Pharmacogn. Res., 2024; 16(4):985-991.
https://www.phcogres.com

Original Article

Antimicrobial, Phytochemical Screening and GC-MS Analysis of *Ruta chalepensis* L. Leaves and Stems Extracts

Sara Hussein Altayeb[1,*] , Fatima Abdalgalill Khleefa[1], Solima Ibrahim Yagoob[1], Rasha Abdelnaser Abdelhadi[1], Omrana Ahmad Kamli[1], Husham Elraih Homeida[2], Asaad Khalid Ahmed[3], Noha Abdullah Arishi[4], Lolah Nasser Ogdi[4]

[1]Department of Physical Sciences, Chemistry Division, College of Science, Jazan University, Jazan, SAUDI ARABIA.
[2]Department of Maxillofacial Surgery and Diagnostic Science, College of Dentistry, Jazan University, Jazan, SAUDI ARABIA.
[3]Health Research Centre, Jazan University, Jazan, SAUDI ARABIA.
[4]Department of Forensic Chemistry, Toxicology Lab Ministry of Health, Jazan, SAUDI ARABIA.

ABSTRACT

Background: *Ruta chalepensis* L. is considered as a medicinal plant with many important secondary metabolites and essential oils which widely used against many diseases. **Objectives:** This study aimed to examine different polar extracts of *Ruta chalepensis* L. leaves and stems to identify different secondary metabolites, evaluate the antimicrobial activity of the prepared extracts and study the active extracts by the Gas Chromatography-Mass Spectrometry (GC-MS) method. **Materials and Methods:** The leaves and stems of *R. chalepensis* were extracted by Soxhlet apparatus using different solvents of varying polarity, were phytochemically screened and, also tested against bacteria namely *Streptococcus faecalis, Staphylococcus aureus,Escherichia coli,Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Salmonella* sp. and one fungus *Candida albicans* using diffusion method. **Results:** The phytochemical screen showed the presence of various secondary metabolites such as flavonoids, alkaloids and coumarins. The antimicrobial assay revealed that the plant extracts exhibited efficacy against only *Salmonella* sp., showing resistance to other tested bacteria and fungus. The methanolic extract of leaves and stems was the most active against *Salmonella* sp., with a high inhibition zone (25±0) mm and (24±0) mm respectively, thus it was subjected to GC-MS analysis which exhibited the presence of approximately six and thirteen phytochemical constituents respectively. **Conclusion:** Both leaves and stems extract showed activity against *Salmonella* sp. The phytochemical screening and GC-MS analysis indicate the presence of various secondary metabolites. Subsequently, the therapeutic efficacy compounds isolated and purified from*Ruta chalepensis* L. could be used as an important source against bacterial ailments in humans and plants.

Keywords: Anti-bacterial, GC-MS, Phytochemical analysis, Ruta *chalepensis* L.

Correspondence:
Mrs. Sara Hussein Altayeb
Department of Physical Sciences, Chemistry Division, College of Science, Jazan University, P.O Box 114, Jazan 45142, SAUDI ARABIA.
Email: saltayeb@jazanu.edu.sa
ORCID: 0009-0002-3237-7438

Received: 19-02-2024;
Revised: 07-03-2024;
Accepted: 13-07-2024.

INTRODUCTION

Ruta chalepensis L. is a medicinal herb belonging to the family *Rutaceae*, it is a small shrub that is most distributed in the Mediterranean region, usually growing on rocky slopes.[1] In the Jazan area (Saudi Arabia), is widespread with a local name "Arabic El-shathap" and is used commonly after braiding it with other aromatic plants in the form of a collar known as "Almikhadara or Alkhador" that men wear on the head as an adornment and for its pleasant scent. It is also used as a medicinal plant against headaches, scarlet fever, measles and heart conditions.[2] *Ruta chalepensis* L. was characterized by its anti-oxidant, anti-cancer and anti-inflammatory properties.[3] Also, the essential oil of *Ruta chalepensis* L. showed antioxidant activity there and might be considered as a potential source of cytotoxic compound and antifungal and herbicidal activity.[4,5] The study conducted on the dried leaves of *Ruta chalepensis* on mice showed the embryotoxic effect of the plant and its harmful use.[6]

Thus, the present study aims to evaluate the *in vitro* antimicrobial activities of different solvent extracts of *R. chalepensis* and to determine the chemical composition of these extracts by GC-MS analysis.

DOI: 10.5530/pres.16.4.112

MATERIALS AND METHODS

Plant material

The *Ruta* plant was bought from a local market in Jazan, Saudi Arabia in December. The plant was identified as *Ruta chalepensis* L. by the taxonomist at the Herbarium of Biology Department,

Science College, Jazan University with specimen number (Voucher no.) JAZUH 1369.

Chemicals and Reagents

Diethyl ether, chloroform, methanol and distilled water. Dragendroff's reagent, Molisch's reagent, Mayer's reagent, Fehling's solution, $FeCl_3$ 10%, KOH (alcoholic 25%), HCl, acetic anhydride, ammonia 25% and sulphuric acid.

Plant extract preparation

Twenty grams of air-dried and powdered leaves and stems of*Ruta chalepensis* were separately extracted using Soxhlet apparatus using three different solvents: diethyl ether, chloroform and methanol successively at temperature below the boiling temperature of each solvent for 4 hr for each. Then the marc was dried and extracted with distilled water in a conical flask for 24 hr. In a rotary evaporator, the extracts were concentrated and then the yields were determined. The extracts were labeled and kept in a refrigerator at 4°C for further study.

Phytochemical screening qualitative analysis

The preliminary phytochemicals {sterols, triterpenes, higher fatty acids, alkaloids, flavonoids, anthracene glycosides, coumarins, tannins, reducing compounds, polyuronides, glucides, starch and saponins} screening at different solvent extracts, were carried out using standard a method as described by Harbone.[7]

Antibacterial activity test

Eight plant extracts were evaluated against seven standard bacterial strains and one fungus. The bacterial strains included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 14153), *Klebsiella pneumoniae* (ATCC 700603), *Staphylococcus aureus* (ATCC 20213), *Salmonella* sp. (ATCC 700623) and *Streptococcus faecalis* (ATCC 29212), however the fungus was *Candida albicans* (ATCC 20231). These strains were obtained from the Department of Microbiology at King Fahad Hospital in Jazan, Saudi Arabia.

Preparation of the Test Organisms

To adjust the turbidity, isolated bacterial colonies were incubated in 4 mL of peptone water at 37°C for 1 hr. The turbidity was adjusted to match the standard of 0.5 McFarland units. Fungal cultures were grown on Sabouraud dextrose agar at 25°C for 4 days. The fungal culture was then washed with 100 mL of sterile normal saline and stored at 4°C until needed.

In vitro Antimicrobial Testing

To assess the antibacterial activity of the plant extracts, the cup plate agar diffusion method was employed with slight modifications. Initially, 1 mL of the standardized bacterial stock suspension containing 105-106 CFU/mL was mixed thoroughly with 100 mL of Muller Hinton agar medium, which was then maintained at 45°C. Subsequently, 20 mL aliquots of the resulting Muller Hinton agar medium were dispensed into sterile Petri dishes and allowed to solidify. Using a sterile cork borer number 4, four wells of approximately 20 mm diameter were created in each plate. The agar discs were removed and the wells were filled with 0.1 mL of plant extracts, allowing them to diffuse at room temperature for at least 2 hr. The plates were then incubated for 18 hr at 37°C. All experiments were performed in duplicate and the diameters of the resulting zone of inhibition were measured, with the mean values being recorded. A similar procedure was followed for testing the extracts for antifungal activity, except that Sabouraud dextrose agar medium was used instead of Muller Hinton agar and the incubation period was 24 hr.[8] Gentamicin and Fluconazole were used as standards for antibacterial and antifungal screening, respectively.

Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) of all extracts (extract 1-extract 11) against the microorganisms was determined using the broth dilution method. Bacterial cultures (100 µL containing 105 CFU/mL) were added to tubes containing varying extract concentrations (0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.078125 and 0625.00390 mg/L) and then incubated overnight at 37°C. MIC values were determined based on the absence of visible growth in the culture tubes. Similarly, the Minimum Bactericidal Concentration (MBC) was determined by subculturing the broth onto freshly prepared Muller Hinton agar medium and incubating at 37°C overnight. The highest concentration of the MIC tubes without any bacterial growth was considered the MBC. (The National Committee for Clinical Laboratory Standards.[9]

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The methanolic extracts of *Ruta* leaves and stems were subjected to GC-MS analysis. The analysis was carried out on a Shimadzu Gas Chromatograph (GC-2010, Germany) instrument fitted with a capillary column TR-5MS (Thermo Scientific TRACE 260F130P, Texas, U.S.A.) (30 m×0.25 mm) and film thickness 0.25 µm. The carrier gas Helium (He) was used at a flow rate of 1.2 mL min^{-1}. The initial temperature was kept at 70°C followed by heating at a rate of 15°C/min to 290°C, which was held for 30 min. The chromatograph was coupled to a Shimadzu QP2010 Ultra MS detector with an electron-ionization system and ionization energy of 70 eV. The ion source temperature was maintained at 230°C. The spectrum of the unknown components was compared with the spectrum of the known components stored in the National Institute of Standards and Technology [NIST] library and the molecular weight and structure of the compound.

RESULTS

Phytochemical Screening of the Leaves and Stems of *Ruta chalepensis* L.

Results of general phytochemical screening of diethyl ether, methanol and water extracts of the leaves and stems of *Ruta chalepensis* were presented in Table 1. Qualitative analysis of the leaves and stems extracts revealed the presence of alkaloids, flavonoids, sterols/triterpenes, higher fatty acids, coumarins, tannins, reducing compounds, polyuronides, glucides and saponins.

Antimicrobial activity of extracts of *Ruta chalepensis* L.

The effectiveness of eight plant extracts against the reference bacteria and fungus was assessed using the disc-diffusion method, with results summarized in Tables 2 and 3. The plant extracts exhibited efficacy against only *Salmonella* sp., showing resistance to the other tested bacteria (Figure 1). The zone of inhibition varied from (10±0) mm in extract 5 to (25±0) mm in extract 3. The results of positive control range from 27±0 to 32±0. However, the disc-diffusion method only indicates bacterial growth inhibition and does not provide evidence of the extract's bacteriostatic or bactericidal action. Therefore, MIC and MBC values were determined to establish the dose specificity and nature of the extract's activity. MIC values were estimated using the broth macro-dilution method, while MBC values were determined by sub-culturing all concentrations (≥MIC) with no detectable growth. The MIC and MBC values of the eight plant extracts ranged from 0.25 to 0.03125 mg/mL and 0.5 to 0.0625 mg/mL, respectively, against all tested bacterial strains and one fungus (Tables 2 and 3).

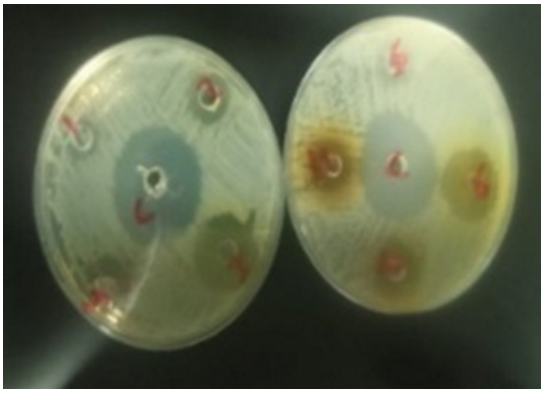

Figure 1: Antibacterial activity of the extracts of *Ruta chalepensis* L. against *Salmonella* sp. '1'-ether extract of stems, '2'-$CHCl_3$ extract of stems, '3'-methanol extract of stems, '10'-aqueous extract of stems, '4'-ether extract of leaves, '5'-$CHCl_3$ extract of leaves, '6'-methanol extract of leaves, '11'-aqueous extract of leaves, 'c'-control.

GC-MS analysis

The methanolic extract of leaves and stems of *Ruta* which exhibited a significant activity against *Salmonella* sp. were moreover analyzed by GC-MS. Figures 2 and 3 represent the gas chromatograms showing the relative abundance of various compounds eluted as a function of retention time. The results summarized in Tables 4 and 5 have demonstrated the presence of about 6 and 13 active phytochemical constituents, which contribute to the pharmacological activity of plant. The major constituents in the methanolic extract of leaves were namely Neophytadiene (peak area 1.19%), Hexadecanoic acid, methyl ester (peak area 1.65%), Benzene, 1-(1,3-dimethyl-3-butenyl)-3-methoxy- (peak area 1.61%), Methyl stearate (peak area 2%), Psoralen, 3-(.alpha.,.alpha.-dimethylallyl)- (peak area 27.04%) and Chalepin (peak area 25.52%). The main constituents in the methanolic extract of stems were namely Dimethyl dl-malate (peak area 2.3%),

Table 1: Qualitative analysis of phytochemicals exists in *Ruta chalepensis* L.

Test for / Extract	Tannins	Reducing reagent compounds	Alkaloids	Anthracene-glycosides	Sterols/ triterpenes	Coumarins	Higher fatty acids	Flavonoids	Saponins	Polyuronides	starch	Glucides
Ether extract of leaves	+	-	+	-	+	+	+	+	+	-	-	-
Ether extract of stem	+	-	+	-	+	+	+	+	+	-	-	-
Methanol extract of leaves	+	+	+	-	+	+	-	+	+	+	-	+
Methanol extract of stems	+	+	+	-	+	+	-	+	+	+	-	+
Aqueous extract of leaves	+	+	+	-	+	+	-	+	+	+	-	+
Aqueous extract of stems	+	+	+	-	+	+	-	+	+	+	-	+

'+'-Present, '-'-Absent.

Table 2: Antimicrobial screening of the extracts with diameter of the zone of inhibition(mm), as well as the (MIC) and (MBC) (mg/ml)

Organism	Extract 1			Extract 2			Extract 3			Extract 4			
	Zone of Inhibition	MIC	MBC	Zone of Inhibition	MIC	MBC	Zone of inhibition	MIC	MBC	Zone of inhibition	MIC	MBC	Con+ (gentamicin)
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	28.05±0.05
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	27.05±0.05
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	30±0
Streptococcus faecalis	-	-	-	-	-	-	-	-	-	-	-	-	31±0
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	29±0
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	27±0
Salmonella sp.	11±0	0.25	0.5	14.95±0.05	0.0625	0.125	25 ±0	0.03125	0.0625	18.05±0.05	0.0625	0.125	32±0
Candida albican	-	-	-	-	-	-	-	-	-	-	-	-	15.5±1.52

Table 3: Antimicrobial screening of the extracts with diameter of the zone of inhibition(mm), as well as the (MIC) and (MBC) (mg/ml)

Organism	Extract 5			Extract 6			Extract 10			Extract 11		
	Zone of Inhibition	MIC	MBC	Zone of Inhibition	MIC	MBC	Zone of inhibition	MIC	MBC	Zone of inhibition	MIC	MBC
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-
Streptococcus faecalis	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella sp.	10±0	0.25	0.5	24±0	0.0313	0.0625	17 ±0	0.0625	0.125	13.05±0.05	0.25	0.5
Candida albican	-	-	-	-	-	-	-	-	-	-	-	-

Table 4: Phytocomponents identified in the methanolic leaves extract of *Ruta chalepensis* L. by GC-MS.

Sample No.	Retention time	Compound name	Molecular formula	Molecular weight	Area%	Similarity index SI
1	10.472	Neophytadiene	$C_{20}H_{38}$	278.5	1.19	97
2	11.128	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	1.65	95
3	12.158	Benzene, 1-(1,3-dim ethyl-3-butenyl)-3-methoxy-	$C_{14}H_{20}O_2$	220.31	1.61	81
4	12.417	Methyl stearate	$C_{19}H_{38}O_2$	298.5	2	96
5	13.12	Psoralen, 3-(. alpha.,.alpha.-dimethylallyl)-	$C_{16}H_{14}O_3$	254.28	27.04	86
6	15.916	Chalepin	$C_{19}H_{22}O_4$	314.4	25.52	85

Table 5: Phytocomponents identified in the methanolic stems extract of *Ruta chalepensis* L. by GC-MS.

Sample No.	Retention time	Compound name	Molecular formula	Molecular weight	Area%	Similarity index SI
1	4.681	Dimethyl dl-malate	$C_6H_{10}O_5$	162.14	2.3	96
2	6.218	Guanosine	$C_{10}H_{13}N_5O_5$	283.24	1.65	92
3	6.4	beta-D-Ribopyranoside, methyl 2,3,4-tri-o-methyl	$C_9H_{18}O_5$	206.24	1.14	93
4	6.742	. beta. -D-Ribopyranoside, methyl	$C_9H_{18}O_5$	206.24	3.58	96
5	6.967	. alpha. -d-Lyxofuranoside, methyl	$C_6H_{12}O_5$	164.16	7.11	93
6	7.18	. beta.-d-Lyxofuranoside, methyl	$C_6H_{12}O_5$	164.06847	2.86	95
7	7.231	Methyl 4,6-ethylidene-. alpha.-d-galactopyranoside	$C_9H_{16}O_6$	220.22	4.11	72
8	7.368	D-Allose	$C_6H_{12}O_6$	180.16	3.06	73
9	8.917	Methyl(methyl 4-O-methyl-. alpha.-d-mannopyranoside) uronate	$C_8H_{16}O_6$	208.21	1.58	85
10	9.02	.beta.-D-Glucopyranose, 4-O-. beta.-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342.2965	1.12	63
11	11.124	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$	270.4507	3.35	95
12	12.417	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	4.09	97
13	15.953	Chalepin	$C_{19}H_{22}O_4$	314.4	1.07	71

Guanosine (peak area 162.14%), beta-D-Ribopyranoside, methyl 2,3,4-tri-o-methyl (peak area 1.14%), beta. -D-Ribopyranoside, methyl (peak area 3.58%), alpha. -d-Lyxofuranoside, methyl (peak area 7.11%), beta. -d-Lyxofuranoside, methyl (peak area 2.86%), Methyl 4,6-ethylidene-. alpha. -d-galactopyranoside (peak area 4.11%), D-Allose peak area 3.06%), Methyl (methyl 4-O-methyl-.alpha.-d-mannopyranoside)uronate (peak area 1.58%), beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl- (peak area 1.12%), Hexadecanoic acid, methyl ester (peak area 3.35%), Methyl stearate (peak area 4.09%) and Chalepin (peak area 1.07%).

DISCUSSION

In this study, the phytochemical screen of extracts of stems and leaves of *Ruta chalepensis* exhibited the presence of various secondary metabolites like flavonoids, alkaloids, sterols/ triterpenes, higher fatty acids, coumarins, tannins, reducing compounds, polyuronides, glucides and saponins. A previous study on the aerial parts of*Ruta chalepensis* L. also supported the presence of different phytochemicals such as coumarins, alkaloids, tannins, flavonoids, volatile oils, sterols and triterpenes, as well as a recent study on the ethanolic extract of the plant using colorimetric assay revealed that it had a good level of flavonoids

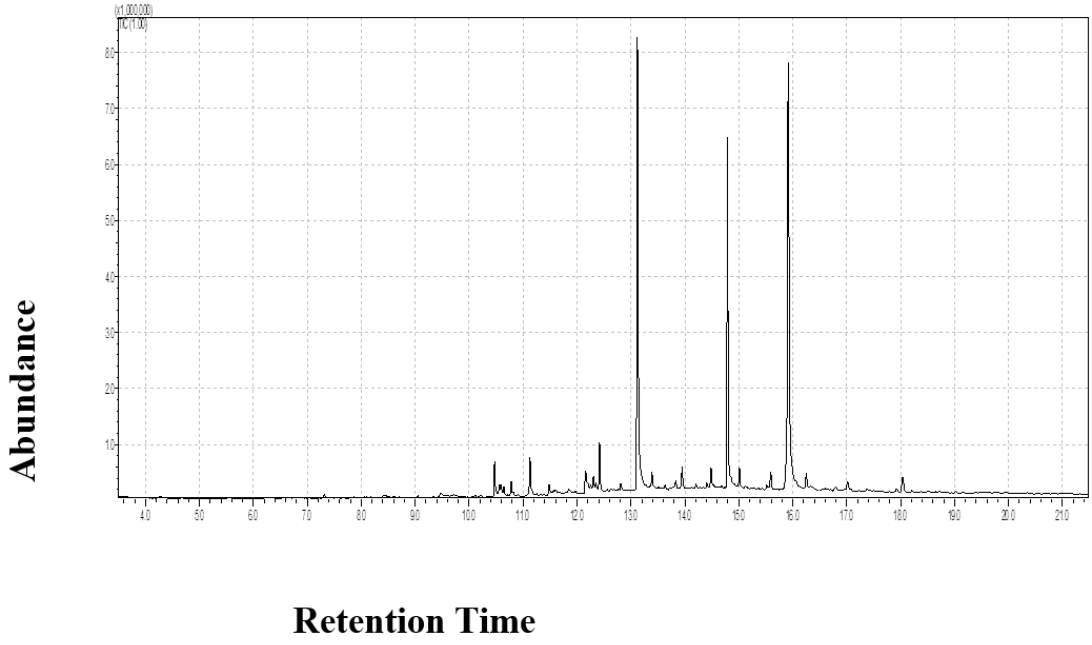

Figure 2: Gas chromatography-mass spectroscopy chromatogram of methanolic leaves extract.

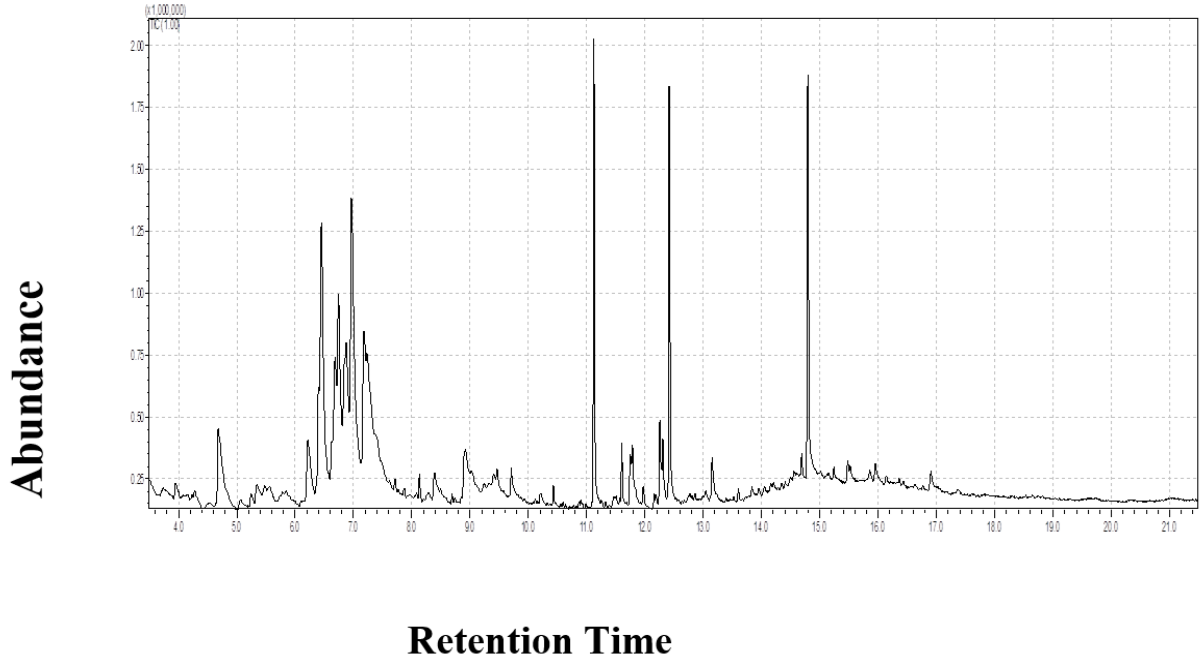

Figure 3: Gas chromatography-mass spectroscopy chromatogram of methanolic Stem extract .

and polyphenols.[10,11] Plant extracts were only effective against *Salmonella* sp. showing resistance to other tested bacteria and fungus, a prior study exhibited marked antibacterial properties of *Ruta chalepensis* extract against human pathogen strains, but the activity varied according to the solvent.[12,13] Another study revealed that flavonoids have been identified as polyphenolic compounds capable of exerting antibacterial activities via various mechanisms of action.[14] A review article also, reported that Chalepin and Chalepensin which were first isolated from the plant *Ruta chalepensis* L. are 3-prenylated bioactive coumarins, their bioactivity includes antimicrobial, antidiabetic, antifertility, antiprotozoal and cytotoxicity.[15] GC-MS analysis revealed the presence of many compounds such as Chalepin, Chalepensin, Hexadecanoic acid, methyl ester and Methyl stearate, while a previous study on the aerial parts of *R. chalepensis* confirmed the presence of ketons, esters, fatty acid and monoterpenes, the different distribution of compounds in the various plant parts depending on the life cycle of the plant.[16]

CONCLUSION

The phytochemical analysis of extracts of *Ruta chalepensis* revealed the presence of different secondary metabolites such as coumarins, alkaloids, flavonoids, glucides, polyuronides, fatty acids, tannins, saponins, sterols and triterpenes. GC-MS analysis reinforced this analysis, which showed the presence of approximately six and thirteen phytochemical constituents in leaves and stems methanolic extracts, respectively. The predominant compounds in leaves extracts were Chalepin and Psoralen, 3-(α, α-dimethylallyl)- other name is Chalepensin, while in stems extract were α-d-Lyxofuranoside, methyl and Methyl stearate.

The extracts also showed antimicrobial activity especially against *Salmonella* sp. further studies into this plant could be more valuable in herbal medicine.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the funding of the Deanship of Graduate Studies and Scientific Research, Jazan University, Saudi Arabia, through Project Number: (RG24-L 05).

FUNDING

This research is funded by the Deanship of Graduate Studies and Scientific Research, Jazan University, Saudi Arabia, (Project Number: (RG24-L05).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has been conducted ethically and responsibly, all participants provided the necessary ethical clearances and informed consent from the beginning of the study.

ABBREVIATIONS

GC-MS: Gas chromatography-mass spectrometry; **MIC:** Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration; **SI:** Similarity index.

SUMMARY

Medicinal and aromatic plants have always been an important source of medicines and treatments against various diseases. The great scientific and technological progress in the fields of separation and analysis has contributed to the manufacture of medicines and treatments scientifically and safely. This study determined a number of phytochemical constituents present in *Ruta chalepensis* L. leaves and stems extracts by phytochemical screening and GC-MS analysis and evaluated the antimicrobial activity which showed that the plant extracts exhibited efficacy against *Salmonella* sp. Thus, the therapeutic efficacy compounds isolated and purified from*Ruta chalepensis* L. could be an important source against bacterial ailments in humans and plants.

REFERENCES

1. Iauk L, Mangano K, Rapisarda A, Ragusa S, Maiolino L, Musumeci R, *et al.* Protection against murine endotoxemia by treatment with *Ruta chalepensis* L., a plant with anti-inflammatory properties. J Ethnopharmacol. 2004;90(2-3):267-72. doi: 10.1016/j.jep.2003.10.004, PMID 15013191.
2. El-shabasy A. Survey on medicinal plants in the flora of Jizan Region, Saudi Arabia. Int J Bot Stud. 2016;2(1):38-59.
3. Khlifi D, Sghaier RM, Amouri S, Laouini D, Hamdi M, Bouajila J. Composition and antioxidant, anticancer and anti-inflammatory activities of *Artemisia herba-alba, Ruta chalpensis* L. and *Peganum harmala* L. Food Chem Toxicol. 2013;55:202-8. doi: 10.1016/j.fct.2013.01.004, PMID 23333573.
4. Althaher AR, Oran SA, Bustanji YK. Phytochemical Analysis, *in vitro* Assessment of Antioxidant Properties and cytotoxic Potential of *Ruta chalepensis* L. Essential oil. J Essent Oil Bear Plants. 2020;23(6):1409-21. doi: 10.1080/0972060X.2020.1871078.
5. Bouabidi W, Hanana M, Gargouri S, Amri I, Fezzani T, Ksontini M, *et al.* Chemical composition, phytotoxic and antifungal properties of *Ruta chalepensis* L. essential oils. Nat Prod Res. 2015;29(9):864-8. doi: 10.1080/14786419.2014.980246, PMID 25553803.
6. Zeichen de Sa RZ, Rey A, Argañaraz E, Bindstein E. Perinatal toxicology of *Ruta chalepensis* (*Rutaceae*) in mice. J Ethnopharmacol. 2000;69(2):93-8. doi: 10.1016/S0378-8741(98)00232-3, PMID 10687865.
7. Harbone JB. Phytochemical method, a guide to modern technique of plants. 3rd ed. Dordrecht: Springer; 1998.
8. Kavanagh F. An approach to accurate diffusion assays. In: Kavanagh F, editor. Analytical microbiology. Cambridge, MA: Academic Press; 1972. p. 31-42.
9. The National Committee for Clinical Laboratory Standards. [NCCLS]. Methods for determining bactericidal activity of antimicrobial agents; approved guideline. NCCLS Document M26-A [ISBN 1-56238-384-1], Clinical and Laboratory Standards Institute. 940 West Valley Road: NCCLS, Suite 1400, Wayne, Pennsylvania: 19087. USA; 1999.
10. Al-Said MS, Tariq M, Al-Yahya MA, Rafatullah S, Ginnawi OT, Ageel AM. Studies on Ruta chalepensis, an ancient medicinal herb still used in traditional medicine. J Ethnopharmacol. 1990;28(3):305-12. doi: 10.1016/0378-8741(90)90081-4.
11. Althaher AR, Oran SA, Awadallah MW, Ameen HH, Shehabi RF, Bourghli LM, *et al.* Chemical Composition, Antioxidant and antibacterial activity of *Ruta chalepensis* L. Ethanolic extract. Chem Biodivers. 2024;21(4):e202400026. doi: 10.1002/cbdv.202400026, PMID 38372467.
12. Ouerghemmi I, Rebey IB, Rahali FZ, Bourgou S, Pistelli L, Ksouri R, *et al.* Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*. J Food Drug Anal. 2017;25(2):350-9. doi: 10.1016/j.jfda.2016.04.001, PMID 28911677.
13. Alotaibi SM, Saleem MS. Al-humaidi JG. Phytochemical contents and biological evaluation of *Ruta chalepennsis* L. growing in Saudi Arabia. Saudi Pharm J. 2018;26(4):504-8. doi: 10.1016/j.jsps.2018.02.008.
14. Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, *et al.* Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. Molecules. 2022;27(4):1149. doi: 10.3390/molecules27041149, PMID 35208939.
15. Nahar L, Al-Majmaie S, Al-Groshi A, Rasul A, Sarker SD. Chalepin and chalepensin: occurrence, biosynthesis and therapeutic potential. Molecules. 2021;26(6):1609. doi: 10.3390/molecules26061609, PMID 33799365.
16. Tedone L, Costa R, De Grazia S, Ragusa S, Mondello L. Monodimensional (GC–FID and GC–MS) and comprehensive two-dimensional gas chromatography for the assessment of volatiles and fatty acids from *Ruta chalepensis* aerial parts. Phytochem Anal. 2014;25(5):468-75. doi: 10.1002/pca.2518.

Cite this article: Altayeb SH, Khleefa FA, Yagoob SI, Abdelhadi RA, Kamli OA, Homeida HE, *et al*. Antimicrobial, Phytochemical Screening and GC-MS Analysis of *Ruta chalepensis* L. Leaves and Stems Extracts. Pharmacog Res. 2024;16(4):985-91.