sterile, molten and cooled media were plated in sterile petri dishes then the plates were rotated slowly to obtain uniform distribution and allowed to solidify on a flat surface. The media are inoculated with the microorganisms (0.5 ml of $1x10^6$ CFU/ml (0.5 Mcfarland turbidity)) in case of the bacterial culture, while the fungal culture concentration was 10^6 CFU/ml) [10]. After solidification, four equidistant and circular wells of 10 mm diameter were carefully punched in the agar medium using a sterile cork borer [11]. One hundred µl of 1.25 mg/ml of antibiotics, antifungal drug and different fractions were added to the wells using micropipette. All plates were incubated overnight at 37°C after that the antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms was measured [12].

2.7.Antimalarial activity

The total ethanolic extract and different fractions of *A. hirtum* leaves were investigated for their *in-vitro* antimalarial activity and were evaluated for their ability to inhibit the chloroquine–sensitive (D6, Sierra Leone) *Plasmodium falciparum* protozoan. The leaves extract and fractions were tested against a suspension of red blood cells infected with *P. falciparum.* A 200 µl, with 2% parasitemia and 2% hematocrit in RPMI-1640 medium supplemented with 10% human serum and 60 µg/ml amikacin was added to the wells of a 96-well plate containing 10 µl of test samples at 15.867 µg/ml in duplicate and the percentage of inhibition was calculated relative to the negative and positive controls. Extract or fractions that showed % inhibition ≥ 50% were further proceeded to the second phase assay.

In the second phase assay, the tested extract and fractions tested at 47.600, 15.867 and 5.289 µg/ml and the tested concentrations that afforded 50% inhibition of the protozoan relative to positive and negative controls (IC_{50}) against the chloroquine –sensitive(D6) and the chloroquine – resistant (W_2) strains are reported. Chloroquine (0.079 µg/ml) was used as the positive control and DMSO was used as a vehicle [13].

2.8.Antileishmanial activity

The antileishmanial activity of the total extract and different fractions of *A. hirtum* leaves was screened against *Leishmania donovani*, a fly-borne protozoan that causes visceral lieshmaniasis. The total extract and different fractions are initially tested in a primary phase assay at 20µg/ml in duplicate. Percentage of inhibition was calculated relative to the negative and positive controls. Extract or fractions that showed % inhibition ≥ 50% were further proceeded to the second phase assay.

In the second phase assay, all samples are tested at 40, 8 and 1.6 µg/ml. IC_{50} as well as IC_{90} (test concentrations that afford 90% inhibition of the protozoan relative to controls) are reported. Samples that have IC_{50}< 1.6 µg/ml in the secondary assay proceed to the tertiary assay where the samples will be tested at 40, 8, 1.6, 0.32, 0.064 and 0.0128 µg/ml, IC_{50} and IC_{90} are reported. Amphotericin B was used as the positive control [14].

3.Results

3.1.Antibacterial activity

3.1.1.Determination of inhibition zones

The *A. hirtum* leaves total extract and different fractions exhibited a moderate inhibitory activity against the tested gram –ve bacterial strains and little or no effect on gram +ve bacteria growth (Table 1). The highest inhibition zones was exhibited by the aqueous fraction against *Pseudomonas aeruginosa* (18 mm), followed by the total extract (17 mm), while crude polysaccharides showed maximum inhibition zone (17 mm) against *E. coli*.

3.1.2.Determination of the Minimum Inhibitory Concentration (MIC)

The total extract exhibited the lowest MIC (11.8 µg/ml) against *P. aeruginosa*. While, the chloroform fraction showed low MIC (59.03, 181.72 and 364.03 µg/ml) against *S. aureus, K. pneumonia*e and *P. aeruginosa* respectively, compared to that obtained by gentamicin (MIC: 777.9, 679.5 and 781.9 µg/ml) (Table 2).

3.2.Antifungal activity

3.2.1.Determination of inhibition of zones

Ethyl acetate showed the highest inhibition zone against *C. albicans* (21 mm) followed by total extract (20 mm), while aqueous fraction showed the maximum inhibition zone (18 mm) against *C. glabrata.* On the other hand, ethyl acetate fraction, crude polysaccharides (20 mm) were most active against *C. krusei* (Table 3).

Table 1. Results of antibacterial activity of total ethanolic extract and different fractions of *A. hirtum* leaves

	Inhibition zone (mm)			
	S. aureus	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
Ampicillin (1.25 mg/ml)	36	30	10	48
Gentamicin (1.25 mg/ml)	35	30	27	41
Unictam (1.25 mg/ml)	40	32	15	40
Total extract (1.25 mg/ml)	NA	NA	NA	17
Pet. ether fraction (1.25 mg/ml)	NA	12	15	14
Chloroform fraction (1.25 mg/ml)	11	NA	NA	14
Ethyl acetate fraction (1.25 mg/ml)	NA	14	13	NA
Aqueous fraction (1.25 mg/ml)	14	NA	14	18
Crude polysaccharides (1.25 mg/ml)	NA	17	14	12

Table 2. MICs of the total ethanolic extract, different fractions of *A. hirtum* leaves and different antibiotics against the tested microorganisms (µg/ml)

	MICs (µg/ml)			
	S. aureus	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
Ampicillin	19.5	16.6	3.89	NA
Gentamicin	777.9	703.9	679.5	781.9
Unictam	4.48	16.6	4.48	912.5
Total extract	1584.5	NA	713.79	11.8
Pet. ether fraction	NA	106.5	NA	NA
Chloroform fraction	59.03	NA	181.72	364.03

NA: Not Active

Table 3. Inhibition zones of the total ethanolic extract and different fractions of *A. hirtum* leaves and ketoconazole against the tested microorganisms.

	Inhibition zone (mm)		
	Candida albicans	***C. glabrata***	***C. krusei***
Ketoconazole (1.25 mg/ml)	46	36	48
Total extract (1.25 mg/ml)	20	17	16
Pet. ether fraction (1.25 mg/ml)	18	NA	18
Chloroform fraction (1.25 mg/ml)	17	NA	NA
Ethyl acetate fraction (1.25 mg/ml)	21	15	20
Aqueous fraction (1.25 mg/ml)	NA	18	15
Crude polysaccharides (1.25 mg/ml)	15	17	20

NA: Not Active

3.2.2. Determination of Minimum Inhibitory Concentration (MIC)

The petroleum ether fraction exhibited the lowest MIC value (3.00 µg/ml) against *C. albicans*, while the total extract showed the lowest MIC (33.11 µg/ml) against *C. glabrata* (Table 4).

3.3. Antimalarial activity

A. hirtum leaves extract and fractions showed a weak antimalarial activity. The chloroform fraction exhibited moderate percentage of inhibition (40%) against *plasmodium falciparum*, followed by petroleum ether fraction and total leaves extract (34 and 29 %, respectively). While the ethyl acetate, aqueous fractions and the crude polysaccharides showed weak activity (11, 6 and 1%, respectively) (Table 5).

3.4. Antileishmanial activity

The chloroform fraction of *A. hirtum* leaves exhibited a potent antileishmanial activity with the highest percentage of inhibition (78%) against *Leishmania donovani* AMASTTHP_Pinh, followed by petroleum ether fraction (38%) and the other fractions showed weak or no antileishmanial activity. Unfortunately the results in the secondary assay were not promising as the chloroform fraction showed IC_{50} > 20 µg/ml (Table 6).

Table 4. MICs of the total ethanolic extract, different fractions of *A. hirtum* leaves and ketoconazole against the tested microorganisms (µg/ml)

	MICs (µg/ml)		
	Candida albicans	***C. glabrata***	***C. krusei***
Ketoconazole	186	186	186
Total extract	NA	33.11	NA
Pet. ether fraction	3.001	600.03	NA
Ethyl acetate fraction	NA	117.4	219.88

NA: Not Active

Table 5. The antimalarial activity of the total extract and different fractions of *A. hirtum* leaves.

Group	% inhibition
Chloroquine (0.079 µg/ml)	100
Total leaves extract (15.867 µg/ml)	29
Petroleum ether fraction (15.867 µg/ml)	34
Chloroform fraction (15.867 µg/ml)	40
Ethyl acetate fraction (15.867 µg/ml)	11
Aqueous fraction (15.867 µg/ml)	6
The crude polysaccharides (15.867 µg/ml)	3

Table 6. The antileishmanial activity of the total extract and different fractions of *A. hirtum* leaves (primary phase assay).

Group	% inhibition		
	*L_donovani*_Pinh	*L_donovani*_AMAST_Pinh	*L_donovani*_AMASTTHP_Pinh
Amphotericin B (0.40 µg/ml)	99	96	81
Total leaves extract (20 µg/ml)	0	4	0
Petroleum ether fraction (20 µg/ml)	38	10	0
Chloroform fraction (20 µg/ml)	16	29	78
Ethyl acetate fraction (20 µg/ml)	3	11	2
Aqueous fraction (20 µg/ml)	4	11	11
Crude polysaccharides (20 µg/ml)	0	10	1

5.Conclusion

The present study dealing with evaluation of antimicrobial and antiparasitic activities of total extract and different fractions of *A. hirtum*. The total extract exhibited potent antimicrobial activity against *P. aeruginosa* and *C. glabrata*. While, the chloroform fraction showed strong antibacterial activity against *S. aureus*. The petroleum ether fraction was effective against *C. albicans*. On the other hand, the chloroform fraction had a potent antileishmanial activity with the highest against *Leishmania donovani* AMASTTHP_Pinh. This study suggests further research in the field of antibacterial and antifungal activities of *A. hirtum* single components to develop new antimicrobial agents of natural sources with their known wide safety margin and lower side effects.

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