

## RESEARCH ARTICLE

**PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS,  
ANTIOXIDANT AND ANTIMICROBIAL EVALUATION OF AERIAL  
PARTS OF *JATROPHA SPINOSA*, *JATROPHA VARIEGATA* AND  
*EUPHORBIA MILII*****Saleh Kassem Algfri<sup>1,\*</sup> , Khaled Saed Ali<sup>2</sup>, Gulia Ahmed Naser<sup>1</sup>, Ahmed Bin Shuaib<sup>1</sup>**<sup>1</sup> Dept. of Pharmacognosy, Faculty of Pharmacy, University of Aden, Yemen<sup>2</sup> Dept. of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Aden, Yemen**\*Corresponding author: Saleh Kassem Algfri; E-mail: [algfri\\_s25@yahoo.com](mailto:algfri_s25@yahoo.com); [algfri\\_s25@pharm.adenuniv.com](mailto:algfri_s25@pharm.adenuniv.com), [algfris25@gmail.com](mailto:algfris25@gmail.com); Mobile No: 967773725084****Received: 13 March 2024 / Accepted: 25 March 2025 / Published online: 31 March 2025****Abstract**

Yemen is renowned for its rich biodiversity, particularly its medicinal flora, which includes numerous endemic species of significant therapeutic value. This study aims to investigate the physicochemical and phytochemical properties of the aerial parts of *Jatropha spinosa*, *Jatropha variegata*, and *Euphorbia milii*, and evaluate their antioxidant and antimicrobial activities. Standard methodologies were used. Physicochemical parameters such as ash value, extractive value and moisture content were evaluated and phytochemicals like phenolics, flavonoids, tannins, terpenes, sterols and carbohydrates were determined in the studied plants. The total phenolic content in the 80% methanolic extracts were 128.12, 63.70, and 50.54 mg GAE/g for *Euphorbia milii*, *Jatropha variegata* and *Jatropha spinosa*, respectively. Antioxidant activity was evaluated using the in vitro DPPH assay. The IC<sub>50</sub> values of the methanolic extract of *Jatropha spinosa*, *Jatropha variegata*, *Euphorbia milii*, and the standard quercetin were  $100.71 \pm 0.43$ ,  $54.62 \pm 0.63$ ,  $12.56 \pm 0.34$ , and  $2.37 \pm 0.23$  µg/mL, respectively. Among the studied extracts, *Euphorbia milii* exhibited the highest antioxidant activity, when compared with the standard quercetin. The antimicrobial activity was assessed using the agar well diffusion method. Methanolic extracts at concentrations of 250 mg/ml showed the highest antimicrobial activity against *Staphylococcus aureus*, with *Euphorbia milii*, followed by *Jatropha variegata* and *Jatropha spinosa*, producing inhibition zone diameters of 22.13 mm, 20.22 mm, and 17.43 mm, respectively. Against *Pseudomonas aeruginosa*, the inhibition zones were 20.22 mm for *Euphorbia milii*, 18.97 mm for *Jatropha variegata*, and 17.77 mm for *Jatropha spinosa*. Similarly, against *Proteus mirabilis*, inhibition zones were 18.32 mm, 17.54 mm, and 15.23 mm for *Euphorbia milii*, *Jatropha variegata*, and *Jatropha spinosa*, respectively. For *Candida albicans*, the inhibition zones were 19.44 mm for *Euphorbia milii*, 16.54 mm for *Jatropha variegata*, and 16.55 mm for *Jatropha spinosa*. The above results indicate that the studied plants, especially *Euphorbia milii*, may be good sources of antioxidants and antimicrobials.

**Keywords:** *Euphorbia milii*, *Jatropha variegata*, *Jatropha spinosa*, Phytochemical, Antioxidant, Antimicrobial.**1. Introduction**

Traditional medicine still plays an important role in health care in Yemen, as in developing countries in general [1, 2, 3], as it includes medical beliefs and practices determined by epidemiological, cultural, historical and economic factors, with plants forming the core of folk medicine [3]. Medicinal plants form the

cornerstone of Yemeni folk medicine due to the country's rich and diverse vegetation, offering a wide range of traditional remedies [3]. Notably, many therapeutic uses of plants in Yemen are unique and not documented elsewhere [4]. The *Euphorbia* family in Yemen, which includes 106 species, has great botanical and medicinal importance. The *Euphorbia* genus includes 62 species,

16 of which are endemic [5, 6]. *Euphorbia* is a vast and diverse genus of flowering plants containing at least 2,000 species globally [7]. Similarly, the genus *Jatropha*, in the Euphorbiaceae family encompasses approximately 170 known species [8]. *Jatropha variegata* is native to Yemen, whereas *Jatropha spinosa* is indigenous to Yemen and neighboring regions, including Saudi Arabia, Somalia, and Djibouti [9]. In Yemeni folk medicine, *J. variegata* and *J. spinosa* have traditionally been used for their antimicrobial, antihemorrhagic properties, and wound-healing capabilities [1, 3]. *Euphorbia milii*, recently identified in Yemen, is native to Madagascar and is widely cultivated as an ornamental plant in tropical and temperate regions [10].

Research on Yemeni species collected from Taiz Governorate has demonstrated that juices of *J. variegata* exhibits antibacterial (against Gram-positive bacteria only) and antioxidant properties, attributed to its bioactive steroids and flavonoids [11]. It has also shown anti-fertility and wound-healing effects [12, 13]. Likewise, *J. spinosa* stems were found to be less effective against tested pathogens compared to the fresh juice extract [14]. Although no scientific studies have been conducted on *E. milii* in Yemen, research in other regions highlights its medicinal potential in treating liver diseases, cancer, and skin conditions [15]. Phytochemical analyses of *E. milii* have identified cardiac glycosides, phytosterols, anthocyanins, proteins, terpenoids, flavonoids, and tannins in its aerial parts [16, 17]. The increasing interest in the antioxidant and antimicrobial properties of plant extracts underscores their potential as sources for novel therapeutic agents [18, 19]. Despite Yemen's rich plant diversity, many species face threats and remain understudied [3, 20]. However, numerous native plants (especially medicinal plants) exhibit promising biological activities with potential global significance [20, 21, 22]. Most studies on the Yemeni *Jatropha spinosa* and *Jatropha variegata* have focused primarily on their stems or juices, leaving many scientific aspects unexplored. Furthermore, there is no research on *Euphorbia milii* to date. Therefore, this study aims to investigate the physicochemical and phytochemical properties of the aerial parts of these three species, collected from Al-Dhalea Governorate, and evaluate their antioxidant and antimicrobial activities.

## 2. Material and Methods

### 2.1. Plant material

Aerial parts of *Jatropha spinosa*, *Jatropha variegata*, and *Euphorbia milii* were collected in April 2024 from Al-Hisha, Al-Dhalea, Republic of Yemen, dried in the shaded area and then manually grinded and stored at room temperature for further analysis. The plant specimens were identified by Prof. Abdul Nasser Algfri, Department of Biology, University of Aden. Voucher

specimens of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii* with No. FPUA 0003, FPUA 0041 and FPUA 0002 respectively, were deposited in the Herbarium Unit of the Pharmacognosy Department, Faculty of Pharmacy, University of Aden for future reference.

### 2.2. Physicochemical analysis

The physicochemical parameters such as loss on drying (moisture content), total ash, acid insoluble ash, water soluble ash, water soluble extractive and ethanol soluble extractive values were carried out in dried powder according to WHO methods [23].

### 2.3. Preparation of the extracts

Dried powders of the aerial parts of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii* (50 g) were extracted separately with 80% methanol (80-90 °C) in a Soxhlet apparatus until complete extraction, which was confirmed by the color of the extracted liquid. The 80% methanolic extracts were filtered and evaporated to dryness by rotary evaporator and the percentage yield of each extract was calculated in terms of air-dried material. The dried crude extracts were stored in a tightly closed bottle at 4°C for further study [24, 25].

### 2.4. Qualitative phytochemical analysis

Phytochemical screening of the 80% methanolic extracts of the aerial parts of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii* was performed according to the standard methods of Harborne [24, 25].

### 2.5. Determination of total phenolic content

The total phenolic content (TPC) of the 80% methanolic extracts of the aerial parts of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii* was determined using the Folin-Ciocalteu reagent, following a slightly modified method of Ainsworth [26]. Gallic acid was used as a reference standard for constructing the calibration curve. For the assay, 0.5 mL of the plant extract (1 mg/mL) was mixed with 2 mL of Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and neutralized with 4 mL of sodium carbonate solution (7.5% w/v). The reaction mixture was incubated at room temperature for 30 minutes with intermittent shaking to allow color development. The absorbance of the resulting blue complex was measured at 765 nm using a UV-VIS spectrophotometer. The total phenolic content was determined using a standard calibration curve prepared with gallic acid at concentrations of 20, 40, 60, 80, 120, and 140 µg/mL. The results were expressed as mg gallic acid equivalent (GAE) per gram of dry extract (mg GAE/g), calculated using the following equation:

$$\text{TPC} = X \text{ mg} \cdot V \text{ ml/Mg (mg GAE/g)}$$

Where:

- $X$  = Concentration obtained from the calibration curve (mg/mL)
- $V$  = Volume of extract used (mL)
- $M$  = Mass of extract used (g)

## 2.6. Thin layer chromatography analysis

The 80% methanolic extract of *Jatropha spinosa*, *Jatropha variegata*, and *Euphorbia milii* (1g of each) was separately extracted with n-hexane (20 mL) to obtain n-hexanic fraction (procedure was repeated three times). The residue was dried, and then extracted with methanol to obtain methanolic fraction as procedure mentioned above. The obtained n-hexanic and methanolic fractions were used for thin layer chromatography to separate compounds and determine their  $R_f$  values, following the method described by Waksmundzka-Hajnos M and Wagner H [27, 28].

## 2.7. Antioxidant activity

The antioxidant activity of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii* was evaluated using the DPPH free radical scavenging method described by Chan et al., with slight modifications [29]. Various concentrations of the extracts and quercetin (5, 20, 60, 100, 140, and 180  $\mu\text{g/mL}$ ) were prepared. A DPPH solution was also prepared by dissolving 6.0 mg of DPPH in 100 mL of methanol. For the assay, 1 mL of each dilution was added to a test tube containing 2 mL of DPPH solution. The mixture was shaken vigorously and left to stand in the dark for 30 minutes. A negative control was prepared by adding 1 mL of methanol to 2 mL of DPPH solution. Quercetin was used as the reference compound. The absorbance of the resulting solutions was measured spectrophotometrically at 517 nm.

The DPPH radical scavenging activity of the extracts was calculated using the following equation:

$$\text{Inhibition \%} = [(A_0 - A_1) / A_0] \times 100;$$

$A_0$  is the absorbance of control and  $A_1$  is absorbance of test.

## 2.8. Calculation of $IC_{50}$ Value

The antioxidant activity of the methanolic extract was expressed as the  $IC_{50}$  value and compared with the standard. The  $IC_{50}$  value, representing the concentration required to scavenge 50% of DPPH radicals, was determined by linear regression analysis of the dose-response curve plotting % inhibition against extract concentration [30]. The results were presented as mean values  $\pm$  standard deviation ( $n = 3$ ).

## 2.9. Antimicrobial activity

### 2.9.1. Microbial strains

The antimicrobial activity of each plant extract was evaluated using four microbial strains. Two strains of

Gram-negative bacteria (*Pseudomonas aeruginosa* and *Proteus mirabilis*), one strain of Gram-positive bacteria (*Staphylococcus aureus*) and one strain of fungi (*Candida albicans*). The microbes were isolated clinically and identified by microbiologist Dr. Abdullah Omar, Head of the Microbiology Department at the National Center for General and Central Laboratories in Aden.

### 2.9.2. Inoculums preparation

Each bacterial strain was subcultured overnight at 37 °C in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, and the turbidity of the bacterial suspension was adjusted to 1.5  $10^8$  CFU/ml by comparison with 0.5 McFarland standard against a sheet of white paper with black stripes. A 0.5 McFarland standard was prepared by mixing 50  $\mu\text{L}$  of 1.175% barium chloride dehydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) with 9.95 ml of 1% sulfuric acid ( $\text{H}_2\text{SO}_4$ ) [31].

### 2.9.3. Antibacterial assay

Antibacterial activity of the 80% methanolic extracts of *Jatropha spinosa*, *Jatropha variegata*, and *Euphorbia milii* was tested by using Agar Well Diffusion method according to [31, 32]. In this method, the Mueller Hinton agar plates were inoculated with respective bacteria (*Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus*) and left to dry for 10 minutes. 5 wells of 6 mm diameter were made on each agar plate using sterile pipette tips. The wells were labeled as test wells and control wells. The test wells were filled with 50  $\mu\text{L}$  of different concentrations of the stock solution prepared from the extracts. The concentrations used were (50, 100, 150, 200 and 250mg/ml). Dimethyl sulfoxide (DMSO) was used as negative control. The antibiotics Augmentine (30  $\mu\text{g/disc}$ ), Ciprofloxacin (5  $\mu\text{g/disc}$ ), Gentamycine (10  $\mu\text{g/disc}$ ), Amikacine (30  $\mu\text{g/disc}$ ) and Imipenem (10  $\mu\text{g/disc}$ ) were used as positive control. The antibacterial activity was determined after 24 hours following incubation of plates at 37 °C, by measuring the diameter of the inhibition zone (in mm) around both the wells and disks using a ruler. All experiments were performed in triplicate.

### 2.9.4. Antifungal assay

Pure culture for fungi was prepared by spreading fungi suspension on Sterile physiological solution on Mueller Hinton Agar. Then the same steps were done as the antibacterial assay. Nystatin (100  $\mu\text{g/disc}$ ) was used as positive control. The experiment was carried out in triplicate [33].

## 2.10. Statistical Analysis

The data were analyzed using Student's t-test for variance assessment. All experiments were performed in triplicate, and the results were expressed as mean  $\pm$

standard deviation (SD). A p-value of less than 0.05 was considered statistically significant.

### 3. Results and Discussion

#### 3.1. Physicochemical analysis

The physicochemical parameters of the aerial parts of *Jatropha spinosa*, *Jatropha variegata*, and *Euphorbia milii* were analyzed using dried powders. Physicochemical analysis provides essential information regarding the authenticity, purity, and quality of crude pharmaceuticals. The percentage yield of 80% methanol of the aerial parts of *J. spinosa*, *J. variegata* and *E. milii* were 10.09%, 18.70%, and 13.37%, respectively. The total ash content was 13.95%, 11.30%, and 12.68% for *J. spinosa*, *J. variegata*, and *E. milii*, respectively, representing the total mineral content. The acid-insoluble ash values were 0.76%, 1.35%, and 0.83%, respectively, indicating minimal siliceous contamination, which ensures plant material quality. The water-soluble ash values were 9.30%, 7.38%, and 6.63%, respectively, supporting the presence of water-soluble inorganic compounds that may have biological significance. Extractive values indicate the solubility of active compounds in different solvents. The high water-soluble extractive values (16.50%, 21.00%, and 32.00% for *J. spinosa*, *J. variegata*, and *E. milii*, respectively) suggest the presence of highly polar compounds. Meanwhile, the ethanol-soluble extractive values (4.80%, 10.60%, and

16.40%, respectively) indicate the presence of moderately polar compounds, such as flavonoids and phenolics. The moisture content was 9.96%, 8.93%, and 10.80%, for *J. spinosa*, *J. variegata*, and *E. milii*, respectively, indicating that the plant material was adequately dried, minimizing the risk of microbial growth. High moisture content in crude drugs can promote spoilage by molds and bacteria and may lead to enzymatic degradation of active constituents [34]. Reducing moisture content enhances the shelf life of crude drugs [35]. Physicochemical analysis plays a crucial role in assessing adulteration, quality, and purity of crude drugs [23, 36, 37]. The analyzed parameters in this study were within acceptable limits (Table 1).

#### 3.1. Qualitative phytochemical analysis

Phytochemicals such as phenolics, flavonoids, tannins, terpenes, sterols and carbohydrates were identified as major chemical components in the studied samples (Table 2). Many references attribute the various pharmacological effects of plants such as anti-inflammatory, antibacterial, antiviral, antioxidant and anticancer effects to the presence of secondary plant metabolites including alkaloids, flavonoids, glycosides, tannins, steroids, etc. [38, 39]. Previous research has shown that the chemical components of Euphorbiaceae family are diterpenoids, triterpenoids, sterols, flavonoids, phenols, and tannins [11, 40, 41], which is consistent with the result of our research.

**Table 1:** Physicochemical parameters (%w/w) of the aerial parts of studied plants

Samples (powder)	Total ash +SD	Acid insoluble ash +SD	Water soluble ash +SD	Moisture content +SD	Water soluble extractive +SD	Ethanol soluble extractive +SD	Percentage yield of 80% methanol
<i>Jatropha spinosa</i>	13.95± 2.31	0.76± 1.61	9.30± 0.31	9.96± 2.12	16.50± 2.33	4.80± 3.01	10.09
<i>Jatropha variegata</i>	11.30± 0.51	1.35± 1.60	7.38± 3.11	8.93± 1.61	21.00± 1.21	10.60± 3.22	18.70
<i>Euphorbia milii</i>	12.68± 3.21	0.83± 1.41	6.63± 3.34	10.80± 3.20	32.00± 3.32	16.40± 0.41	13.37

**Table 2:** The phytochemical screening of 80% methanol extracts of the aerial parts of studied plants

Phytochemicals	Tests	<i>Jatropha spinosa</i>	<i>Jatropha variegata</i>	<i>Euphorbia milii</i>
Carbohydrates	Benedict's test	+	+	++
Phenols/Tannins	Ferric chloride test	+++	+++	+++
Flavonoids	Shinoda test	+++	+++	+++
	Sodium hydroxide Test	++	++	++
	Lead acetate test	+++	+++	+++
	Aluminum chloride test	+++	+++	+++
Saponins	Foam test	-	-	-
Sterols/ Triterpenes	Salkowski test	+++	+++	+++
	Liebermann-Burchard test	+++	+++	+++
Alkaloids	Wagner's test	-	-	-
	Mayer's test	-	-	-
	Dragendorff's test	-	-	-

+++ = Most intense, ++ = moderately intense, + = Least intense, - = absent.



### 3.2. Determination of the total phenolic content (TPC)

The present study has been carried out for quantification of the total phenolic content of 80% methanolic extract of the aerial parts of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii*. The content of the phenolic compounds in the selected extracts determined from regression equation of the calibration curve ( $y=0.0024x-0.1024$ ,  $R^2 = 0.992$ ) of gallic acid (20–140  $\mu\text{g/mL}$ ) and expressed in mg Gallic acid equivalent (GAE) per gram dry extract. The results were 128.12, 63.70 and 50.54 mg/g equivalent (GAE) in 80% methanolic extracts of *E. milii*, *J. variegata* and *J. spinosa* respectively, indicating the high phenolic content in *E. milii*. The identification of phenolic compounds is important because they possess diverse biological activities, e.g., antiulcer, anti-inflammatory, antioxidant, anti-cytotoxic, antispasmodic, angiogenic and antitumor activities [11, 42]. Here we point out the importance of intensifying chemical and pharmaceutical research to isolate important compounds from these plants and determine their pharmacological activity. The calculation of the total phenolic content (TPC) is

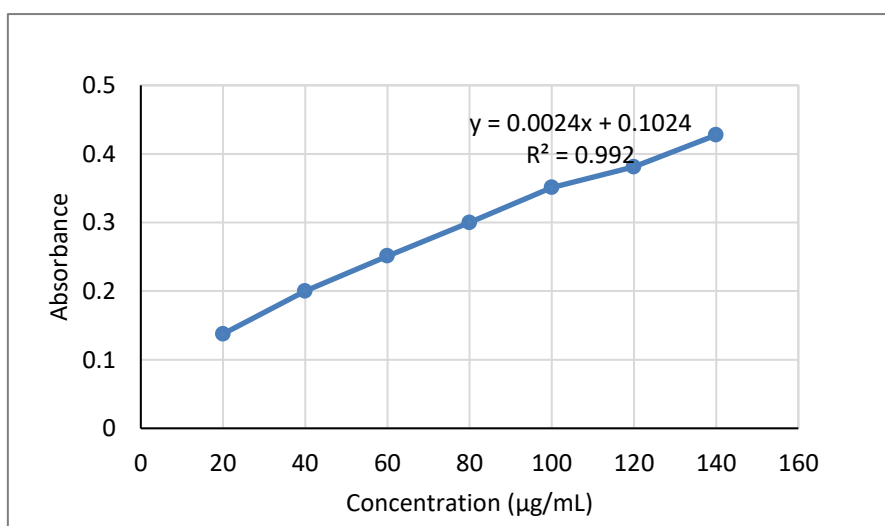
represented in Table 3, and the calibration curve of gallic acid shown in Figure 1.

### 3.1. Thin Layer chromatography analysis

The methanol and n-hexane fractions of 80% methanolic extracts of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii* were subjected to TLC test. TLC plate (silica gel G 60 F254 with layer thickness 0.2mm, Merck- Germany) and solvent system toluene: ethyl formate: formic acid (8:4:0.5) was used to obtain the best separation of compounds. TLC is used as a rapid and reliable screening method to confirm the presence of phytoconstituents [27, 28]. By TLC examination, in the methanol fraction, a large number of spots were found in the *E. milii* extract (13 spots), while in the n-hexane fraction a large number of spots were found in the *J. spinosa* extract (11 spots). Photos of the plates were taken in UV chamber (365 nm) and  $R_f$  values of developed spots of different extracts were calculated (Table 4 and Figure 2).

**Table 3:** Calculation of the total phenolic content (TPC) of 80% methanolic extracts of studied plants.

Sample	Absorption	Regression equation	X ( $\mu\text{g}$ )	X (mg)	TPC=X mg.V ml /M g	TPC +SD
E milii	0.4099	$y=0.0024x+0.1024$	128.125	0.128125	TPC=0.12812mg. 0.5ml/.0005 g	128.12 $\pm$ 2.43
J variagata	0.2553		63.708	0.063708	TPC=0.063708mg.0.5ml/0, 0005	63.70 $\pm$ 1.31
J spinosa	0.2237		50.541	0.050541	TPC=0.050541mg.0.5ml/0, 0005	50.54 $\pm$ 1.42

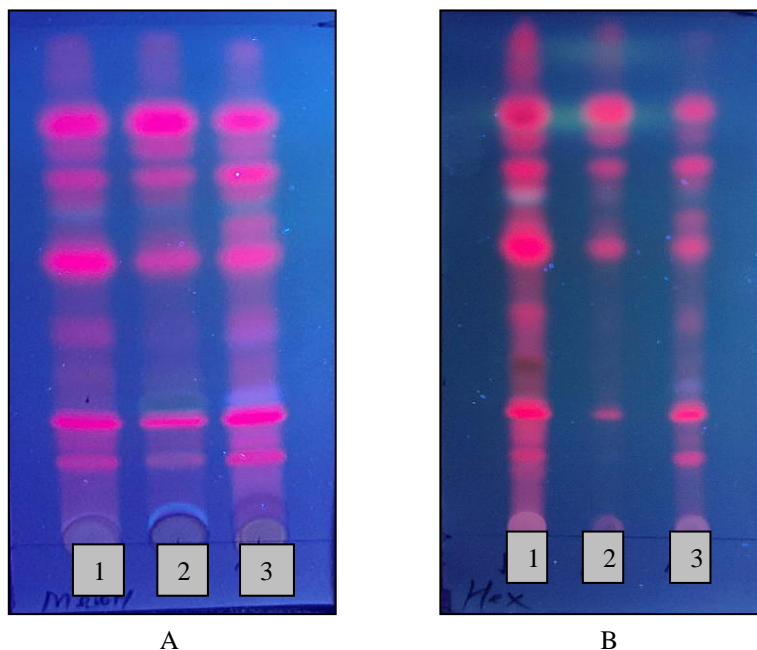


**Fig. 1:** Calibration curve of gallic acid

**Table 4:** TLC profile of methanol and n-hexane fractions in toluene- ethyl formate - formic acid (8:4:0.5).

Spots under 365 nm		Methanol fractions of:			n-Hexane fractions of:		
R <sub>f</sub> values	colour	Jatropha spinosa	Jatropha variegata	Euphorbia milii	Jatropha spinosa	Jatropha variegata	Euphorbia milii
0.96	Very light pink	+	+	+	+	+	-
0.92	Very light pink	+	+	-	+	-	-
0.82	Deep pink	+++	+++	+++	+++	+++	+++
0.77	Light pink	+	+	+	+	+	
0.72	Deep pink	++	++	++	++	++	++
0.65	Blue	++	+	+	++	+	-
0.61	Light pink	-	-	+	-	-	+
0.56	Deep pink	+++	+++	+++	+++	++	++
0.42	Light pink	+	-	+	+	-	-
0.40	Blue	-	-	+	-	-	-
0.32	Light pink	+	-	+	-	-	+
0.30	Red	-	-	-	++	-	-
0.28	Blue	-	-	++	-	-	+
0.26	Deep pink	+++	+++	+++	+++	+	++
0.17	Pink	++	++	++	++	-	+
0.06	Blue	-	++	-	-	-	-
Total spots		11	10	13	11	7	8

+++ = Most intense, ++ = moderately intense, + = Least intense, - = absent.

**Fig. 2:** TLC plates of methanol fraction (A) and n hexane fraction (B) of *J. spinosa* (1), *J. variegata* (2) and *E. milii* (3), in toluene - ethyl format: formic acid(8:4:0.5) under UV 365

### 3.2. The antioxidant activity

The antioxidant activity of 80% methanolic extracts of the aerial parts of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii* was determined by using in-vitro DPPH free radical scavenging method. Different concentrations of the sample extracts and standard

quercetin viz. 5 µg/ml, 20 µg/ml, 60 µg/ml, 100 µg/ml, 140 µg/ml and 180 µg/ml were used in the experiment. The free radical DPPH in solution is purple and gives a strong absorption maximum at 517 nm. DPPH turns from purple to yellow when the added electron pairs with the hydrogen from the antioxidants that scavenge free radicals to form the reduced DPPH-H.

Data of % Inhibition (scavenging capacity) and IC<sub>50</sub> values of the methanolic extracts of *J. spinosa*, *J. variegata* and *E. milii* and standard quercetin at different concentrations are represented in Table 5 and Figure 3. At a concentration of 180 µg/mL, the scavenging activity of *J. spinosa*, *J. variegata* and *E. milii* was 84.30± 0.42, 84.49± 0.41, and 84.81± 0.31%, respectively, while at the same concentration, the scavenging activity of the standard quercetin was 96.70± 0.24%. The IC<sub>50</sub> values of the methanolic extracts of *J. spinosa*, *J. variegata*, *E. milii* and quercetin were 100.71 ± 0.43, 54.62 ± 0.63, 12.56 ± 0.34 and 2.37 ± 0.23 µg/ml, respectively, indicating the high activity of *E. milii* extract among other extracts, and thus the free radical scavenging activity of different extracts and quercetin was in the following order: Quercetin > *Euphorbia milii* > *Jatropha variegata* > *Jatropha spinosa*. According to [43], the antioxidant strength level is divided into four levels: very strong (IC<sub>50</sub> < 50 µg/ml), strong (IC<sub>50</sub>: 50-100 µg/ml), moderate (IC<sub>50</sub>: 101-150 µg/ml), and weak (IC<sub>50</sub>: 250-500 µg/ml). These results indicate that 80% of the methanolic extracts of the studied plants have high antioxidant activity due to the presence of flavonoids, glycosides, tannins and steroids which were identified in the studied plants and thus are in agreement with

previous research [38, 39]. Also, our results regarding the antioxidant activity of *J. variegata* are in agreement with those of previous researches [11, 12], and the antioxidant activity of Indian and Pakistani *E. milii* is in agreement with our results [16, 17].

### 3.1. The antimicrobial activity

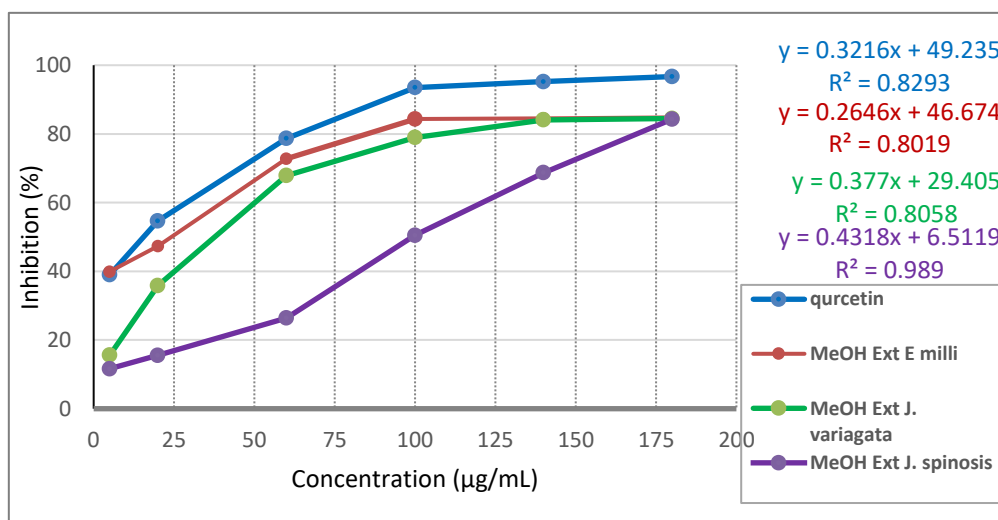
#### 3.1.1. The agar well diffusion assay

The results of the antimicrobial activity of the investigated extracts are shown in Table 6 as well as Figures 4, 5, 6 and 7. The antimicrobial activity was determined by the presence or absence of an inhibition zone around the wells. All of tested plants show antimicrobial activity against used strains *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus* and *Candida albicans*.

The growth of microbial strains inhibited very effectively by *Euphorbia milii* aerial part, followed by *Jatropha variegata* then *Jatropha spinosa*. *J. spinosa* extract recorded the smallest inhibition zone (10.12 mm) from the lowest concentration of 50 mg/ml against *C. albicans* with the diameters of the inhibition zone increasing with increasing solution concentrations.

**Table 5:** Free radical DPPH scavenging activity (% inhibition) and IC<sub>50</sub> value of extracts of studied plants and quercetin.

Concentration (µg/mL)	Quercetin +SD	J. spinosa +SD	J. variagata +SD	E. milii +SD
5	39.00± 0.13	11.65± 0.43	15.57± 0.33	39.93± 0.35
20	54.70± 0.21	15.56± 0.21	35.87± 0.21	47.33± 0.26
60	78.70± 0.22	26.40± 0.53	67.86± 0.43	72.78± 0.34
100	93.50± 0.34	50.50± 0.33	78.94± 0.35	84.31± 0.33
140	95.20± 0.14	68.70± 0.52	84.10± 0.26	84.49± 0.25
180	96.70± 0.24	84.30± 0.42	84.49± 0.41	84.81± 0.31
IC <sub>50</sub> (µg/mL)	2.37± 0.23	100.71± 0.43	54.62± 0.36	12.56± 0.34



**Fig. 3:** The DPPH radical scavenging activity of 80% methanolic extracts of studied plants, and standard quercetin

The methanolic extracts at concentrations of 200 and 250 mg/mL exhibited the highest antimicrobial activity against *S. aureus*, with *E. milii* showing the strongest inhibition, followed by *J. variegata* and *J. spinosa*, producing inhibition zone diameters of 20.22 and 22.13 mm, 18.11 and 20.22 mm, and 16.33 and 17.43 mm, respectively. Against *P. aeruginosa*, the inhibition zones at 250 mg/mL were 20.22 mm for *E. milii*, 18.97 mm for *J. variegata*, and 17.77 mm for *J. spinosa*. Similarly, against *P. mirabilis*, inhibition zones measured 18.32 mm, 17.54 mm, and 15.23 mm for *E. milii*, *J. variegata*, and *J. spinosa*, respectively, at 250 mg/mL. For *C. albicans*, the inhibition zones at the highest concentration (250 mg/mL) were 19.44 mm for *E. milii*, 16.54 mm for *J. variegata*, and 16.55 mm for *J. spinosa*.

By comparing the results of the current study with previous research on Yemeni species, notable similarities and differences in antimicrobial activity were observed. For instance, *J. variegata* has been reported to exhibit antibacterial activity exclusively against Gram-positive bacteria, including multidrug-resistant strains [11]. The leaf extract of *J. variegata* demonstrated moderate antibacterial activity against *S. aureus*, with an inhibition zone of 10.60 mm [13]. Additionally, the methanolic extracts of *J. spinosas* were found to be less effective against tested pathogens compared to the fresh juice extract [14]. In contrast, the 80% methanolic extract in our study exhibited the strongest inhibition. Furthermore, both the methanolic stem extract and the fresh stem juice of *J. spinosa* were ineffective against *C. albicans* at any

concentration [14]. However, our findings demonstrated significant inhibition at a concentration of 250 mg/mL. These discrepancies may be attributed to several factors, including the plant part used, extraction methods, solvent type, and environmental conditions. Several studies have linked the antimicrobial and antioxidant activities of various *Euphorbiaceae* family to the presence of secondary metabolites such as biologically active steroids, tannins, phenolics, and flavonoids [11, 12, 44]. Our findings align with this understanding, further supporting the role of these bioactive compounds in antimicrobial activity. Therefore, in-depth research, such as bioactivity-guided fractionation, is needed to isolate compounds with good biological activity from the plants under study.

### 3.1.2. The sensitivity test of studied microbes against selected antibiotics

The results presented in Table 7 indicate the sensitivity of all tested bacterial strains to the antibiotics used, with some exceptions. *P. aeruginosa* showed no inhibition in response to Augmentin or Ciprofloxacin, while *P. mirabilis* exhibited no inhibition against Imipenem. The inhibition zone diameters of the tested antibiotics ranged from 17.00 mm to 40.00 mm. *P. mirabilis* demonstrated the lowest sensitivity to Gentamicin, with an inhibition zone of 17.00 mm, whereas it exhibited the highest sensitivity to Ciprofloxacin, with an inhibition zone of 40.00 mm. Additionally, *C. albicans* showed susceptibility to Nystatin, with an inhibition zone of  $25.67 \pm 0.58$  mm.

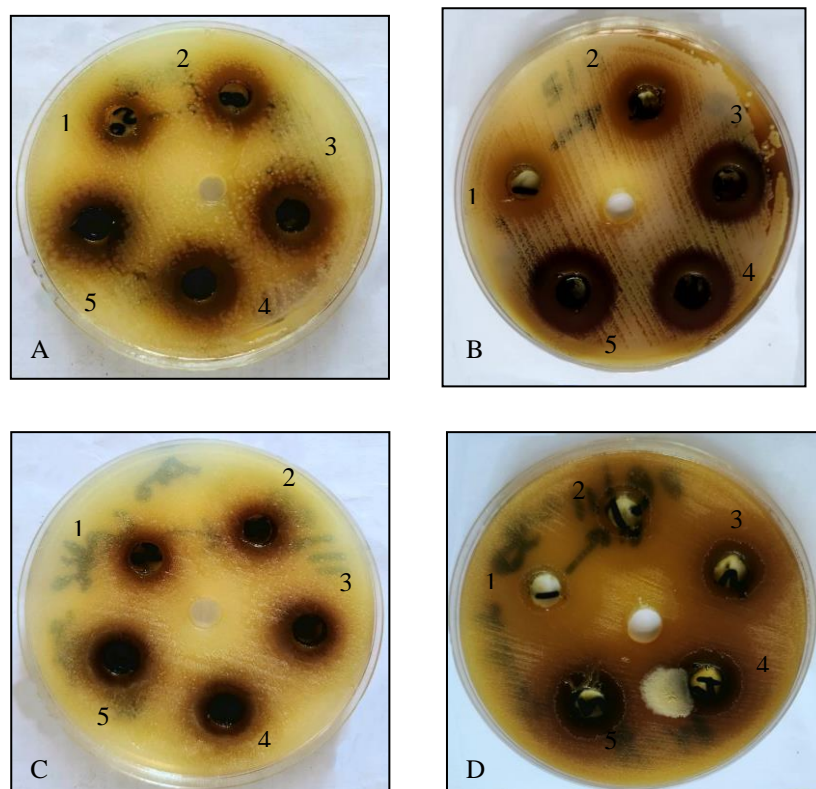
**Table 6:** Inhibition effect (mm) of aerial parts of studied plants against microbial tested by Agar well diffusion at concentrations (50, 100, 150,200, 250mg/ml).

Microorganisms	Zone of inhibition (mm)					
	Methanolic extract of <i>Euphorbia milii</i>					
	50 mg	100 mg	150 mg	200 mg	250 mg	DMSO
<i>Proteus mirabilis</i>	11.55	13.65	14.22	16.43	18.32	-
<i>Pseudomonas aeruginosa</i>	12.33	14.45	16.44	18.76	20.22	-
<i>Staphylococcus aureus</i>	15.33	17.44	19.45	20.22	22.13	-
<i>Candid albicans</i>	11.55	13.33	14.21	16.65	19.44	-
Methanolic extract of <i>Jatropha variegata</i>						
<i>Proteus mirabilis</i>	10.65	12.45	13.22	14.77	17.54	-
<i>pseudomonas aeruginosa</i>	10.33	12.34	14.98	16.77	18.97	-
<i>Staphylococcus aureus</i>	11.33	15.77	16.88	18.11	20.22	-
<i>Candid albicans</i>	11.34	13.55	14.65	15.11	16.54	-
Methanolic extract of <i>Jatropha spinosa</i>						
<i>Proteus mirabilis</i>	10.44	12.34	13.43	14.65	15.23	-
<i>pseudomonas aeruginosa</i>	11.56	12.54	13.33	14.76	17.77	-
<i>Staphylococcus aureus</i>	10.77	13.22	15.12	16.33	17.43	-
<i>Candid albicans</i>	10.12	12.43	13.32	15.43	16.55	-

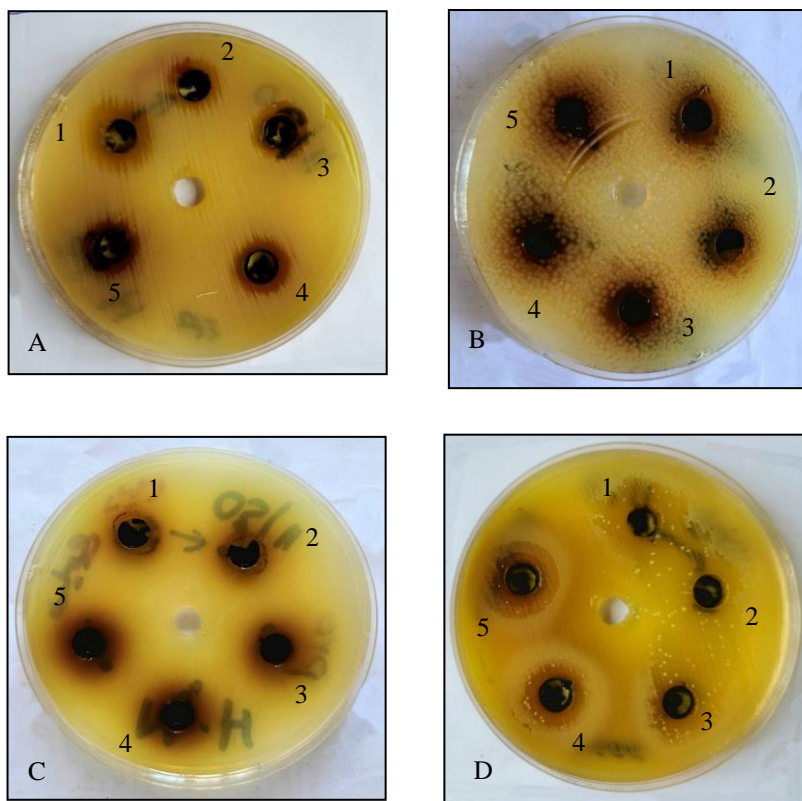


**Table 7:** Sensitivity test (mm) of bacterial tested against some antibiotics.

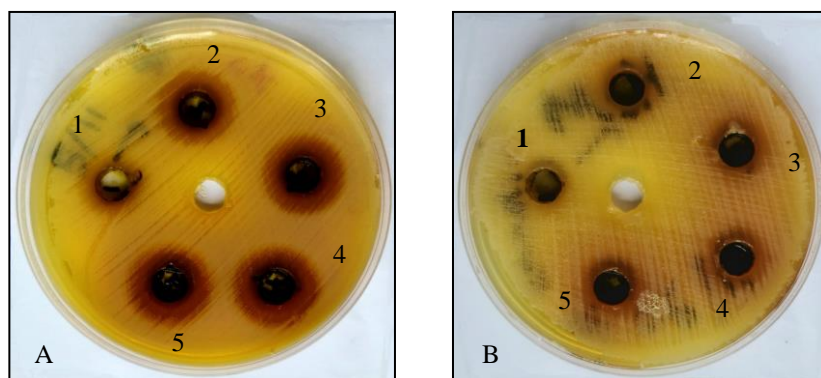
Antibiotics Microorganisms	Augmentine (Amc)	Ciprofloxacin (Cip)	Gentamycine (CN)	Amikacine (Ak)	Imipenem (IPM)	Nystatin
<i>pseudomonas aeruginosa</i>	Intermediate resistance	resistance	20.00 ± 0.5	16.00 ± 0.2	33.00 ± 0.3	-
<i>Staphylococcus aureus</i>	21.00 ± 0.3	30.00 ± 0.58	25.00 ± 0.3	23.00 ± 0.4	39.00 ± 0.2	-
<i>proteus mirabilis</i>	21.00 ± 0.1	40.00 ± 0.29	17.00 ± 0.5	18.00 ± 0.3	resistance	-
<i>Candid albicans</i>	-	-	-	-	-	25.67 ± 0.58



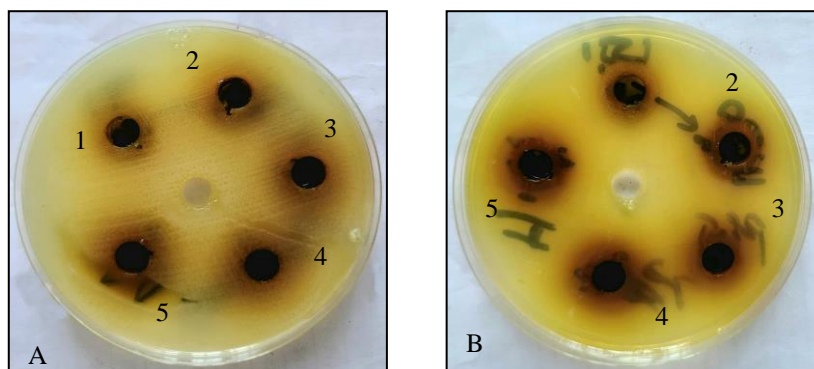
**Fig. 4:** A culture plate showing diameter of zones of inhibition of microbial growth for methanol extract of *Euphorbia milii* A: *Pseudomonas aeruginosa*, B: *Staphylococcus aureus*, C: *Proteus mirabilis* and D: *Candid albicans* with concentrations of 1 = 50 mg/ml 2 = 100mg/ml 3 = 150 mg/ml 4= 200 mg/ml 5= 250 mg/ml



**Fig. 5:** A culture plate showing diameter of zones of inhibition of microbial growth for methanol extract of *Jatropha spinosa* A: *Staphylococcus aureus*, B: *Pseudomonas aeruginosa*, C: *Proteus mirabilis*, D: *Candida albicans* with concentrations of 1 = 50 mg/ml 2 = 100mg/ml 3 = 150 mg/ml 4= 200 mg/ml 5= 250 mg/ml



**Fig. 6:** A culture plate showing diameter of zones of inhibition of microbial growth for methanol extract of *Jatropha variegata* A: *Staphylococcus aureus*, B: *Candida albicans*, with concentrations of 1 = 50 mg/ml 2 = 100mg/ml 3 = 150 mg/ml 4= 200 mg/ml 5= 250 mg/ml



**Fig. 7:** A culture plate showing diameter of zones of inhibition of microbial growth for methanol extract of *Jatropha variegata* A: *Pseudomonas aeruginosa*, B: *Proteus mirabilis*, with concentrations of 1 = 50 mg/ml 2 = 100mg/ml 3 = 150 mg/ml 4= 200 mg/ml 5= 250 mg/ml

## Conclusion

The present study was conducted on *Jatropha variegata*, which is native to Yemen, and *Jatropha spinosa*, which is native to Yemen and neighboring areas, in addition to *Euphorbia milii*. The physicochemical parameters, such as total ash, acid-insoluble ash, water-soluble ash, moisture content, water-soluble extractive and ethanol-soluble extractive values were performed. Phenolics, flavonoids, tannins, terpenes, sterols and carbohydrates were identified as major chemical components in the studied samples. The study showed that 80% of the methanolic extracts of the aerial parts of *Jatropha spinosa*, *Jatropha variegata*, and *Euphorbia milii* exhibited antioxidant activity using the DPPH method, as well as antimicrobial activity using the agar diffusion method. This is the first study conducted on *E. milii* cultivated in Yemen, and it can provide a simple comparison between three species of the Euphorbiaceae family, as well as contribute to documenting and expanding knowledge of Yemeni medicinal plants. However, further chemical and pharmacological studies are needed to isolate compounds with good biological activity from the plants under study.

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## التحليل الفيزيائي والكميائي النباتي، وتقييم مضادات الأكسدة ومضادات الميكروبات للأجزاء العلوية للنباتات *JATROPHA SPINOSA*, *JATROPHA VARIEGATA* AND *EUPHORBIA MILII*

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### المُلخَص

تشتهر اليمن بتنوعها البيولوجي الغني، وخاصة نباتاتها الطبية، التي تضم العديد من الأنواع المتوطنة ذات القيمة العلاجية الكبيرة. تهدف هذه الدراسة إلى دراسة الخصائص الفيزيائية والكيميائية النباتية للأجزاء العلوية من نباتات الجاتروفا سبينوزا (ديمع شوكي)، والجتروفا فاريجاتا (ابكي)، واليوفوربيا ميلي، وتقييم أنشطتها المضادة للأكسدة والميكروبات. وقد استُخدمت منهجيات قياسية. نتائج الدراسة: تم تقييم المعايير الفيزيوكيميائية مثل قيمة الرماد، والقيمة الاستخلاصية ومحتوى الرطوبة. كما تم تحديد المواد الكيميائية النباتية، مثل الفينولات، الفلافونويدات، العفص، التربينات، الستيرولات والكربوهيدرات. نتيجة إجمالي محتوى الفينول في المستخلصات الميثانولية كانت 50.54, 63.70, 128.12 ملغرام مكافئ ل حمض الجالك لكل من *Euphorbia milii*, *Jatropha variegata* و *Jatropha spinosa* على التوالي، مما يشير إلى ارتفاع ملحوظ في محتوى الفينول في *Euphorbia milii*. أيضا تم تقييم النشاط المضاد للأكسدة باستخدام اختبار DPPH في المختبر. بلغت قيم  $IC_{50}$  للمستخلصات الميثانولية لنباتات *Jatropha spinosa* و *Jatropha variegata* و *Euphorbia Milii* والكورسيتين القياسي  $100.71 \pm 0.43$  و  $54.62 \pm 0.63$  و  $12.56 \pm 0.34$  و  $2.37 \pm 0.23$  ميكروغرام/مل، على التوالي. ومن هذه النتيجة أظهرت *Euphorbia milii* أعلى نشاط مضاد للأكسدة، مقارنةً بالكورسيتين القياسي. تم تقييم النشاط المضاد للميكروبات باستخدام طريقة انتشار بئر الأجار. أظهرت المستخلصات الميثانولية بتركيز 250 ملغ/مل أعلى نشاط مضاد للميكروبات ضد *Staphylococcus aureus* مع *Euphorbia milii*، تليها *Jatropha variegata* و *Jatropha spinosa*، حيث بلغت أقطار مناطق التثبيط 22.13 مم، و 20.22 مم، و 17.43 مم على التوالي. أما بالنسبة *Pseudomonas aeruginosa*، فكانت أقطار مناطق التثبيط 20.22 مم لـ *Euphorbia milii*، و 18.97 مم لـ *Jatropha variegata*، و 17.77 مم لـ *Jatropha spinosa*. وبالمثل، كانت أقطار مناطق التثبيط ضد *Proteus mirabilis* 17.54 مم، و 15.23 مم لـ *Euphorbia milii* و *Jatropha variegata* و *Jatropha spinosa*، على التوالي. بالنسبة *Candida albicans*، كانت مناطق التثبيط 19.44 مم لـ *Euphorbia milii*، و 16.54 مم لـ *Jatropha variegata*، و 16.55 مم لـ *Jatropha spinosa*. تشير النتائج المذكورة أعلاه إلى أن النباتات المدروسة وخاصة نبات الفربيون ميلي قد تكون مصادر جيدة لمضادات الأكسدة ومضادات الميكروبات.

الكلمات المفتاحية: يوفوربيا ميلي، جاتروفا فاريجاتا (ابكي)، جاتروفا سبينوزا (ديمع شوكي)، كيمياء نباتية، مضاد للأكسدة، مضاد للميكروبات.

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