Effectiveness of Phytochemicals extracted from *Mirabilis jalapa* (L) Roots on Some Antibiotic resistance Bacteria

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Abstract

This study was conducted in the laboratories of the College of Science / University of Kufa from October 2021 to March 2022, the roots of *Mirabilis jalapa*(L.) plant was extracted by Soxhlet and Vortex methods using methanol solvent 96% with three temperatures (30,40 and 50 C°) degrees to obtain the best alcoholic extract. GC-MS analysis was conducted to identify the quality and quantity of phytochemical compounds in crude extracts 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,. Two types of extracts (Soxhlet +40C° and Vortex +50C°) were chosen as separate treatments in the subsequent experiments.

Bacteria resistant to antibiotics *Staphylococcus aureus* (48.57%) and *Pseudomonas aeruginosa* (37.14%).were obtained from the post Graduate Microbiology Laboratory / College of Science / University of Kufa.Bacterial samples were diagnosed by morphological, microscopic and biochemical tests. The diagnosis was confirmed using the Phytek device,

Three concentrations of roots extract 1, 5 and 10 mg/ml were prepared for each of the selected extraction methods and Temperature degree to evaluate the antibacterial activity at three replicates for each concentration using the well diffusion method.

The qualitative analysis was studied for the detection phytochemicals using GC-MS analysis was conducted to identify the quantity of phytochemical compounds in crude extraction methods used in this study. The higher phytochemicals reported 46 peaks indicating in roots of *M.jalapa* plant extract used Soxlate apparatus at 40°C by GC-MS chromatography. The results showed that all extract concentrations gave bacterial growth inhibition and all species were more affected by increasing these concentration study Also, the results in current study reported that the highest growth inhibition rate of *Pseudomonas aeruginosa* was for the methanol extract by soxlat methods at 40C° with an inhibition rate (23.33mm), while the lowest inhibition growth rate was for the alcoholic extract using Vortex methods at 30 C° an inhibition rate (12.41mm) for the *Staphylococcus aureus* bacteria. Also, the grater bacteria affected by crude plant extract was *Pseudomonas aeruginosa* at 10 mg/ml.

1. Introduction

In the fields of medicinal Plants are widely used by our people for therapy and healing [1]. According to international records about 80% of the world's population has use of traditional medicine of plant parts. 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. [2]. 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. There are several methods established for the extraction of secondary metabolites from plant materials have evolved the preparation of various pharmaceutical drugs. [3].

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 roots were crushed by an electric grinder and store in refrigerator at a temperature of 4°C until use.

Extraction by Soxhlet apparatus

The ground roots of *M. jalapa* plant (10 g) were extracted with 200 ml of methanol (99% purity) during 6-8 hours at a temperature of (30,40,50) °C respectively in a Soxhlet 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. [4].

Vortex-assisted extraction

Root 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. [4].

Phytochemicals detection using GC-MS technology.

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In this study, phytochemicals in roots of *M.japala* extracts have been studied using (GC-Mass). GC-MS 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. [5].

Preparation of plant extracts concentrations

The higher phytochemicals reported 46 peaks indicating in roots of *M.jalapa* plant extract used Soxlate 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 [6].

3. 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. All bacterial isolates were identified according to colony morphology such as color, shape and size of colonies [7].

Antibiotic Susceptibility Test

The efficacy of the antibacterial susceptibility test was studied according to the disc diffusion method. 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).

4. 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. The results were analyzed using the program Genstat was used to organize and extract rates.

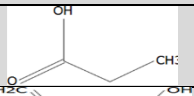
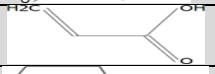
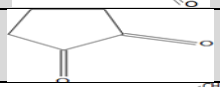
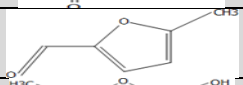
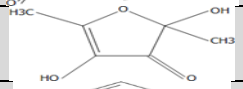
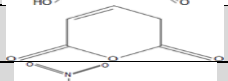
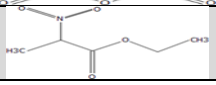
Results and Discussion:

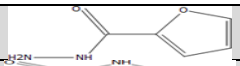
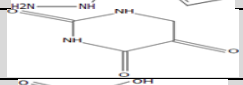
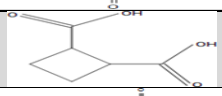
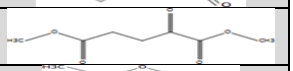
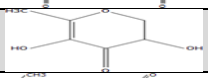
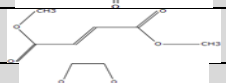
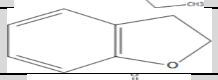
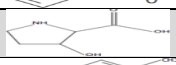
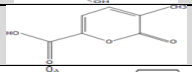
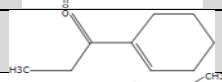
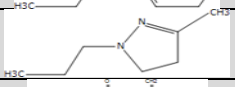
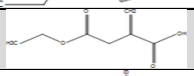
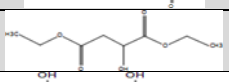
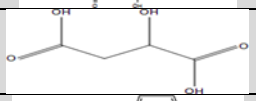
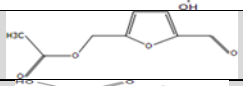
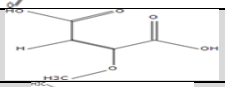
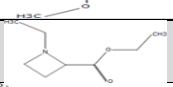
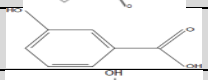
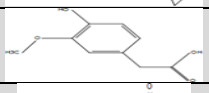
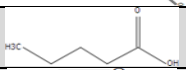
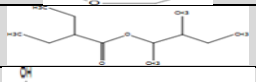
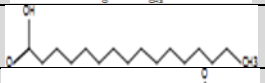
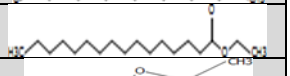
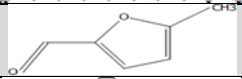
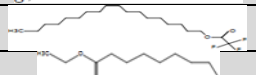
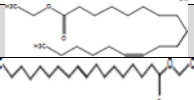
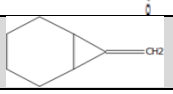
Phytochemicals detection in *M.jalapa* roots extract used Soxlet apparatus at 40°C by GC-MS chromatography

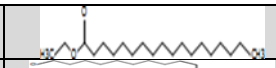
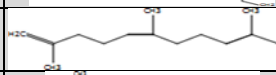
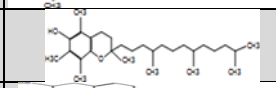
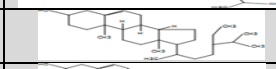
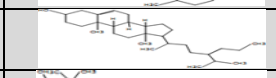
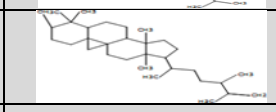
To make the chosen of the best extract with best of temperature degree for later work study about the antibacterial capacity, were chosen from the followed methods depending on the amount of active compounds in each extract diagnosed by GC-MS. Phytochemical analysis of *M.jalapa* roots extracts by methanol 96% extract used Soxlate apparatus at 40 °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 46 peaks indicating in roots of *M.jalapa* plant extract.

Results in Table (1) and figure (1) show that 46 peaks indicating. The major phytochemical contents are 1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl- with ratio (14.833%) as antibacterial properties [8] followed by beta.-D-Glucopyranose, 1,6-anhydro- with ratio (6.740 %) could be a good source for inhibiting the neuropsychiatric disorders, third consitituent is 2-Furancarboxaldehyde, 5-methyl- with ratio of (6.641%) the antibacterial, antiradical and carcinopreventive efficacy [9] and 3-Furaldehyde with ratio (5.397%) and Oleyl alcohol, trifluoroacetate with ratio (3.623%) and. Also, Propenoic acid, ethenyl ester with ratio (2.09 %) demonstrated that antibacterial, antifungal and antioxidant and antidiabetic activities [10, 11]. followed by consitituent is Stigmasterol with ratio of (2.26 %) a member of the phytosterols. has antinociceptive action [12].

Table(1) Qualitative Compound report in roots of *M.jalapa* plant extract used soxlate apparatus at 40°C

Name	Formula	RT	%	Chemical structure
2-Propenoic acid	C3H4O2	4.209	0.191	
3-Furaldehyde	C5H4O2	6.799	5.397	
1,1'-Carbonyldiimidazole	C7H6N4O	9.372	1.530	
2-Furancarboxaldehyde, 5-methyl-	C6H6O2	9.715	0.822	
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C6H8O4	10.039	0.310	
2H-Pyran-2,6(3H)-dione	C5H4O3	10.34	0.238	
Ethyl 2-nitropropionate	C5H9NO4	11.351	0.191	

2-Furancarboxylic acid, hydrazide	C5H6N2O2	11.966	0.728	
2,4,5-Trihydroxypyrimidine	C4H4N2O3	12.013	0.388	
1,2-Cyclobutanedicarboxylic acid, trans-	C6H8O4	12.268	0.200	
Pentanedioic acid, 2-oxo-, dimethyl ester	C7H10O5	13.098	0.595	
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	13.141	2.776	
But-2-enedioic acid, dimethyl ester	C6H8O4	13.812	0.888	
1,3-Dioxolane, 2-butyl-2-ethyl-	C9H18O2	13.903	0.604	
Benzofuran, 2,3-dihydro-	C8H8O	14.094	0.330	
trans-3-Hydroxyproline	C5H9NO3	14.246	0.854	
3-Methyl-2-oxo-2H-pyrane-6-carboxylic acid	C7H6O4	14.254	0.613	
1-Propanone, 1-(1-cyclohexen-1-yl)-	C9H14O	14.687	2.866	
1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl-	C7H14N2	14.707	14.833	
4-Ethyl hydrogen itaconate	C7H10O4	14.778	0.508	
Butanedioic acid, hydroxy-, diethyl ester	C8H14O5	14.857	0.364	
Malic Acid	C4H6O5	15.337	1.520	
5-Acetoxymethyl-2-furaldehyde	C8H8O4	15.444	0.217	
Methoxymaleic acid	C5H6O5	16.912	0.581	
N-Ethyl-2-carbethoxyazetidine	C8H15NO2	17.479	0.284	
Benzoic acid, 3-hydroxy-	C7H6O3	18.332	0.284	
.beta.-D-Glucopyranose, 1,6-anhydro-	C6H10O5	18.494	6.740	
Homovanillic acid	C9H10O4	19.803	0.355	
Pentanoic acid	C5H10O2	19.854	2.454	
1,3,5,7-Tetroxane	C4H8O4	19.927	0.732	
2-Ethylbutyric acid, 3-methylpent-2-yl ester	C12H24O2	22.323	0.198	
n-Hexadecanoic acid	C16H32O2	22.974	1.958	
Hexadecanoic acid, ethyl ester	C18H36O2	23.277	2.246	
2-Furancarboxaldehyde, 5-methyl-	C6H6O2	23.379	6.641	
Oleyl alcohol, trifluoroacetate	C20H35F3O2	24.669	3.849	
Linoleic acid ethyl ester	C20H36O2	24.848	1.270	
(E)-9-Octadecenoic acid ethyl ester	C20H38O2	24.904	3.623	
Bicyclo[4.1.0]heptane, 7-methylene-	C8H12	24.918	0.282	

Octadecanoic acid, ethyl ester	C20H40O2	25.106	0.235	
9,17-Octadecadienal, (Z)-	C18H32O	29.146	1.927	
1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	C14H24	30.112	0.420	
dl-.alpha.-Tocopherol	C29H50O2	32.395	2.720	
Ergost-5-en-3-ol, (3.beta.)-	C28H48O	33.352	0.791	
.gamma.-Sitosterol	C29H50O	34.411	18.513	
Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	C30H50O	34.508	0.217	
9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-	C31H52O	35.963	0.213	
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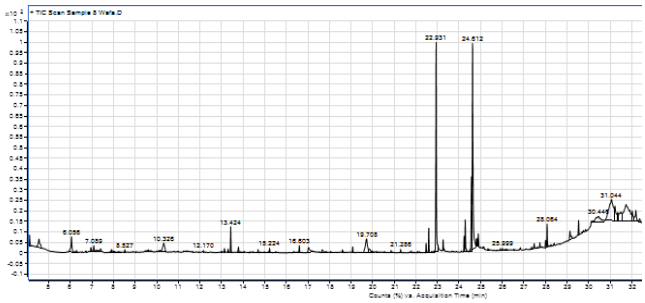

Figure(1) Chemical Compounds in M.jalapa roots by GC-MS chromatography

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 [13]. The inhibition zones were measured by the ruler in millimeters and compared with CLSI (2020). Table (2) 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 [14, 15] 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 [16] where mentioned in his study that all isolates of *S.aureus* were resistant to Oxacillin and sensitive to trimethoprim, Naimi et al. [17] mentioned that *S.aureus* was susceptible to ceftriaxone, doxycycline, trimethoprim.

Table (2) Evaluation of antibiotics activity against studied bacterial isolates

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

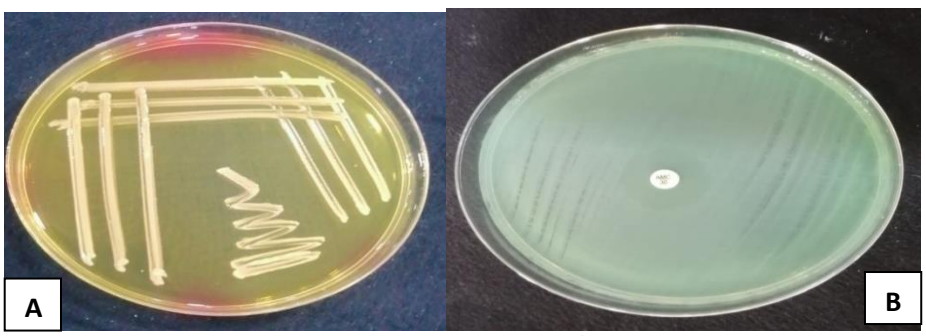

Figure (2) Antibiotics sensitivity test for bacteria study

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

The antibacterial activity of *M. jalapa* roots extracts against *S. aureus* and *P.aeruginosa* 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 (3) for the antibacterial activity of roots extracts against *S. aureus* found that the methanolic root extracts in the Soxhlet device at 40 °C found significant differences between all concentrations compared with the control group and the highest rate of inhibition area (20.12mm)

at a concentration of 10 mg / ml compared to other concentrations (1 and 5) mg / ml (17.22 mm)) and (18.44mm), respectively. While, data in same figure and table found that root extracts in the vortex device with Temp. 50°C were significant differences between all concentrations compared with the control group.The highest rate of inhibition area (14.36 mm) at a concentration of 10 mg / ml compared to the other concentrations (1 and 5) mg / ml with (12.41 mm)) and (13.21±mm), respectively. While, vortex device with Temp. 50°C were significant differences between all concentrations compared with the control groups, the highest rate of inhibition area (14.36 mm) at a concentration of 10 mg / ml compared to the other concentrations (1 and 5) mg / ml with (12.41 mm)) and (13.21mm), respectively.

Meanwhile, for the antibacterial activity of roots extracts against *P.aeruginosa* bacteria in the Soxlat device at 40 °C found significant differences between all concentrations compared with the control group and the highest rate of inhibition area (23.33mm) at a concentration of 10 mg / ml compared to other concentrations (1 and 5) mg / ml (20.22 mm)) and (22.13mm), respectively. While, data in same figure and table found that root extracts in the vortex device with Temp. 50°C were significant differences between all concentrations compared with the control group.The highest rate of inhibition area (18.32mm) at a concentration of 10 mg / ml compared to the other concentrations (1 and 5) mg / ml with (15.32mm)) and (17.11mm), respectively.

Results of this study were compatible with Rashad [18] 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. [19] used clove extracts against oral bacteria including *S.mutans* 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 Pandey et al. [20] mentioned that all concentrations of *S.aromaticum* extracts showed inhibition zones against many bacterial isolates including *S.aureus*.

Table (3) Antibacterial activity of *M. jalapa* roots extracts against *S. aureus.*

Concentration Mg/ml	*S. aureus*		*P.aeruginosa*	
	MIC (mm)			
	Soxelate Temp. 40 °C	VortexTemp. 40°C	Soxelate Temp. 40 °C	Vortex Temp. 40 °C
Con.	00	00	00	00
1	17.22	12.41	20.22	15.32
5	18.44	13.21	22.13	17.11
10	20.12	14.36	23.33	18.32
LSD(0.5)= 1.138	LSD(0.5) = 0.805		LSD(0.5) = 0.805	

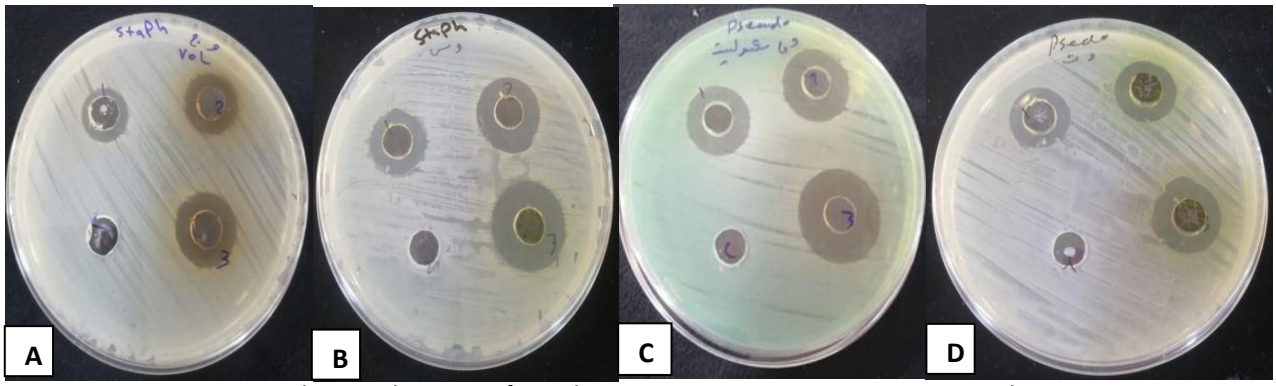

Figure (3) Antibacterial activity of M. jalapa roots extracts against S.aureus and P.aeruginosa.
A: Soxlate 40°C B: Vortex 50°C (S.aureus)
C: Soxlate 40°C D: Vortex 50°C (P.aeruginosa)

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