Identification and Evaluating of Phytochemicals Extracted from Mirabilis Jalapa (L) Leafs on Some Antibiotic Resistance Bacteria

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Abstract
This study was conducted in the laboratories of the faculty of Science / University of Kufa from October 2021 to March 2022, the Mirabilis jalapa(L.) plant was obtained from some gardens in Nagif City , A specific weight (10) grams of leaf powder was extracted separately by Maceration and vortex method using methanol 96% with three temperatures (30,40 and 50°C) degrees to obtain the best alcoholic extract . The qualitative analysis was studied for the detection phytochemicals using chemical reagents and GC-MS analysis was conducted to identify the quality and quantity of phytochemical compounds in crude extraction methods used in this study. The extracts were chosen from the followed methods depending on the amount of active compounds in each extract diagnosed by GC-MS. Where the results of chemical tests for the alcoholic extract showed positive results for alkaloids using, phenols, terpenes, alkaloids and glycosides .Result show the alcoholic extract of the leaves of the plant using the Maceration method and a temperature of 30 °C showed the highest rate of the chemical compounds diagnosed using GC-MS and were chosen as the best treatments in the subsequent experiments. Bacteria resistant to antibiotics study were obtained from the post Graduate Microbiology Laboratory / College of Science / University of Kufa. 15 bacterial samples were diagnosed by morphological, microscopic and biochemical tests. The diagnosis was confirmed using the Phytek device, and the percentage of gram positive was as follows S.aureus (48.57%), followed by P.aeruginosa (37.14%). Three concentrations of leafs extracts by Maceration 30°C as 1, 5 and 10 mg/ml were prepared for each of the selected extraction methods to evaluate the antibacterial activity at three replicates for each concentration using the geavure diffusion method.

Keywords: Mirabilis jalapa; Phytochemicals extracted; Leafs

1. Introduction

In the fields of medicinal Plants are widely used by our people for therapy and healing (Subin et al., 2011). According to international records about 80% of the world's population has use of traditional medicine of plant parts (Elamaran and Sivananthan, 2013). The excessive use of antibiotics against microbe , which lead to the emergence of signs of antibiotic resistance in pathogenic bacteria, particularly when used for long periods of time. (El-mahmood and Amedh, 2007). Several Studies show that medicinal herbs and their secondary metabolites are effective antimicrobials. M. Jalapa medicinal plant are rich source in natural compounds such as Alkaloids phenols, Terpenoids , Glycosides, Volatile oils which is traditionally use in many treatments as antibacterial, antifungal, antiviral, antispasmodic. (Tiwari et al., 2011). There are several methods established for the extraction of secondary metabolites from plant materials have evolved the preparation of various pharmaceutical drugs. (Bussmann et al. 2011).
The aims of this study was to screening :
To screen the phytochemical constituents of roots extract using GC-MS spectrometry technique, Then obtain the best methods for extraction of M.jalapa roots with and suitable temperature and to screen these phytochemical for antimicrobial activity against Staphylococcus aureus and Pseudomonas aeruginosa antibiotic bacteria resistance .

2. Materials and Methods

Collection and preparation of plant Samples

M. jalapa plant were collected randomly from some garden of Najaf City, the dry leafs were crushed by an electric grinder and store in refrigerator at a temperature of 4°C until use.

Extraction by Maceration apparatus

The ground leafs of M. jalapa plant (10 g) were extracted with 200 ml of methanol (99% purity) during 24 hours at a temperature of (30,40,50) °C respectively in a Maceration device, then the extract was evaporated by Oven dried at 40°C then kept in the refrigerator at a temperature of 4°C until use in later experiments. (Shtayeh and Abu Ghadeib,1999).

Vortex-assisted extraction

Leafs plant powder(10) g were placed in 200 ml of methanol solvent (99% purity), then put it in a water bath for an hour at a temperature (30,40,50) °C sequentially , then extract it with a vortex device for half hour , then dry the extract using a device Oven at 40°C. The dried extract was stored in a refrigerator at 4°C until use. (Shtayeh and Abu Ghadeib,1999).

Phytochemicals detection using GC-MS technology.

In this study, phytochemicals in leafs of *M.japala* extracts have been studied using (GC-Mass) . GC-MS

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analysis was performed on a Perkin Elmer Turbo mass spectrometer (Norwalk, CTO6859, USA) which included a Perkin Elmer XLGC. The column used was a Perkin Elmer Elite-5 capillary column measuring 30 × 0.25 mm with a film thickness of 0.25 mm and consisting of 95% dimethylpolysiloxane. The carrier gas used was helium at a flow rate of 0.5 ml/min.Thus, the names, molecular weights, and structure of the components of the test materials were ascertained. (Ullah et al,2019)[1].

Preparation of plant extracts concentrations

The higher phytochemicals reported 48 peaks indicating in leafs of *M.jalapa* plant extract used Maceration apparatus at 40°C by GC-MS chromatography that make it the chosen of the best extract for later work study about the antibacterial capacity. Three concentrations of each methods extract were prepared separately, weighed 100 mg, 500 mg and 1000 mg of the dry extract powder beside control treatment D.W, each weight was dissolved in 100ml of distilled water directly, and thus the final concentration for each solvent would be 1 mg/mL, 5 mg/mL and 10 mg/mL respectively were used in inhibition of bacterial growth (Packiyalakshmi *et al.*, 2017).

Bacteria culture media

All the swabs S. aureus, and P. aeruginosa bacteria collected were incubated in different culture media for 24 hours at a temperature of 37C° under aerobic and anaerobic conditions and activation growth of bacteria has been used brain heart infusion agar (Lagier et al., 2015). All bacterial isolates were identified according to colony morphology such as color, shape and size of colonies (Habib et al., 2015).

Antibiotic Susceptibility Test

The efficacy of the antibacterial susceptibility test was studied according to the disc diffusion method (Le et al., 2015). Antibiotic discs have been placed on the inoculated culture medium by sterile forceps (distributed at the same distances). The antibiotic activity has been determined by measuring the diameter of the inhibition zone in (millimeters) for each antibiotic disk then the results were compared with standard tables according to CLSI, (2020)[2].

3. Statistical analysis

The study experiments were carried out according to a completely randomized design (C.R.D.). Factorial experiments were used according to the design of CRD and RCBD to implement experiments with two factors. The Least Significant Difference (LSD) test was conducted at the level of significance at 0.05 to compare the results of laboratory experiments and pot experiments (Al-Rawi and Khalaf Allah, 2000). The results were analyzed using the program Genstat was used to organize and extract rates.

4. Results and Discussion

Phytochemicals detection in M.jalapa leafs extract used Maceration apparatus at 30°C by GC-MS chromatography

Phytochemical analysis of M.jalapa leafs extracts by methanol 96% extract used Maceration apparatus at 30 °C were subjected to analysis using gas chromatography-mass spectrometry analytical techniques as the pest extract. The higher phytochemicals results by GS-MS technology reported 48 peaks indicating in leafs of M.jalapa plant extract .

Phytochemicals detection in Leafs of M.jalapa plant extract used Maceration at 30°C by GC-MS chromatography.

Phytochemical analysis of M.jalapa leaves extracts used Maceration at 30 °C were subjected to analysis using gas chromatography-mass spectrometry analytical techniques.

Results in Table (2) and figure (1) show that chemical components identified in GC-MS analysis of Maceration at 50°C from Leafs of M.jalapa plant extract there are 48 peaks indicating. By comparison with mass spectra these phytochemicals were characterized and identified . The major phytochemical contents are Ergosta-5,8,22-trien-3-ol, (3.beta.,22E)- with ratio (11. 75) exhibited immune promoting properties antitumor and antibacterial properties, (Wasser and Weis 1999) followed by 1,8,11-Heptadecatriene, (Z,Z)- (figure 2 D) with ratio (3.94 %) exhibited antibacterial properties (Arrabala et al,2011) , third constituents is Myo-Inositol, 4-C-methyl- (figure 2 C) with ratio of (2.75 %) , followed by constituents is Stigmasterol with ratio of (2.26 %) a member of the phytosterols. has antinociceptive action (Walker et al., 2017).Also, Propenoic acid, ethenyl ester with ratio (2.09 %) demonstrated that antibacterial, antifungal and antioxidant and antidiabetic activities (Dracheva et al., 2009; Berzosa et al., 2011). Linoleic acid ethyl ester with ratio (1.471%) as natural antioxidants (YADAV et al,2018) and 9-Octadecenoic acid (Z)-, methyl ester with ratio (1.47%) as natural product against bacterial (E. coli, P. aeruginosa, B. subtilis, S. aueus)(Mustapha and Runner,2016)Followed by Ethane, 1,1,2,2-tetramethoxy- with ratio (1.63%) as antibacterial agent. (Aeed et al,2021)[3].

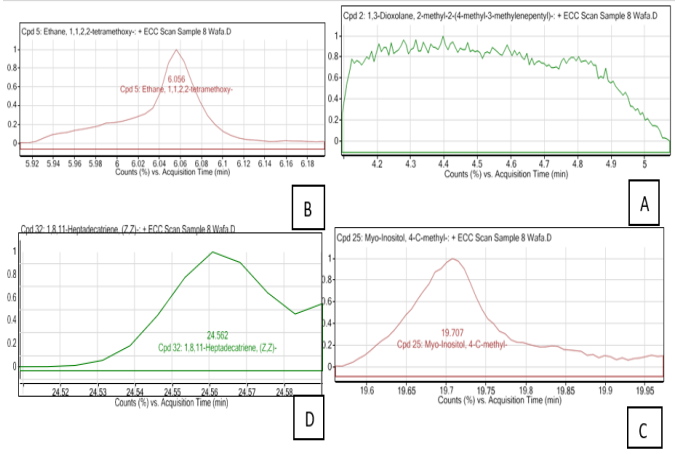
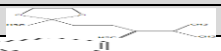
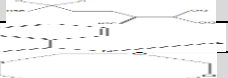
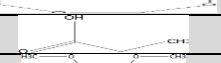
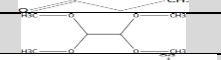
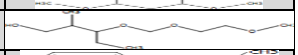
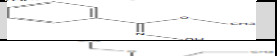
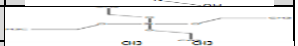
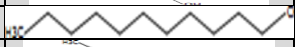
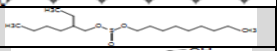
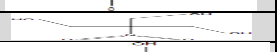
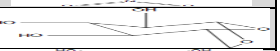
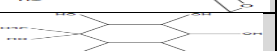
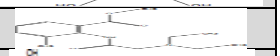
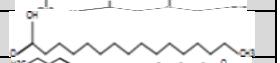
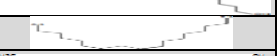
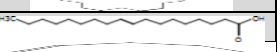
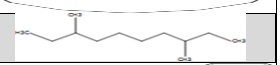
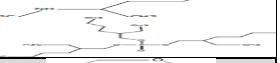
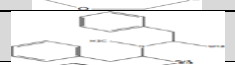
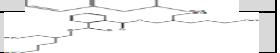
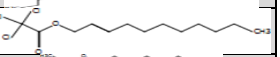
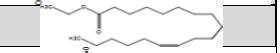
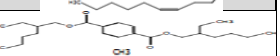
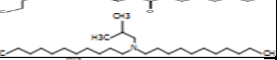
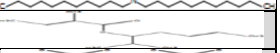
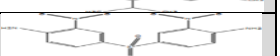
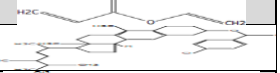


Figure (1) Chemical Compounds in M.jalapa leafs by GC-MS chromatography

Table(1) Qualitative compound report in of M.jalapa Leafs plant ethanolic 96% extract used Maceration at 30°C				
Name	Formula	RT	%	Chemical structure
1,3-Dioxolane, 2-methyl-2-(4-methyl-3-methylenepentyl)-	C11H20O2	4.396	1.01	
Benzoyl isothiocyanate	C8H5NOS	4.551	0.91	
1,3,5,7-Tetroxane	C4H8O4	4.633	0.64	
2-Propenoic acid	C3H4O2	4.209	1.14	
Ethane, 1,1,2,2-tetramethoxy-	C6H14O4	6.056	1.63	
1-propanol, 1,3,3-trimethoxy-, acetate	C8H16O5	6.284	0.14	
3-(2-Methoxyethoxymethoxy)-2-methylpentan-1-ol	C10H22O4	6.956	0.34	
Ethylbenzene	C8H10	7.216	0.16	
p-Xylene	C8H10	7.907	0.34	
Oxime-, methoxy-phenyl-_	C8H9NO2	8.524	0.15	
Tetraethyl silicate	C8H20O4Si	10.266	0.36	
Octane, 3,5-dimethyl-	C10H22	10.324	1.59	
Heptane, 4-ethyl-2,2,6,6-tetramethyl-	C13H28	12.167	0.17	
Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	C10H18O	13.136	0.20	
Dodecane	C12H26	13.784	0.35	
Sulfurous acid, 2-ethylhexyl nonyl ester	C17H36O3S	16.601	0.35	
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	C4H9NO5	17.032	0.97	
beta.-D-Glucopyranose, 1,6-anhydro-	C6H10O5	17.668	0.31	
Myo-Inositol, 4-C-methyl-	C7H14O6	19.707	2.75	
Tolycaine	C15H22N2O3	22.465	0.41	
Hexadecanoic acid, methyl ester	C17H34O2	22.588	1.05	
Methyl 13,14-octadecadienoate or 13,14-18:2	C19H34O2	24.223	0.62	
9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	24.274	1.47	
1,8,11-Heptadecatriene, (Z,Z)-	C17H30	24.562	3.94	
Octadecanoic acid	C18H36O2	24.794	0.45	
1,5-Cyclododecadiene, (Z,Z)-	C12H20	24.832	0.34	
Decane, 3,8-dimethyl-	C12H26	25.138	0.14	
Propylamine, N,.alpha.-dimethyl-.gamma.-phenyl-	C11H17N	27.363	0.18	
Phosphoric acid, tris(2-ethylhexyl) ester	C24H51O4P	27.463	0.25	
1,3,5,7-Tetroxane	C4H8O4	19.927	0.322	
N,.alpha.,.alpha.'-Trimethyldiphenethylamine	C19H25N	27.993	0.32	
Bis(2-ethylhexyl) phthalate	C24H38O4	28.065	1.05	
Trichloroacetic acid, undec-2-enyl ester	C13H21Cl3O2	29.121	0.70	
Linoleic acid ethyl ester	C20H36O2	24.848	1.47	
1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)	C24H38O4	29.522	0.73	
Diundecylisobutylamine	C26H55N	30.155	0.43	
Hepten-2-yl tiglate, 6-methyl-5-	C13H22O2	30.327	1.04	
Bis[3-nitro-4-aminophenyl]sulfone	C12H10N4O6S	31.037	0.39	
2-Propenoic acid, ethenyl ester	C5H6O2	31.059	2.09	
Anthraergostatetraenol p-chlorobenzoate	C35H45ClO2	31.206	0.49	
.beta.-Tocopherol	C28H48O2	31.716	2.09	
Benzene, 1,3,5-trimethyl-2-(1,2-propadienyl)-	C12H14	32.004	0.43	
Stigmasta-3,5-diene	C29H48	32.173	0.60	
5-Aminoisophthalic acid	C8H7NO4	32.504	0.71	
Ergosta-5,8,22-trien-3-ol, (3.beta.,22E)-	C28H44O	33.125	11.75	
Campesterol	C28H48O	33.332	1.76	
Stigmasterol	C29H48O	33.649	2.26	
9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-	C31H52O	35.943	1.17	
Total= 48 compounds				

A: 1,3-Dioxolane, 2-methyl-2-(4-methyl-3-methylenepentyl)- :(1.01%)
B: Ethane, 1,1,2,2-tetramethoxy- :(1.63 %)
C: Myo-Inositol, 4-C-methyl- :(2.75 %)
D: 1,8,11-Heptadecatriene, :(3.94 %)

Identification of bacterial study isolates by Vitek 2 compact.

Pathogenic bacterial isolates under study have been determined according to cultural, morphological and biochemical tests. The Vitek2 compact was used for purpose of the final validation of the diagnosis. Identification Using VITEK2 automated compact system with Gram-negative-ID and Gram positive-ID cards with 64 biochemical tests. The isolates were identified after 6 hours as *S. aureus* with probability of 95% *P. aeruginosa* with probability of 91% . Antibiotic susceptibility test (AST) was achieved for tested isolate using VITEK2 compact system.

Antibiotics sensitivity test for bacteria study

Study of bacterial isolates resistance to antibiotic is one of the most serious medical issues, making it more difficult to selective the best therapeutic drugs (Laxminarayan *et al.*, 2013). The inhibition zones were measured by the ruler in millimeters and compared with CLSI (2020). Table (3) and figure (A) and (B) investigated that two types of bacteria study were resistant to the antibiotics tested using disc diffusion method, namely Ceftriaxone (CRO) 10 µg, Cephalexin (CL) 30 µg. This study is compatible with Jiang *et al*, (2020); Hennequin and Robin, (2016) where they mentioned the *S.aureus* showed sensitivity to ceftriaxone, , while *P. aeruginosa* was resistant to the Cephalexin , where were inactive on the isolated bacteria and without inhibition zones. These results were compatible with Lee, (2003) where mentioned in his study that all isolates of *S.aureus* were resistant to Oxacillin and sensitive to trimethoprim, Naimi *et al*, (2017) mentioned that *S.aureus* was susceptible to ceftriaxone, doxycycline, trimethoprim and

Table (1) Evaluation of antibiotics activity against studied bacterial isolates

Bacteria Antibiotics discs	Dose	*S.aureus*	*P. aeruginosa*
		Inhibition zone (mm)	
Ceftriaxone	10 µg	R	R
Cephalexin	30 µg	R	R

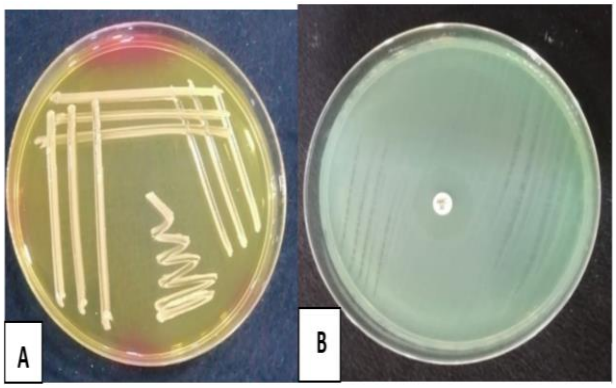


Figure (2) Antibiotics sensitivity test for bacteria study

Evaluation of antibacterial activity of M. jalapa leafs extracts against S.aureus and P. aeruginosa.

The antibacterial activity of M. jalapa leaves extracts against S. aureus bacteria was determined and has been examined at different concentrations of methanolic extract as well as a control group by using disc diffusion method.

Results in the Table (2) found that the methanolic leafs extracts with two types of extraction methods used showed strong antibacterial activity against S. aureus bacteria. The antibacterial activity increases with increasing extract concentration and shows presence of significant differences among all concentrations compared with the control group . Data in the table(2) investigated that the highest inhibition zone was found at concentration 10 mg/ml using Maceration extract methods at Tem.30 °C which recorded (22.39 mm) and show significant differences with among all concentrations compared with the control group .

Also , in the same table for leafs extracts in the vortex device at 50 °C found significant differences between all concentrations compared with the control group and the highest rate of inhibition area (18.31 mm) at a concentration of 10 mg / ml compared to other concentrations (1 and 5) mg / ml b (15.13mm) and (17.21mm), respectively.

Meanwhile, the less antibacterial activity against S. aureus bacteria show in leafs crud extract used Vortex method with different temperature degree.This study showed that there is a great potential for using M. jalapa extracts as natural antimicrobials to control pathogenic bacteria due to the phytochemicals present in the M. jalapa due to its biological effect compared to other chemical antibiotics.

Results of this study were compatible with Rashad, (2008) as mentioned that the aqueous extract of S.aromaticum was an effective antibacterial agent for S.mutans because of the extract activity in penetrating the bacterial cell wall. Phaiboon et al, (2019) concluded from their study that methanolic extracts of M. jalapa have antibacterial and anticarcinogenic activities against S. aureus. Gupta et al, 2019 used clove extracts against oral bacteria including S. aureus and confirmed the main antibacterial effect factor was concentration. This study is compatible with my study which proved that antibacterial activity increases with increasing concentration for all extract types. Also, These results compatible with the findings of many researchers, including Pandey and Singh, (2011) mentioned that all concentrations of M. jalapa extracts showed inhibition zones against many bacterial isolates including S.aureus.

Table (2) Antibacterial activity of *M. jalapa* leaves extracts against *S. aureus.*				
ConcentrationMg/ml	P.aeruginosa		S. aureus.	
	MIC (mm)			
	Maceration Temp.. 30 °C	Vortex Temp. 50 °C	Maceration Tem. 30 °C	Vortex Temp. 50 °C
Con.	0	00	0	0
1	15.32	15.33	18.11	15.13
5	17.11	16.41	20.12	17.21
10	18.32	17.23	22.39	18.31
LSD(0.5)= 1.138	LSD(0.5) = 0.805		LSD(0.5) = 0.805	

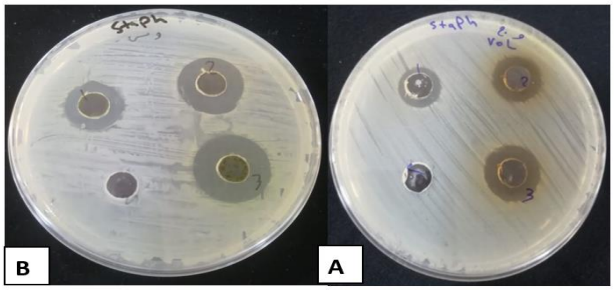


Figure (3) Antibacterial activity of M. jalapa leafs extracts against S.aureus. A: Maceration 30°C B: : Vortex 50°C

Renaults in the same Table and same figure Show that the leaf extracts by Maceration methods against *P.aeruginosa* there were significant differences between all concentrations compared to the control group, and the highest rate of inhibition area was (18.32 mm) at 10 mg/ml compared with the other concentrations (1 and 5) mg/ml (15.33mm) and (16.41 mm), respectively.

Also, the highest inhibition growth rate was at a concentration of 10 mg/ml in the vortex device at Temp. 50 °C was significant differences between all concentrations compared with the control group and the highest rate of inhibition area (17.23 mm) at a concentration of 10 mg / ml compared to other concentrations (1 and 5) mg / ml (15.33 mm)) and (17.11 mm) respectively.

Results in the table (4-10) reported that the leaves plant extracts recorded the highest antibacterial activity against P.aeruginosa by Maceration method at Temp. 30°C in compare with other methods used in cruds extract at a concentration of 10 mg/ml .Meanwhile, the less antibacterial activity against P.aeruginosa show in leafs crud extract used Vortex method.

This study showed that there is a great potential for using M. jalapa extracts as natural antimicrobials to control pathogenic bacteria due to the phytochemicals present in the M. jalapa due to its biological effect compared to other chemical antibiotics.

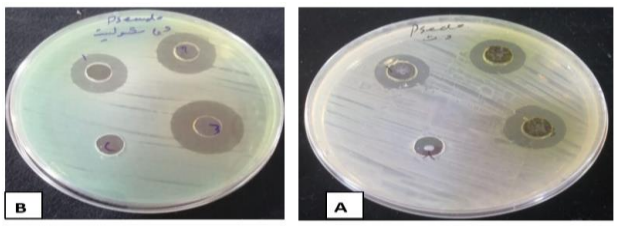


Figure (4) Antibacterial activity of M. jalapa leafs extracts against P.aeruginosa A: Maceration 30°C B: Vortex 50 °C

Where the table shows the size of the main effect of the independent variables to choose the main factor depending on the value of L.S.D. and upon interaction between the factors, it was found that the main effect factor was the concentration because it gave the highest value of L.S.D.

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