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The Inhibitory Effect of *Euphorbia Hirta* Extracts against Some Wound Bacteria Isolated From Yemeni Patients

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Abstract

The search for novel natural products from medicinal plants against bacterial strains resistant to multidrug is promising and urgent need to overcome the resistance complication. This study was carried out to investigate the antibacterial activities of Euphorbia hirta extracts against some bacteria isolated from surgical wounds of the hospitals located at Aden City, Yemen. The methanolic and aqueous extracts of the leaves and stems of Euphorbia hirta were determined for their antimicrobial activity by agar well diffusion method against *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis*, and *Staphylococcus aureus*. The phytochemical screening was conducted to determine the plant constituents. The results showed that the extracts have a significant inhibitory effect on most bacterial species, especially *P. mirabilis* bacteria. Also, the *E. hirta* methanolic extracts were more efficient than aqueous extracts in the inhibition of tested bacteria at a concentration of 150 µL. However, the results revealed that the ciprofloxacin antibiotic had a higher inhibitory effect than other antibiotics used. Also, both of methanolic and aqueous extracts of *E. hirta* were showed more effect than antibiotics on most bacterial species, especially when concentration at 150 µL. The phytochemical screening revealed the presence of reducing sugars, terpenoids, alkaloids, steroids, tannins, oils, saponin, cardiac glycosides, anthroquinones, and flavanoids. The present findings showed that *E. hirta* possesses interesting inhibitory properties against bacteria associated with surgical wounds infection.

Keywords: *Euphorbia hirta; Phytochemical; Aqueous and Methanolic Extracts; Antimicrobial Activity*

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Introduction

Wounds, resulting from microbial infection, are the most common public health problems that are responsible between 70 to 80% of mortality in the world [1,2]. The common wound pathogens includes bacteria, fungi, protozoa and viruses among which the most common are *Streptococcus pyogens, Staphylococcus aureus, Pseudomonas aeuruginosa, Proteus, Escherichia coli* and *Enterococcus* [3,4]. These microorganisms are present in hospital and community acquired infections, decreasing antibiotic therapy options especially *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Due to increasing of resistance against several antimicrobial drugs, searching for new therapeutic alternatives using medicinal plants play a significant role for obtaining new drugs [5,6]. Medicinal plants are part and parcel of human

society to combat diseases, from the dawn of civilization. They usually contain many biological active ingredients and are used primarily for treating mild or chronic ailments. According to world health organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs [7]. Therapeutical properties of medical plants are very useful in healing various diseases owing to their attributes having wide biological and medicinal activities [8]. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell [9,10]. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains [11]. In this study, it was investigated the antibacterial activities of the E. hirta aqueous and methanolic extracts against four bacteria isolated from surgical wounds of Yemeni patients.

Materials and Methods

Collection of plant material

The fresh plant *Euphorbia hirta* that used in this study was collected from a different area in Dhi Al-Sufal located in Ibb City, Yemen. The taxonomical identification of the plant was confirmed by Dr. AbdulNaser Al-Gifri of the Biology Department, Faculty of Science, and Aden University, Yemen.

Preparation of plant extracts

Aqueous extract

A weighed quantity of 40 g of plant powder (in each of leaves and steams) was extracted in 400 mL of sterile water and soaked for two days (48h). After that, the mixture was filtered through double layered of clean muslin cloth and then Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using an oven at 45ºC to remove the excess solvent. Subsequently, the solution was filter sterilized with 0.45 µm of mixed cellulose ester membranes (Millipore USA). The extracts were kept in a sterile bottle under refrigerated condition (4ºC) until needed for use [12]. 1g of extract was dissolved in 10 mL of sterile water and this gave 100 mg/mL. Thereafter three serial dilutions were made from the original stock of 10 mL (containing 100 mg/mL), according to the method described by Akujobi., *et al*. [13], using the solvents to obtain the following concentrations: 5 mg/mL (50 µL), 10 mg/mL (100 µL), and 15 mg/mL (150 µL) which were used for the antimicrobial sensitivity test.

Methanolic extract

The preparation of methanolic extraction followed the Naga., et al. [14], method. 40g of dried powder (in each of leaves and steams) plant was soaked into a flask containing 400 mL of methanol 96% and shaken for 6 hours. The mixture was filtered by Whatman No.1 filter paper and evaporated to dryness using a rotary evaporator at 45ºC and pressure 25 mm Hg [15]. 1g of extract was dissolved in 10 mL of Daimethylsulfoxide to gives 100 mg/mL and the solution was filter sterilized with 0.45 µm of mixed cellulose ester membranes (Millipore USA). After that, the prepared different concentrations (50, 100, 150 mg/mL) were prepared of the standard solution with a concentration of 100 mg/Ml. Then, the extracts were stored in the refrigerator in airtight containers until use [12].

Source of bacteria species

Four types of bacteria species used in this study are *E. coli, P. aeruginosa, P. mirabilis,* and *S. aureus* which have been isolated from the wounds of surgeries for patients from different hospitals located in Aden City, Yemen. The Isolated bacteria were subjected to standard microbiological identification tests based on morphological characteristics for the colony, and biochemical tests to confirm their identity/purity according to the criteria of Bergey's Manual of Systematic Bacteriology, 2nd edition [16].

Antibiotics susceptibility testing of extracts

The method described by Emeruwa [17] was used. In briefly, 100 µL of a 20-hour culture of bacteria adjusted to 1.0×10^8 CFU/mL was spread using a sterile glass spreader onto a Mueller Hinton agar plate. The wells approximately 6 mm in diameter and 2.5 mm deep

were bored on the surfaces of the agar medium using a sterile cork borer. Each concentration of extract was pipetted individually into one of the holes. The plates were allowed to stand for one hour for pre-diffusion of the extracts to occur before incubating overnight at 37ºC for 24h. The zones of inhibition that developed were measured in mm (millimeter) and the results were recorded [17].

Phytochemical screening of the plant material

Phytochemical screening was carried out on the powdered plant material for the presence of bioactive components such as terpenoids, tannins, alkaloids, cardiac glycosides, carbohydrates, anthroquinones, steroids, oils, flavonoids, and saponins [17-19].

Results

Antibacterial activity of plants extract

The present study showed that the extract of leaves and stems of *E. hirta*, both aqueous and methanol, observed the inhibitory effects on all bacterial species isolated. The results of the antibacterial screening of the different concentrations of the aqueous extract of leaves on the test isolates are shown in Table (1) and Figure (1).

The aqueous extract of plant leaves was showed the minimum effect on growth of *S. aureus* and *E. coli* bacteria at 50 µL and 100 µL in two diameters inhibitory of 6.1 mm, while it was showed the maximum effect on the *S. aureus* and *P. mirabilis* at 150 µL in diameters inhibitory of 12 mm and 12.8 mm, respectively, (Table 1).

However, the aqueous extracts of the stems were showed the minimum effect on the S. aureus bacteria at 50 µL in inhibitory diameter of 7 mm and a maximum effect of P. mirabilis when at 150 µL in diameter of 20.3 mm (Table 1). The results showed that the increase in the concentration of aqueous extract increased the zone of growth inhibition of tested bacteria.

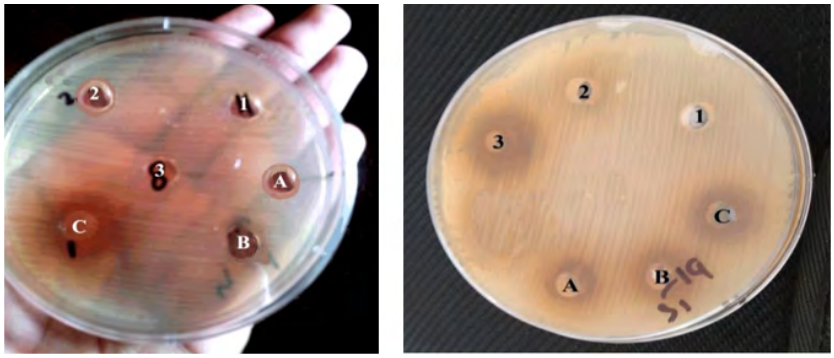
Pathogen organisms	Aqueous extract					
	Leaves			Stems		
	50µL (5mg)	100µL (10mg)	150µL (15mg)	50µL (5mg)	100µL (10mg)	150µL (15mg)
E. coli	6.1	6.1	9.6	15,1	16.3	17.7
P. mirabilis	6	9.7	12.8	13.9	16.3	20.3
P. aeruginosa	6	6.3	9.9	7.3	9.7	13.1
S. aureus	6.1	9.1	12	7	9.4	13.3

Table 1: *effect of aqueous extract of E. hirta on the isolated bacteria.*

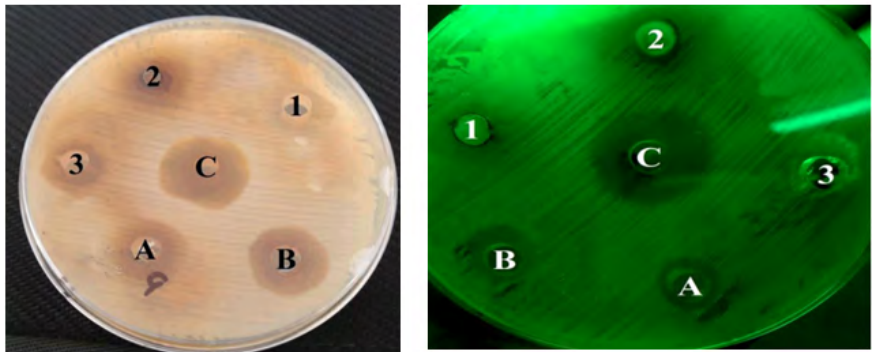
In the methanolic extract of leaves, it was showed the minimum effect on the *S. aureus* bacteria when at 50 µL with an inhibitory diameter of 7.1 mm and the maximum effect of *P. mirabilis* bacteria at 150 µL was recorded with an inhibitory diameter of 13.9 mm as shown in Table (2) and Figure. (2).

While, in the methanolic extract of the stems was showed the minimum inhibitory effect on growth of *P. aeruginos* and *S. aureus* bacteria at 50 µL in inhibitory diameters of 7.3 mm and 7.9 mm, respectively, and the maximum effect of the P. mirabilis at 150 µL in inhibitory diameter of 21.1 mm (Table 2).

P. mirabilis *E. coli*



S. aureus *P. aeruginosa*



Concentration of extracts

Stems: (A)= 50 µL, (B)=100 µL, (C)=150 µL Leaves: (1)=50 µL, (2)=100 µL, (3)=150 µL

Figure 1: *The effect of E. hirta methanolic aqueous on isolated bacteria.*

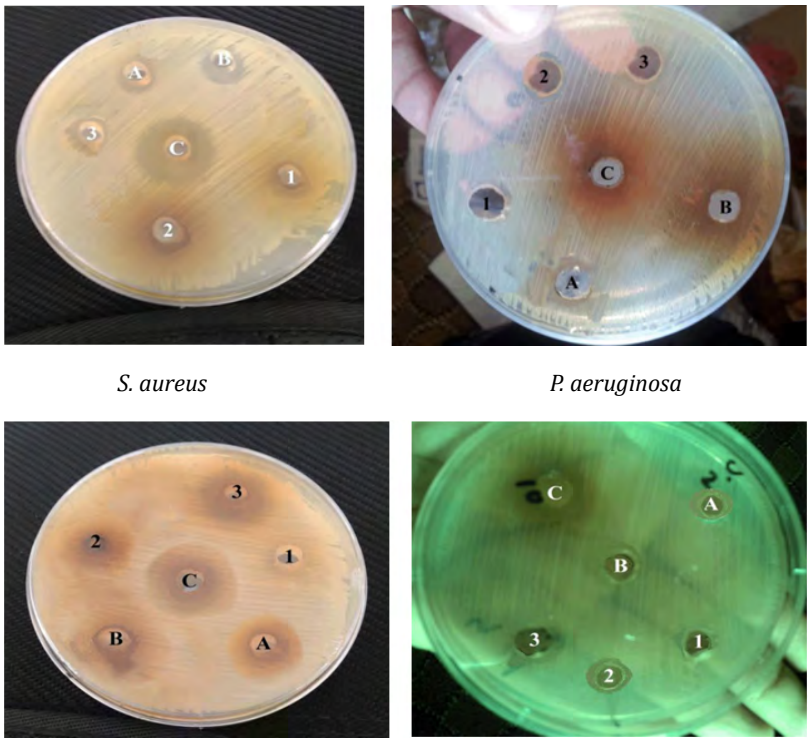
Pathogen organisms	Methanolic extract					
	Leaves			Stems		
	50µL (5mg)	100µL (10mg)	150µL (15mg)	50µL (5mg)	100µL (10mg)	150µL (15mg)
E. coli	6	7.3	9.7	14.6	16.1	19.7
P. mirabilis	6.3	9.3	13.9	10.4	16.7	21.1
P. aeruginosa	6.2	7.1	10.3	7.3	9.6	10.9
S. aureus	7.1	13	13	7.9	10.6	13.7

Table 2: *Effect methanolic extract of E. hirta on the isolated bacteria.*

Phytochemical constituents

It was observed that the methanol extract contained all the active compounds excepting the anthraqunones and saponins, while the anthroquinones, oil, and saponins were not detected in the aqueous extract as showed in the table (3).

P. mirabilis *E. coli*



S. aureus *P. aeruginosa*

Concentration of extracts

Stems: (A)= 50 µL, (B)=100 µL, (C)=150 µL Leaves: (1)=50 µL, (2)=100 µL, (3)=150 µL

Figure 2: *The effect of E. hirta methanolic extract on isolated bacteria.*

Chemical compounds	Name of the test	Aqueous extract		Methanolic extract	
		Leaves	Stems	Leaves	Stems
ALKaloids	Hager's	+	+	+	+
Anthroquinones	Chloroform layer	-	-	-	-
Cardiac glycoside	Killer-Killani's	+	+	+	+
Flavonoids	Ammonia (modified)	+	+	+	+
Oils	Solubility	-	-	+	+
Reducing sugar	Fehling's	+	+	+	+
Saponins	Frothing	-	-	-	-
Steroids	Salkowski	+	+	+	+
Tanins	FeCl3	+	+	+	+
Terpenoids	Salkowski (modified)	+	+	+	+

- = Compound not detected; + = compound detected

Table 3: *Phytochemical screening of E. hirta extract.*

Discussion

The results obtained showed that all the bacteria tested were susceptible to both methanolic and aqueous extracts at different concentrations of *E. hirta*. It was observed that the methanolic extracts are more effective than aqueous extracts. Also, the steam extracts more effective than the leaf extracts. These findings are consistent with results of Ogueke., *et al*. [20] who indicated that the *E. hirta* extract inhibited the growth of *S. aureus, E. coli* and *P. aeruginosa* in varying degrees. Also, the study by Nazeer, [21] showed that the fresh latex extract of *E. hirta* was inhibited the growth of *E. coli and P. aeriginosa,* while the *S. aureus* is resistant. *S. aureus* produces the enzyme penicillinase which converts the antibiotic penicillin to penicillinic acid which is no longer inhibitory to its growth [22]. Other workers have also shown that extracts of *E. hirta* inhibit the growth of various microorganisms at different concentrations [13, 23-25]. The current study raveled that the phytochemical screening of extract was found to contain the tannins, flavonoids, alkaloids and cardiac glycosides and free of saponins. These results are in accordance obtained from the extraction of *E. hirta* with different solvents by many workers [20,24]. Some workers have also identified tannins, flavonoids, and alkaloids in the extracts of the plant [23, 26-27].

Plants are rich in a wide variety of secondary metabolites such as alkaloids, tannins, terpenoids, and flavonoids having been found in vitro since they have antimicrobial properties and may serve as an alternative, effective, cheep, and safe antimicrobial for the treatment of microbial infections [28]. The observed antibacterial properties corroborate its use in traditional medicine. Traditionally extracts of the plant are used in sore and wound healing, as ear drop for boils in the ear and treatment of boils. They are also used in the control of diarrhea and dysentery [29,30].

Conclusions

The aqueous and ethanol extracts of *E. hirta* show promise and can form a primary platform as an alternative therapy in the control of wounds pathogens. Further phytochemical and Pharmacological studies will be required to isolate the active constituents and estimate the antimicrobial activities against a wide range of bacterial pathogens.

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