

Evaluation of The Antibacterial Activity of Some Yemeni Euphorbiaceae species

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Abstract

Extracts (Petroleum ether, chloroform, ethylacetate, 96% methanol, and water) from different parts of fifteen Yemeni Euphorbiaceae species: *Croton lobatus* L., aerial part; *Euphorbia ammak* Shweinf., succulent stems; *Euphorbia cuneata* Vahl., aerial part; *Euphorbia fruticosa* Forssk., succulent stems; *Euphorbia heterophylla* L., whole plant; *Euphorbia hirta* L., whole plant; *Euphorbia inaequilatera* Sond., aerial part; *Euphorbia inarticulata* Schweinf., aerial part; *Euphorbia schimperi* Presl., succulent stems; *Euphorbia uzmuk* S. Carter and J.R.I.Wood, whole plant; *Flueggea virosa* (Roxb.ex Willd) Voight, aerial part; *Jatropha curcas* L., leaves and twigs; *Jatropha spinosa* Vahl, aerial part; *Jatropha variegata* (Forsk) Vahl., aerial part; and *Ricinus communis* L., leaves were screened for antibacterial activity against the following standard bacterial strains: *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), using a qualitative agar diffusion test. Twenty-seven extracts out of 51 tested extracts were found active against one or more of the tested microorganisms. Extracts from *Euphorbia cuneata* and *Flueggea virosa* were active against all tested bacteria. Extracts of *Euphorbia ammak* (96% methanol, water) and of *Euphorbia uzmuk* (ethyl acetate, water) were the most active against (*E. coli* and *B. subtilis*) and against (*S. aureus*, *S. epidermidis* and *E. coli*) respectively.

Introduction:

Yemen has a rich and diverse flora, which is characterized by strong endemism due to the geographical structure and various climates of the country (1). It supplies the Yemeni traditional medicine with a large and various medicinal flora used by the indigenous people in traditional healing of various diseases, and provides us with an excellent source to be explored for new therapeutic agents without the disadvantages of growing resistance and toxicity of the currently available commercial antibiotics. Determination of the antimicrobial activity of traditionally used drugs can also help to provide scientific justification for the use of those drugs, and can pave the way for their involvement in the public

health system to cover the basic health needs especially for people living in remote areas in developing countries.

Euphorbiaceae is one of the most important families in Yemen, characterized by a large number of endemic species (2). Euphorbiaceae species are used in Yemeni traditional medicine for the treatment of various diseases including skin and other infectious diseases (3, 4). The antimicrobial efficacy of many of these species however has not been investigated. Screening the antibacterial activity of different extracts obtained from different parts of 15 Yemeni plants belonging to the family Euphorbiaceae was the purpose of this work. Furthermore, a preliminary phytochemical screening of

the methanol extracts of the tested plants was performed. This work constitutes a part of the ongoing investigation of the antimicrobial activity of Yemeni medicinal plants with the aim of finding new effective antibacterial compounds.

Materials and Methods

Materials

Plant materials

Plants used in this study were the *Croton lobatus* L., aerial part; *Euphorbia ammak* Schweinf., succulent stems; *Euphorbia cuneata* Vahl., aerial part; *Euphorbia fruticosa* Forssk., succulent stems; *Euphorbia heterophylla* L., whole plant; *Euphorbia hirta* L., whole plant; *Euphorbia inaequilatera* Sond., aerial part; *Euphorbia inarticulata* Schweinf., aerial part; *Euphorbia schimperii* Presl., succulent stems; *Euphorbia uzumuk* S. Carter and J.R.I.Wood, whole plant; *Flueggea virosa* (Roxb.ex Willd) Voight, aerial part; *Jatropha curcas* L., leaves and twigs; *Jatropha spinosa* Vahl, aerial part; *Jatropha variegata* (Forsk) Vahl., aerial part; and *Ricinus communis* L., leaves. They were collected in Taiz province, in the autumn (September – October). The collection and authentication was made under the supervision of Dr. A. Al Khulaidi and Abdulhabib Al-Kadasi-Agriculture Research Centre in Dhamar. Voucher specimens were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Sana'a.

After collection, the plant materials were subsequently shade dried at ambient temperature and then in an oven (35°C). The dried plant materials were then ground in a grinder.

Microorganisms

The following bacterial strains were used for determining antibacterial activity: *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), and *Escherichia coli* (ATCC 10536).

Methods

Preparation of extracts

50g from each sample of ground plant material were extracted with hot 96% methanol (500ml) for 4 hours; the extract was shaken during the extraction, and at the end of the extraction period was filtered through Whatman No.1 filter paper. The residue was extracted with 96% methanol for an additional 2 times. The filtered extract was then concentrated to dryness under reduced pressure.

Methanol extracts found to be antibacterial active were suspended in distilled water and extracted successively with petroleum ether (3 x), chloroform (3 x) and finally with ethyl acetate (3 x). The collected petroleum ether extract, chloroform extract and ethyl acetate extract were evaporated to dryness in vacuum. The aqueous phase was freeze-dried.

Antibacterial assay

A modified agar diffusion method (5, 6) was used to determine antibacterial activity. Sterile Mueller- Hinton agar (38 g agar / 1 distilled water) was inoculated with bacterial cells (200 µl of bacterial cell suspension in 20ml medium) and poured into petri dishes to give a solid plate. Twenty-five µl of the test extract (equivalent to 5 mg of the dried extract) was applied on sterile paper disc (5mm in diameter). After removing the solvent from the paper discs (under laminar airflow cabinet), the discs were deposited on the surface of inoculated agar plates. Paper discs impregnated with ampicillin (10µg), gentamicin (10µg), and erythromycin (15µg) used as positive controls and those impregnated with the corresponding solvents (Petroleum ether, chloroform, ethyl acetate, 96% methanol and water) and dried, served as negative controls, were placed on the agar surface. Plates were then incubated for 24 hrs at 37°C.

The inhibition of bacterial growth was determined by measuring the diameter of the clear zone around each disc at the end of the incubation time. An average zone of inhibition was calculated for three replicates.

Phytochemical screening of the methanolic extracts

The phytochemical screening of the methanol extracts for detection of chemical constituents present in them was carried out by using procedures (chemical methods and thin layer chromatography) described earlier (7, 8).

Statistics

The assay was performed three times. All values were expressed as mean \pm standard deviation.

Results

The present article reports the antibacterial activity of a number of Euphorbiaceae species. A summary of their ethnobotanical data collected from folklore reports and literature reviews (3, 4, 9, 10, 11, 12, 13, 14, 15, 16) is presented in (Table 1). The results of the phytochemical screening of the methanol extracts revealed the presence of alkaloids, flavonoids, saponins, sterols, trepenoids and tannins (Table 2). Out of 51 tested extracts (Petroleum ether, chloroform, ethyl acetate, 96% methanol and water) obtained from 15 plants, 27 extracts belong to 9 plants showed variable degrees of antibacterial activity against one or more of the tested microorganisms (Table 2).

The 96% methanol extract of *E. cuneata* was found to possess antibacterial activity against all tested microorganisms (Table 2). The inhibition of *B. subtilis* by the aqueous extract of *E. ammak* was equal to that produced by the positive control (Table 2). The 96% methanol and the aqueous extracts of *E. ammak* as well as the aqueous extract of *E. uzumuk* demonstrated antibacterial activity against *E. coli* approaching as well as exceeding those exhibited by the positive controls (Table 2, Figure 1). Noteworthy to mention is the inhibition of *S. epidermidis* and *S. aureus* by the ethyl acetate extract of *E. uzumuk* (Table 2).

The test microorganisms showed different sensitivity to the plant extracts. *S. aureus* was the most sensitive; it was inhibited by 63% of the tested extracts.

Discussion

Euphorbiaceae (with 106 species) is an important family in Yemen with a large number of endemic species (31 species). *Euphorbia* is the largest and the most diverse genus inhabit in the Yemen flora. (2, 17). This study describes the antibacterial activity of 27 different extracts found active among 51 tested extracts obtained from different parts of 15 Euphorbiaceae species growing in Yemen. A number of Euphorbiaceae species are utilized in Yemeni traditional medicine mainly externally to cure various skin diseases. Some species however are used internally for the treatment of constipation (3) (Table 1). Several species of Euphorbiaceae have been used by local population of many other countries in folk medicine as remedies for several diseases such as malaria, ulcers, tumors, warts, diabetes, gastrointestinal disturbances, respiratory tract complaints, cardiac, hepatic, nephrotic, urinary tract, rheumatic, neurologic, and ophthalmic diseases (18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28).

The microbial growth inhibition demonstrated by the polar extracts of *E. ammak* such as 96% methanol and water extracts against *E. coli* (Figure 1) and water extract against *B. subtilis* (Table 2) implies that this plant could contain antibacterial active constituents useful in treating infections diseases caused by *E. coli* and by *B. subtilis*, which is now being recognized as a bacterial pathogen (29, 30, 31, 32, 33). Moreover, these results provide some scientific confirmation for the use of *E. ammak* in Yemeni traditional medicine for the treatment of skin diseases.

The broad spectrum of antibacterial activity demonstrated by the 96% methanol extract of *E. cuneata* and by the different extracts of *F. virosa* as well as the magnitude of the antibacterial activity exhibited by the ethyl acetate and water extracts of *E. uzumuk* against *S. aureus*, *S. epidermidis* and *E. coli* respectively (Table 2) suggest that these plants could be utilized for the treatment of infectious diseases. This is

the first report about the antibacterial activity of *E. ammak*, *E. cuneata* and *E. uzumuk*, as no data regarding the antibacterial activity of those plants were found. On the other hand, a study (16) presented that *F. virosa* growing in Uganda was active against *S. aureus*, *S. epidermidis* and *B. subtilis* supports our results.

The growth inhibitory effect of different extracts of *E. hirta* against the tested gram-positive bacteria especially *S. aureus* indicates that several compounds (polar and nonpolar) may be involved in the antibacterial activity of the plant. A number of studies (34, 35, 36, 37, 38, 39, 40) have demonstrated that different extracts (Petroleum ether, ethyl acetate, ethanol and water) of *E. hirta* were active against a number of bacteria including *S. aureus*, *B. subtilis* and *E. coli*. Although *E. hirta* is not used in Yemen for the treatment of infectious diseases, the observed antibacterial activity of *E. hirta* extracts in our study lends some scientific justification for the traditional use of this plant in other countries (11,12,13,14,15), and at the same time suggests that this plant could be used in Yemeni traditional medicine for the treatment of infectious diseases.

Although *J. varigata* was found to possess weak antibacterial activity, the usefulness of this plant in the Yemeni traditional medicine as antiseptic may be due to the application of a great amount of the extract on the infected area. Hence, the observed antibacterial activity

of this plant against gram-positive bacteria may afford some validation for the use of this plant in the traditional medicine. Despite the rule of Euphorbiaceae species in folk medicine, it has been indicated that a number of other Euphorbiaceae species contain skin irritants; (41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51,52) toxic (53, 54) and cancer promoting (45, 51,55) substances.

Several studies (56, 57, 58, 59) have demonstrated that the antibacterial active constituents found in higher plants belong to the commonly secondary metabolites such as alkaloids, coumarins, chromans, flavonoids, quinines, saponins, terpenoids and tannins. Consequently the results of our phytochemical screening (Table 2) suggest that alkaloids, saponins, terpenoids, flavonoids and tannins detected in the tested extracts may be involved in the antibacterial activity of the investigated plants.

Conclusion

The results presented in this study provide some scientific justification for the use of some plants frequently utilized in Yemeni traditional medicine as a local treatment of skin diseases. They also reveal the importance of some other plants such as *E. ammak*, *E. cuneata*, and *E. uzumuk* as promising antibacterial active candidates for a further investigation for new effective antibacterial compounds.

Table 1. Ethnobotanical data of the tested plants

Plant species	Part used	Vernacular name	Traditional uses
<i>Croton lobatus</i> L.	-	-	Malaria (9)
<i>Euphorbia ammak</i> Schweinf.	Lat	Amaq عمق	Camel mange, verruca (3)
<i>Euphorbia cuneata</i> Vahl	-	Shinzib شنزيب	-
<i>Euphorbia fruticosa</i> Forssk	WP	Zaqum زقوم Shurur شورور	Antiparalytic (3)
<i>Euphorbia heterophylla</i> L.	L	-	Analgesic (10), worms (11)
<i>Euphorbia hirta</i> L.	Sa, WP, L	Labbayn, lubain لبين، أم الحليب	Verruca(4), fever, inflammation, diarrhea, asthma, colic, cough, genitor- urinary diseases, sterility, false teeth, worms and bowel complaints (11, 12, 13, 14, 15)
<i>Euphorbia inaequilatera</i> Sond.	-	Libanah لبنان	-
<i>Euphorbia inarticulata</i> Schweinf.	-	Syayb, Qasas قصاص، صياب	-
<i>Euphorbia schimperi</i> Presl.	-	Duhun دهن، جعدان، رميد عشيق	-
<i>Euphorbia uzumuk</i> S. Carter and J.R.I.Wood.	-	Uzmuk عزمق	-
<i>Flueggea virosa</i> (Roxb.ex Willd) Voight.	R	Nahaf نهف، شجرة الذبين	Hydrocele in children, gonorrhoea, hernia, migraine and abortion(11)
<i>Jatropha curcas</i> L.	L, R, S	Sharib شرب زيت	Laxative(3), hernia, wounds, promote labour, retained placenta, painful menstruation, fatigue, Boil (11)
<i>Jatropha spinosa</i> Vahl.	Sa	Anab diab عنب ثنب	Antiseptic, wound healing (4)
<i>Jatropha variegata</i> (Forsk) Vahl.	Sa	Ibki ابكي	Antiseptic, haemostatic (3)
<i>Ricinus communis</i> L.	S,R, L	Kurua خروع، تبشع	Laxative(3), miscarriage, dislocation, uterine fibroids, snake bite, premature ejaculation, antenatal, haemorrhoids (11), Abdominal pain, Appetizer (16)

L: leaves, R: Roots, S: seeds, WP: Whole plant, Lat: latex, Sa: sap, -: no data available

Table 2. Results of the antibacterial activity and phytochemical screening of the tested plant extracts

Plant species/ Tested Extract (% yield)1	Microorganism				Phytochemical screening
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>B. subtilis</i>	<i>E. coli</i>	
	(Diameter of inhibition zone, mm)				
<i>Euphorbia ammak</i> Succulent stems extracts					
Chloroform (0.1)	0	0	7(±0.6)	0	Flavonoids, saponins, sterols, terpenoids, tannins
96% Methanol (13.4)	0	0	0	14(±1.5)	
Water (2.0)	0	0	17 (±1.5)	15 (±1.5)	
<i>Euphorbia cuneata</i> Aerial part extracts					
Ethyl acetate (0.7)	0	0	7(±1.5)	0	Alkaloids, flavonoids, saponins, sterols, terpenoids, tannins
96% Methanol (7.4)	11(±1.5)	10(±1.5)	12(±2)	12(±1)	
<i>Euphorbia fruticosa</i> Succulent stems extracts					
Chloroform (0.1)	0	0	7(±0.6)	0	Flavonoids, sterols, terpenoids
96% Methanol (2.5)	9(±1)	9(±1.2)	0	0	
<i>Euphorbia heterophylla</i> Whole plant extracts					
96% Methanol (6.1)	0	0	7(±0.6)	0	Flavonoids, saponins, sterols, terpenoids, tannins
<i>Euphorbia hirta</i> Whole plant extracts					
Petroleum ether (0.2)	7(±1)	0	0	0	Alkaloid, flavonoids, saponins, sterols, terpenoids, tannins
Chloroform(0.2)	7(±0.6)	8(±1.2)	0	0	
Ethyl acetate (0.1)	7(±1.2)	10(±1.5)	7(±0.6)	0	
96% Methanol (5.5)	11(±1.5)	0	0	0	
Water (1.0)	8(±1)	0	0	0	
<i>Euphorbia uzmuk</i> Whole plant extracts					
Ethyl acetate (0.1)	13(±1.2)	20(±2)	0	7(±0.6)	Flavonoids, saponins, sterols, terpenoids, tannins
96% Methanol(7.5)	11(±1)	6(±0.6)	0	0	
Water (0.6)	9(±0.6)	0	0	13(±1.5)	
<i>Flueggea virosa</i> Aerial part extracts					
Petroleum ether (2.4)	0	8(±1)	10(±1)	0	Alkaloid, flavonoids, sterols, terpenoids, tannins
Chloroform (0.4)	0	0	0	11(±1)	
96% Methanol (11.1)	12(±1.5)	0	0	0	
<i>Jatropha variegata</i> Aerial part extracts					
Petroleum ether (0.4)	0	7(±0.6)	0	0	Flavonoids, saponins, sterols, terpenoids, tannins
Chloroform (0.4)	7(±0.6)	0	0	0	
96% Methanol(6.9)	11(±1)	0	0	0	
Water (0.9)	9(±1)	6(±0.6)	0	0	
<i>Ricinus communis</i> Leaves extracts					
Petroleum ether (2.4)	8(±1)	10(±0.6)	0	0	Alkaloid, flavonoids, sterols, terpenoids, tannins
Chloroform(0.6)	7(±0.6)	0	0	0	
Ethyl acetate (0.8)	6(±0.6)	6(±0.6)	0	0	
96% Methanol(10.5)	0	10(±0.6)	0	0	
Solvent2	0	0	0	0	
Ampicillin (10µg/disc)	31(±1)	34(±1.5)	16(±1.5)	15(±1)	
Gentamicin (10µg/disc)	19(±1.5)	23(±1.5)	19(±2)	17(±1.5)	
Ervthromvcin (15ug/disc)	28(±1.7)	28(±0.6)	31(±1.5)	13(±1.5)	

¹Percentage extract yield (w/w) was estimated as dry extract weight/dry starting material weight × 100.

²Sovent control: 25 µl of the corresponding solvent (Petroleum ether, chloroform, ethyl acetate, 96% methanol and water).

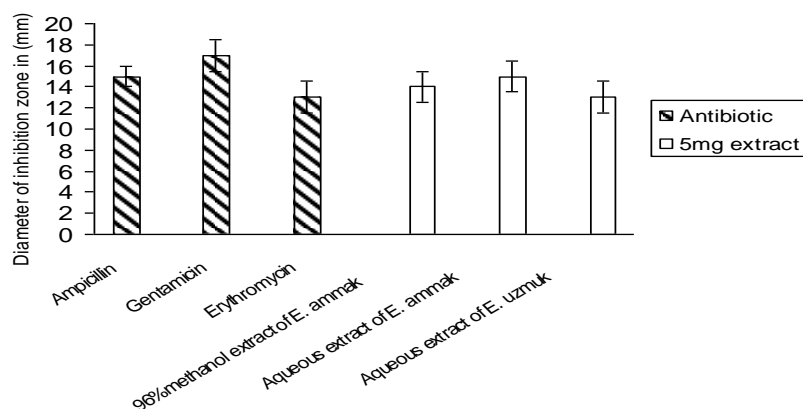


Figure 1: Antibacterial activity of the 96% methanol and aqueous extracts of *Euphorbia ammak* (*E. ammak*) and of the aqueous extract of *Euphorbia uzumuk* (*E. uzumuk*) against *Escherichia coli*

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