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Phytochemical Screening, Antioxidant and Antimicrobial Activities of red and yellow flowered *Mirabilis jalapa*

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Abstract

The phytochemical content, antioxidant capacity, and antimicrobial efficacy of leaf extracts from two forms of *Mirabilis jalapa (M. jalapa)* - red and yellow flowered- were investigated. Using the cold maceration method with 70% methanol, the presence of alkaloids, flavonoids, carbohydrate, protein, amino acid, phenol, tannins, saponins, , and steroids was confirmed. Fourier-transform infrared (FT-IR) spectrum analysis detected hydroxyl groups (OH), aromatic and aliphatic C-H bonds, aromatic C=C bonds, carbonyl group (C=O), amines (-C-N), and C-O group in both forms red and yellow leaves of *M. jalapa*.

The antioxidant activity assessed using the1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method showed higher free radical scavenging activities for the red -flowered leaves compared to the yellow-flowered ones at different concentrations. Four human pathogenic microorganisms; *Staphylococcus aureus(S. aureus), Escherichia coli(E. coli)*, *Pseudomonas aeruginosa(P. aeruginosa)*, and *Candida albicans(C. albicans)* were evaluated for antimicrobial activity by agar well diffusion method.The red-flowered form exhibited higher activity against the tested pathogens compared to the yellow form, likely due to its higher content of phenols and flavonoids in the methanol extracts. The minimum inhibitory concentration (MIC) was found to be 0.195 mg/ml *against S. aureus* and 0.049 mg/ml against *E. coli* and *P. aeruginosa*; for both forms, it was 0.025 mg/ml against *C. albicans*.

Article Info

Keywords:
Yemeni *Mirabilis jalapa*, phytochemical screening, FT-IR spectrum analysis antioxidant activity, Antimicrobial activities

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1. INTRODUCTION

The genus *Mirabilis,* which is mostly native to tropical America, is a member of the Nyctaginaceae family and is referred to as the "four-o'clock flower". The genus *Mirabilis* has more than fifty species, most of which are found in tropical and temperate regions of the world. *Mirabilis* himalaya is the sole species that is indigenous to the Himalayas rather than the Western Hemisphere [1]. There is a great deal of disagreement on where it originated (tropical America, most likely of Peru, Mexico, Chile, or India). Today it was widely distributed in the tropical regions of the world [2]. Furthermore; this geus is represented in Yemen by one species; *M. jalapa* Linn. specially in Taiz ,Assayani ,Ibb (J. Sumara, AlQae'ida, J. Dhisufal, Annajd Al Ahamer, Jibla, Mutheikhira, Alu'deyn, J.Bura ,and J.Raymah) [3].

Under optimum conditions, *M. jalapa* develops quickly, taking 10 weeks to reach full maturity from seed to seed. The leaves are ovate shape, green, and bitter with a characteristic odour. It possesses various flower colors which include yellow, white, magenta and pink **(Figure 1)**. The flower's unique feature is that it blooms fragrantly

(a) (b)

Figure 1. General view of Mirabilis jalapa, A:Red form ; B:Yellow form

in the early evening and collapses the following morning with a fragrant smell [4].

Different components of *M. jalapa* have been studied by a number of researchers. It has been demonstrated that various portions of *M. jalapa* contain a wide range of phytochemical compounds, including phenols, alkaloids, glycosides, flavonoids, saponins, steroids, tannins, triterpenes, and anthraquinones [5].

The various parts of *M. jalapa* are also commonly utilized as food coloring, ornamentation,and medicinal purposes [6]. Morover is also known for its healing properties. Folk medicine in various countries applies it to treat dysentery, diarrhea, inflammations, muscular pain, diabetes, and urogenital disorders.

Furthermore, there is evidence of the various biological activities of the plant extracts. These include properties that stimulate the immune system, reduce inflammation, fight bacteria, fight microbes, fight cancer, inhibit nociception, fight infections, fight diabetes, fight helminth, fight malaria, and reduce stress [5].

As mentioned, about genus *Mirabilis* has many unique biological features and medicinal importance. Several are extensively used as a medicinal plant in almost all folklore remedies worldwide for treating various diseases. However, The studies have evidenced its antimicrobial, antiviral, and antioxidant activities in all the world except in Yemen. Therefore, this study aims to investigate the pharmaceutical properties of *M. jalapa* by determining the Phytochemical.

composition and investigating its anti-oxidant and antimicrobial activities.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The plant materials were collected from Wadi-Bana (Ibb-Yemen) during the period 15/6/2023 to 20/8/2023. The samples were identified by Dr.Hassan Ibrahim at the Biological Sciences Department, Faculty of Science, Sana'a University. More than one sample of each category was given a Herbarium number and was stored at the Faculty of Science Herbarium as a reference. Fresh, monitored leaves were cleansed with tap and deionized water, dried in a shadow case, then crushed using an electric blender. [7, 8, 9].

2.2. Preparation of *Mirabilis jalapa*, two forms leaf extracts

The extracts were prepared in this investigation: The methanol leaf extracts of the two *M. jalapa* forms (red and yellow flowered) were prepared by soaking 20 grams of the dried leaf powder of *M. jalapa* individually with 200 ml of solvent (methanol and water 70:30 %) in brown bottles at room temperature (25-30 ^{o}C) in a dark place for 7 days and shake for 4 hours with a shaker gently every day. The mixtures were filtrated by the suction pump and Buchner funnel throw filter paper. Then, methanol was evaporated at reduced pressure using a rotary evaporator (Buchi, Switzerland). Ultimately, the extracts were allowed to dry in Petri dishes until completely dry [10]. The extracts were stored in sterile containers and refrigerated until use [11]. The percentage yields were calculated for the dry extracts of two forms, which were 12.5%.

2.3. Method

The extractions were used for the chemical investigations, including qualitative testes, IR spectrophotometer, and biological investigations, which included antioxidant (DPPH) and antimicrobial activities of methanolic leaf extracts of the two forms of (red and yellow flowered) *M. jalapa*.

2.4. Qualitative Preliminary of Phytochemical Analysis

By using established techniques, the freshly prepared leaf methanolic extracts were utilized to qualitatively examine a variety of phytoconstituents, including alkaloids, glycosides, steroids, saponins, phenolic compounds, tannins, flavonoids, carbohydrates, and proteins [12, 13, 14, 15].

2.5. Determination of functional groups by Fourier transform infrared (FTIR) assay

The functional groups of the two forms of *Mirabilis jalapa* leaves extracts were determined by IR spectra. Employing a mortar and pestle, a fraction of the powdered crude extract was combined with Potassium Bromide (KBr) salt (individually). The powder was compacted into a thin pellet by using a KBr press. Pellets were put in the Perkin-Elmer FT-IR spectrometer 410 compartment and scanned throughout the IR range between 4000 and 400 cm^{-1} [16, 17, 18].

2.6. Biological Evaluation of Extracts

2.6.1. Antioxidant Activity Evaluation

Determination of Plant Antioxidant Activity by 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH) assay: The antioxidant activity of the methanolic leaves extract of two forms red and yellow flowered of *M. jalapa* was determined by 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH) assay [19, 20]. In this analysis, ascorbic acid was used as a standard antioxidant agent and DPPH solution (2.5 mg DPPH/25 ml of methanol). 1 ml of a methanolicsolution of DPPH was added to different concentrations (0.002 m g/ml, 0.004 mg/ml, 0.006 mg/ml, 0.008 mg/ml and 0.010 mg/ml) of ascorbic acid and different concentrations (0.20, 0.40, 0.60, 0.80 and 1 mg/ml) of *M. jalapa* leaf extracts of two forms red and yellow flowered individually (ascorbic acid and both extracts were dissolved in methanol). Control and treated samples with DPPH solution (ascorbic acid and both extracts) were incubated in the dark at room temperature for 30 min; then, the absorbance was recorded at 517 nm by Genova Life Science Analyzer Protein. The radical scavenging effect was calculated in percentage by the formula: Radical scavenging effect (%) = Ac – As/Ac × 100, where Ac = absorbance of the control and As= absorbance of the test (ascorbic acid and both extracts) sample [19].

2.7. Antimicrobial assays

2.6.2.1. Test Microorganisms used :
The antimicrobial activity of *M. jalapa* was examined against three types of bacteria (one gram-positive bacteria, namely *Staphylococcus aureus*) and two gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, and one fungus: *Candida albicans*, which was obtained as fresh pure cultures from the National Central of Public Health Laboratory (NCPHL)-Sana'a.

2.6.2.2. Determination of Antimicrobial Activities:
The agar well-diffusion method on Mueller Hinton Agar (MHA) media and Sabouraud Dextrose Agar (SDA) were applied to determine the antibacterial and antifungal activities of the two forms (red and yellow) leaf extract, respectively. A stock solution of *M. jalapa* leaf extracts with a concentration of 100% was prepared by dissolving 0.3 g of both forms in 3 ml of DMSO. A serial dilution was prepared; 100.0, 50.00, 25.00, 12.50, 6.250, 3.120, 1.560, 0.780, 0.390, 0.195, 0.098, 0.049, 0.025 and 0.012 mg/ml from the stock solution of each extract.100 μl of each concentration; from each extract were pipetted into a well (individually), while, 100μl of DMSO was used as a negative control [19, 21, 22]. Above preparation form determined antibacterial and antifungal in different media. However, Ciprofloxacin, Ampicillin, Amikacin, Amphotericin, Miconazole**,** and Nystatin antibiotics were used as a positive control for antibacterial and antifungal activities, then incubated at 37 ℃ for 24 h and 72 hrs, respectively. The inhibition zone diameter was measured, then procedures were carried out in triplicate, and the data of inhibition zone diameter(mm) were calculated as a mean [21, 22, 23].In addition, the minimum Inhibitory Concentration (MIC) was assigned [19, 21, 22].

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

The results of the phytochemical analysis in (Table 1) indicate that the leaf methanol extracts of two forms, red and yellow flowered of *M. jalapa*, contain active pharmacological components such as alkaloids, flavonoids, carbohydrate, protein, amino acid, phenol, tannins, saponins, and steroids were detected in *M. jalapa* leaf methanol extract. Furthermore, the results of the phytochemical analysis shown in (Table 1) in the plant studied correlated with other reports[2, 5, 24, 25].

3.2. Definition of chemical groups by Fourier transform infrared (FT-IR) analysis

According to the FT-IR analyses, the chemical/functional groups in the leaves methanol extracts of the two forms (red and yellow flowered) of *M. jalapa* are exhibited in

Table 1. Determination of chemical qualitative constituent of the leaves methanol extracts of two forms red and yellow flowered *M. jalapa*

NO	Phytochemical compositions	Test reagent	Note	leaves methanol extracted of *M. jalapa*	
				Red M.j	Yellow M.j
1.	Alkaloids	Dragendroffs	Orange-red precipitates	ve+	ve+
		Hager's (picric acid)	Yellow ppt	ve+	ve+
2.	Flavonoids	Lead acetate	Yellow ppt	ve+	ve+
3.	Carbohydrate	Molisch	Purple ring	ve+	ve+
4.	Amino acid	Ninhydrine	Blue-purple color	ve+	ve+
5.	Protein	Folin _ciocalteau reagent	Blue color	ve+	ve+
6.	Phenolic compound	Ferric chloride	dark green	ve+	ve+
		$K_2Cr_2O_7$ 10%	Orange color	ve +	ve +
7.	Tannines	Green ppt	Ferric chloride	ve+	ve +
8.	Saponin	Foam test	small bubbles (foam)	ve+	ve+
9.	Terpinoides	Salkowski	golden yellow color	ve +	ve +

Table 2 and Figures 2 & 3; the peaks at 3628.41-3400.85 cm^{-1} and 3629.37-3398.00 cm^{-1}refer to the Hydroxyl group (O-H) which indicates the attendance of pharmaceutical substances such as phenols, alcohol, and their derivatives in the leaf extract of the two forms (red and yellow flowered) of *M. jalapa*, respectively. Also, peaks 2935.13 cm^{-1} and 2926.45 cm^{-1} belong to the methylene (-CH) function group of aliphatic compounds, while 1725.98 and 1726.94 cm^{-1} refers to the attendance of the C=O group in both forms Consecutively. In addition,the absorptions at the wavenumbers 1384.64 cm^{-1} and 610.36 cm^{-1} confirm the occurs of -C-N and -C-H in both forms of plant, respectively. Moreover, the absorption occurs in pairs at 1456.96 cm^{-1}, 1608.34 cm^{-1} and 1456.36 cm^{-1}, 1636.3 cm^{-1} showing the presence of C=C in the methanol leaf extracts of the two forms (red and yellow flowerd) *M. jalapa* Conse-cutively. Furthermore; the peaks at 1074.16 cm^{-1}, 1120.44 cm^{-1} and 1075.12 cm^{-1} , 1119.48 cm^{-1} indicate the attendance of -C-O and -C-H in both forms (red and yellow flowerd) of *M. jalapa*, respectively (figure 2 and 3).

3.3. Determination of Plant Antioxidant Activityby1,1-Diphenyl-2picrylhydrazyl free radical (DPPH)

This method is based on the reduction of the stable free radical purple-colored DPPH in the presence of an antioxidant to the nonradical form of yellow-colored DPPH. Figure 4 summarizes the free radical scavenging activity of the two forms (red and yellow flowered) of *M. jalapa* extracts. It is clear that the methanol leaf extract of the red flowered *M. jalapa* (figure 4) exhibited high antiradical activity towards DPPH radical; 22.24%, 34.79%, 44.498%, 58.14%, and 84.14% at concentrations; 0.20 mg/ml, 0.40 mg/ml, 0.60 mg/ml, 0.80 mg/ml and 1 mg/ml respectively.

However, the methanol leaf extract of yellow-flowered *M. jalapa* (figure 4) shows lower free radical scavenging activities when compared with the other form(red flowered *M. jalapa*) methanol leaf extract at the same concentrations (18.123%, 26.54%, 36.17%, 55.42% ,and 65.615 % correspondingly).On the other hand, the scavenging activity of the pure antioxidant standard (ascorbic acid) (figure 4) was 15.94% at a lower concentration of 0.002 mg/ml followed by 33.01 %,52.51 %,68.69 %, and 91.42 % at concentrations 0.004 mg/ml, 0.006 mg/ml, 0.008 mg/ml and 0.010 mg/ml ,respectively. Overall, the red-flowered *M. jalapa* leaves exhibited higher free radical scavenging activities compared to those from yellow-flowered *M.jalapa* across various concentrations. Numerous studies have confirmed the antioxidant properties of *M. jalapa* extracts; they possess moderate antioxidant capabilities [26]. Research on the in vitro antioxidant potential of methanolic extracts from *M.jalapa* has highlighted their significant antioxidant activity in both aerial parts and roots while elucidating their mechanisms of action.

Zachariah et al.'s findings indicate that methanolic extracts from *Mirabilis* also exhibit potential antioxidant activity [27]. The total flavonoid content within these extracts has been identified as a key active compound responsible for this antioxidant effect; it may act as a free radical inhibitor or scavenger. Further experiments have validated that extracts from *M.jalapa* demonstrate moderate to potent antioxidant activity [28, 29].

3.4. Determination of Antimicrobial Activities:

Table 3,4 and Figure 5 illustrate the anti-microbial activities of the leaf extract of *M. jalapa* two forms at concentrations of 100.00; 50.00; 25.00, 12.50, 6.250, 3.120, 1.560, 0.780, 0.390, 0.195, 0.098, 0.049,0.025 and 0.012 mg/ml. The extracts demonstrate antibacterial effects against specifically *S. aureus*, with mean inhibition zones

Table 2. FT-IR Spectrum of the leaves methanol extracts of two forms red and yellow flowered *M. jalapa*

No.	Function group	Wave number of Absorption cm^{-1}	
		Red $M._j$	Yellow $M._j$
1.	-OH	3628.41-3400.85	3629.37-3398
2.	-C-H (Alkanes/Aliphatic)	2935.13	2926.45
3.	Overtone/Combination-Aromatic ring	1868.68, 1845.54	1869.65, 1845.54
4.	-C=O	1725.98	1726.94
5.	-C=C	1456.96, 1608.34	1456.96, 1636.30
6.	C-N	1384.44	1384.64
7.	-C-O	1074.16	1075.12
8.	-C-H (out of plane -oop)	610.36	610.36
9.	N-H	1120.44	1119.48

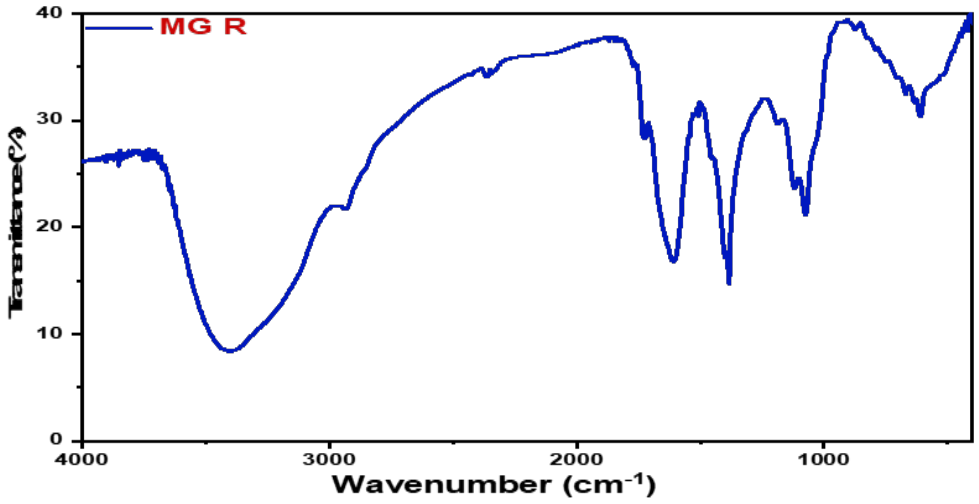

Figure 2. FT-IR Spectrum analysis of the leaf methanol extract of red flowered *M. jalapa*

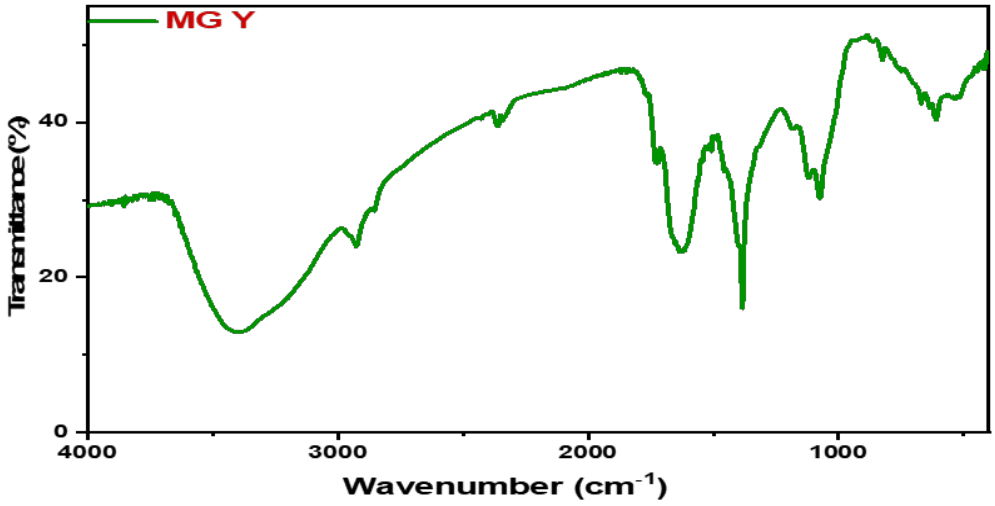

Figure 3. FT-I Spectrum analysis of the leaf methanol extract of yellow flowered *M. jalapa*

measuring (in mm) as follows: red-flowered *M. jalapa* exhibited inhibition zones of 17, 16, 15, 15, 15, 14, 14, 13, 12, and 10 mm respectively; while the yellow-flowered form showed

inhibition zones of 16, 16, 15, 15, 14, 14, 14, 13, 11, and 10 mm at the same concentrations. Moreover, the leaf extract of *M. jalapa* red-flowered shows antibacterial activity against selected gram-negative pathogens, *E. coli* (mean inhibition zone of 14, 14, 14, 14, 13, 12, 11, 10 ,10, 10, 10, 10 mm sequentially) and *P. aeruginosa* (mean inhibition zone of 17, 15, 15, 14, 14, 13, 13, 12, 11, 11, 11, 11 mm correspondingly) while the leaf extract of *M. jalapa* yellow-flowered shows (mean inhibition zone of 12, 13, 14, 14, 13, 13, 12, 10, 10, 10, 10, 10 mm and 16, 15, 14, 14, 14, 14, 13, 13, 12, 11, 11, 11, 11mm sequentially at the same concentra- tions and bacterial pathogens. Although the leaf extract of yellow-flowered *M. jalapa* exhibits antifungal activity against *C. albicans*

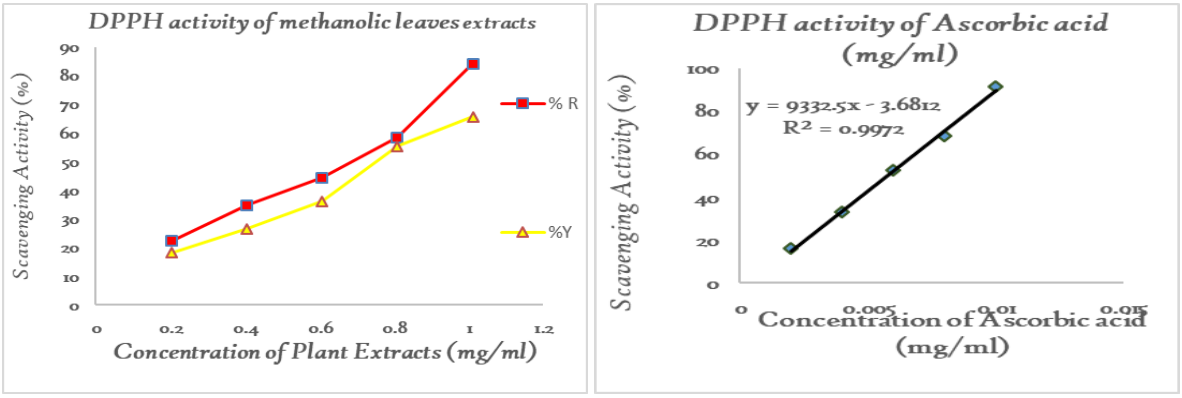

Figure 4. Free radical scavenging activity % of the leaves methanol extracts red and yellow flowered M. jalapa and Ascorbic acid.

Table 3. Inhibition zone diameters (mm) of the leaves methanol extracts of red and yellow flowered *M. jalapa*

Test microorganisms (Bacterial Pathogens)	Type	Extraction concentration (mg/ml)													Negative Control (DMSO)	Positive Control (Antibiotic)			MIC
		Extract Mirabilis jalapa Red																	
		100	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.098	0.049	0.0245		Amp(0.01 mg)	AMk(0.05 mg)	CPR(0.05 mg)	
		Concentration														Inhibition zone diameter (mm)			
S.aureus	+G	17	16	15	15	15	14	14	13	12	10	-	-	-	-	14	18	25	10
E. coli	-G	14	14	14	14	13	12	11	10	10	10	10	10	-	-	-	19	30	10
Pseudomonas	-G	17	15	15	14	14	13	13	12	11	11	11	11	-	-	-	16	36	11
		Extract Mirabilis jalapa Yellow																	
S.aureus	+G	16	16	15	15	14	14	14	13	11	10	-	-	-	-	14	18	25	10
E. coli	-G	12	13	14	14	13	13	12	10	10	10	10	10	-	-	-	19	30	10
Pseudomonas	-G	16	15	14	14	14	14	13	13	12	11	11	11	-	-	-	16	36	11

G +ve = Gram-positive; G -ve = Gram-negative

Antibiotics: AMP = Ampicillin; AMK= Amikacin and CIP= Ciprofloxacin

(Table 4) with an average inhibition zone of 15, 16, 17, 18, 18, 19, 20, 20, 17, 15, 13, 12, 11 mm at concentrations of 100; 50; 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, 0.098, 0.049, 0.0245 and 0.01225 mg/ml, respectively. Moreover, the yellow-flowered *M.jalapa* displays an inhibition zone of 13, 15, 16, 17, 17, 18, 19, 18, 16, 14, 12, and 12, 1 mm at the same concentrations ,consecutively.

According to Tables 3,4, and Figure 5 the gram-positive pathogens, including *Staphylococcus aureus* and *Candida albicans* are more significantly affected by the methanolic extract of *M. jalapa* leaves compared to gram-negative bacteria. This difference may be attributed to the distinct cell wall structures of gram-positive bacteria and *C. albicans* on one side and gram-negative bacteria on the other. Gram-negative bacteria possess an outer membrane that acts as a protective barrier against various compounds, particularly antibiotics [30, 31].

The minimum inhibitory concentration (MIC) (Figure 6) for both red and yellow flowered forms of *M.jalapa* was determined to be (0.195 mg/ml) against *S.aureus*, with corresponding inhibition zones of 10 mm each, whereas, for both *E.coli* and *P. aeruginosa*, it was found to be (0.049 mg/ml) with inhibition zones measuring 10 mm and 11 mm ,respectively.

For antifungal activity against *C.albicans*, the MIC was established at (0.0245 mg/ml), yielding inhibition zones of 11 mm and 10 mm, respectively, for red and yellow flowered forms.

The results presented in Tables 3 & 4 align closely with previous studies that investigated the antimicrobial properties of various leaf extracts from different forms of *M.jalapa.* These studies reported that methanolic extracts from white-flowered varieties exhibited the highest antibacterial activity, followed by those from pink-flowered forms and then yellow and orange forms [2, 32].

4. CONCLUSION

Many plants thrive in Yemen and exhibit a variety of colors. One such plant is *Mirabilis jalapa*, from which therapeutic substances beneficial to human health are derived from its leaves. Phytochemical investigations and FT-IR spectrum analyses revealed the presence of several medicinal compounds, including alkaloids, flavonoids, carbohydrates, proteins, amino acids, phenols, tannins, saponins, and steroids, in the methanol

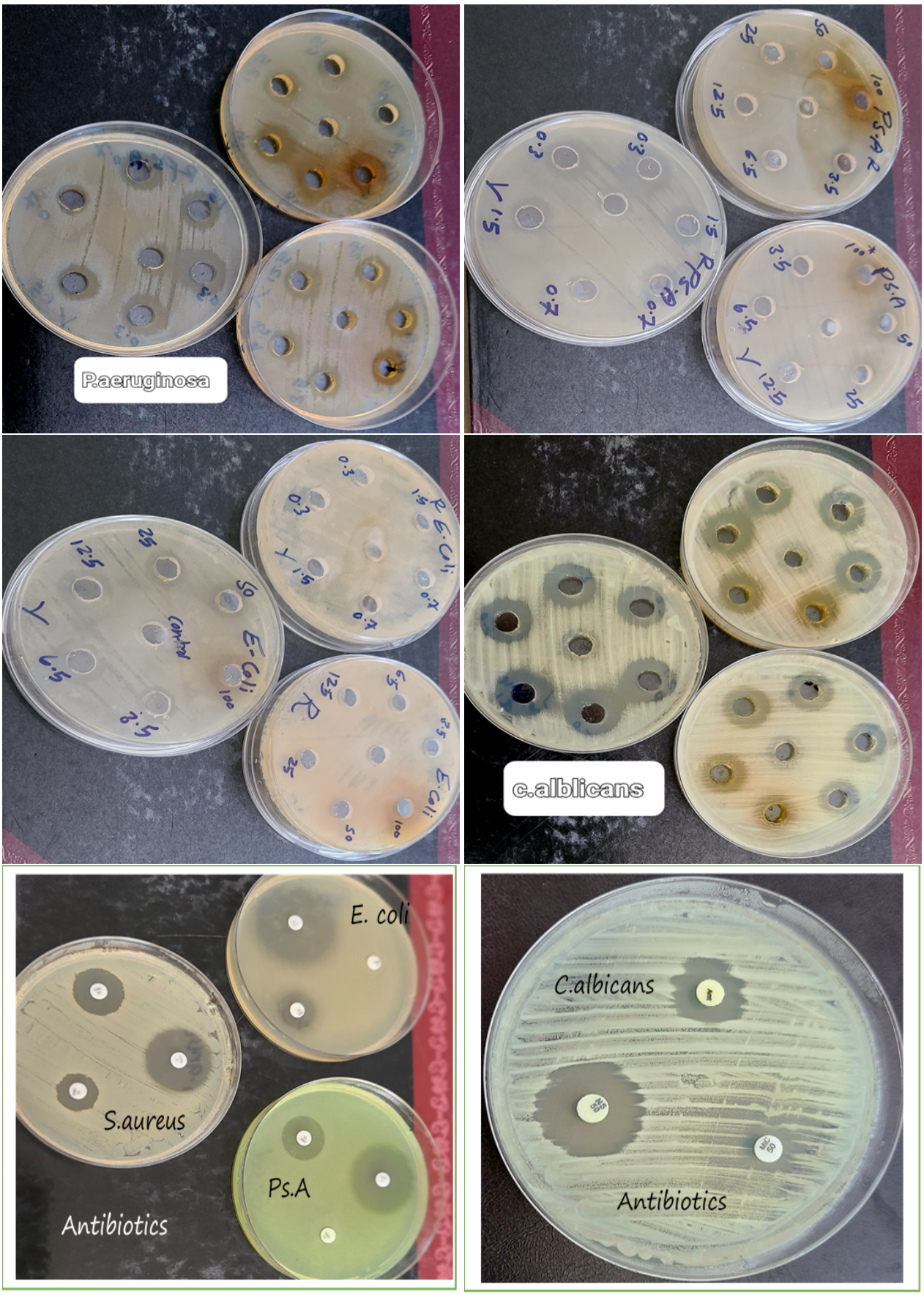

Figure 5. The antimicrobial activity of the leaf methanol extracts of red and yellow flowered *M. jalapa* and antibiotics. **Note** :1 represent high concentration at 100 mg/ml.

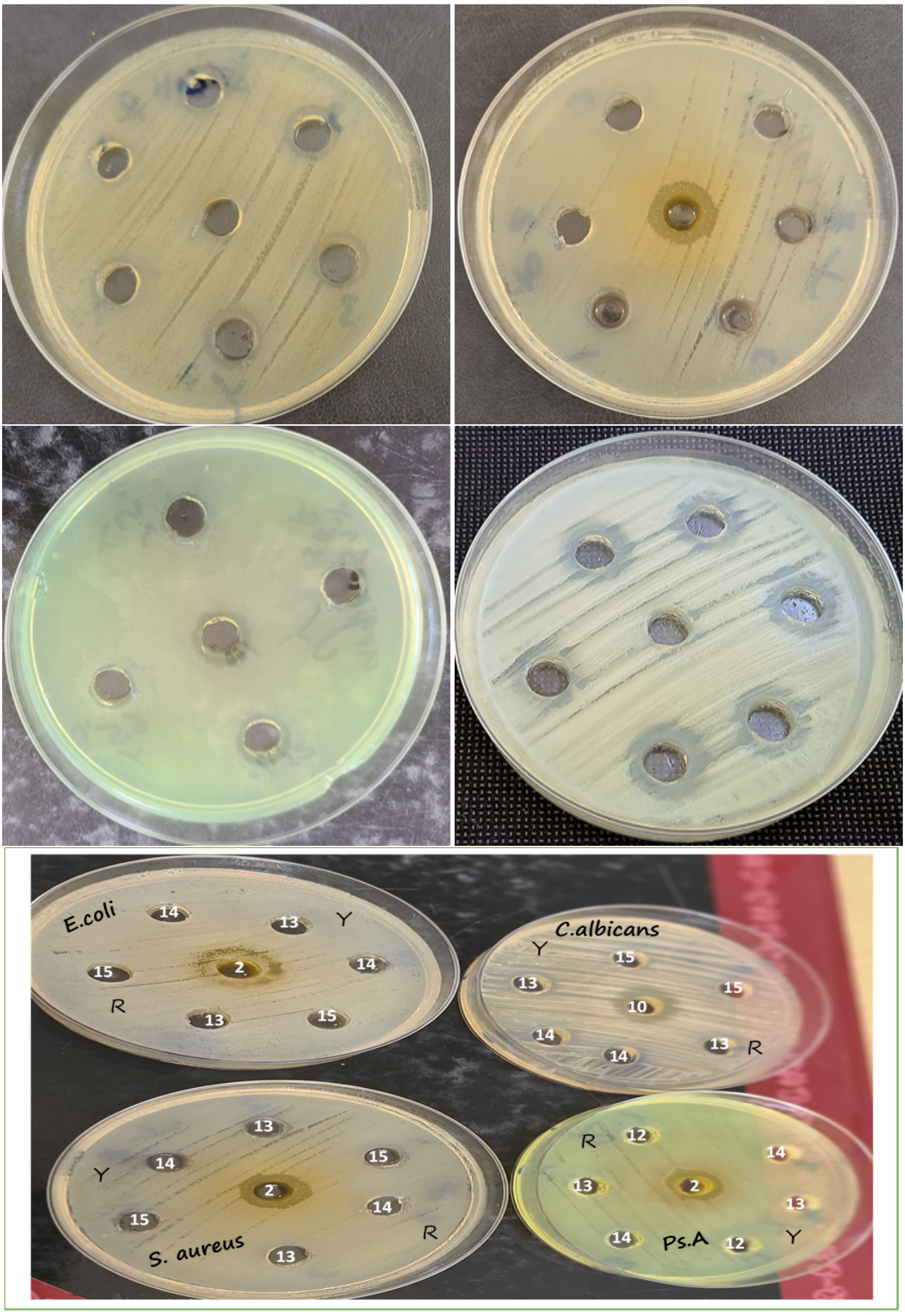

Figure 6. The minimum inhibitory concentration (MIC) of the leaf methanol extracts of red and yellow flowered *M. jalapa*.

Table 4. Inhibition zone diameters (mm) of the leaves methanol extracts of red and yellow flowered *M. jalapa*

Test microorganisms (Fungal Pathogens)	Extraction concentration (mg/ml)														Positive Control (Antibiotic)			MIC
	100	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.098	0.049	0.0245	0.01225	Amt(0.01 mg)	Mz(0.05 mg)	NS(0.05 mg)	
	Concentration																	
Mj red extract	15	16	17	18	18	19	20	20	17	15	13	12	11	-	16	11	20	11
MG yellow extract	13	15	16	17	17	18	19	18	16	14	12	12	10	-				10

Antibiotics: Amt = Amphotericin ,Mz =Miconazole = and NS= Nystatin

leaf extracts of both red and yellow-flowered *M. jalapa*. Notably, the methanol extract from the red-flowered *M. jalapa* demonstrated higher antioxidant and antimicrobial activities than compared to its yellow-flowered counterpart. This difference may be attributed to the higher concentration of potent antioxidant compounds such as phenols and flavonoids in the red form than in the yellow form of the same plant.

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