

Revealing The Phytochemical Constituents And Biological Evaluation Of *Ruta Chalepensis L* (MK828113)

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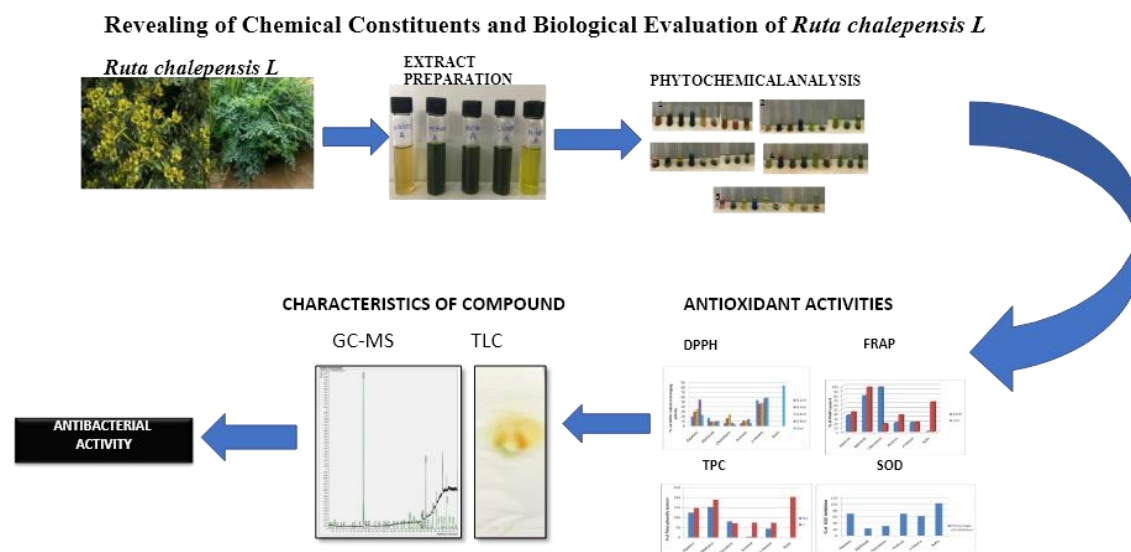
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Abstract

Ruta chalepensis L is commonly known as rue, is used in the traditional medicine of many countries for the treatment of various diseases. Recent pharmacological activities like antioxidant, anticancer, and anti-inflammatory effects are reported in this species. The aim of the study is to evaluate the biological constituents of *Ruta chalepensis L* (MK828113) plant extracts. The phytochemical screening tests were conducted as per the standard procedure to identify the compounds present in the leaf extracts of *Ruta chalepensis*. The leaves of this plant revealed the presence of alkaloids, flavonoids, phenols, quanines, and steroids with different bioactive compounds. The antibacterial activities of these extracts were examined using *E.coli*, *B.subtilis*, *S.aureus*, and *P.aeruginosa* to find the microbial inhibiting activities of the bioactive compounds. The bioactive compounds of these plants are great interest in medicinal chemistry. The antioxidant activity such as DPPH, FRAP, and SOD was carried out and evaluated according to the dose-dependent manner. The compounds were further characterized using TLC and GC-MS analysis.

Keywords: *Ruta chalepensis L*, Phytochemical, Antioxidant, TLC, GC-MS and Antibacterial.

GRAPICAL ABSTRACT



1. Introduction

Nowadays, the global interest in the use of herbal remedies has created a huge need for information about the uses and the therapeutic properties of the plants(1). Most medicinal plants are sources of bioactive compounds used mainly for medicinal purposes. In recent years, human pathogenic microorganisms developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases reported. Mostly in this situation, the undesirable side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobials to overcome the challenges (2).

Ruta chalepensis L. (Rutaceae) is commonly known as Rue or fringed rue is a wild perennial herbaceous shrub which widely distributed in the Mediterranean Sea regions were seen. It usually grew on the rocky slopes of mountains and hills. Mainly it has glabrous, alternate bipinnatisect leaves with narrow oblong lanceolate or obviates segments and cymose inflorescence were studied.(3). (4) stated that *R.augustifolia*, *R.chalepensis*, *R graveolens* are morphologically very similar and virtually impossible to differentiate in their parts. Within the context of the phylogenetic relationships supported by molecular data, *Ruta*, the type genus of the family can only be diagnosed by using a combination of plesiomorphic, homoplasious, and autapomorphic morphological characters (5).

The bioactive compounds of traditional medicinal plants are mostly obtained from plant leaves (6). Oil glands are present in leaves, having a strong deterrent odour. *R. chalepensis* is the most frequently used plant in folk medicine for its emmenagogue, anthelmintic, anti-inflammatory, and spasmolytic effects are shown by (7,8). Earlier the leaves of this plant infused with vinegar are given to children for the treatment of convulsion and other nervous disorders. In fact, it was shown that ethanol extract of the aerial parts of *R. chalepensis* produces a significant central nervous system depressant activity in mice were explained by (9). Different species of Rutaceae have already been using a tonic, febrifuge, against inflammatory and microbial processes and in the treatment of malaria (10).

Medicinal plants are known to owe their curative potentials and authenticate certain biologically active substances including terpenes, flavonoids, bioflavonoids, benzophenone, xanthenes as well as some metabolites such as tannins, saponins, cyanates, and oxalate increased all over the world and a large number of evidence shown were collected to show the immense potential of medicinal plants used in various traditional systems (11).The variations present in phytochemical groups in any plant can be used as promising support for the possible presence of biological activities were determined by (12). *Ruta chalepensis* L collected in the Chebba region (Mahdia, Tunisia), was found to possess potent antimicrobial activities against gram-positive and gram-negative strains, in conjunction with the presence of phenolic compounds (flavonoids,ortho-diphenols, tannins, and flavonol components) confirmed (13). Antioxidants may also contribute to diverse biological activities such as anti-inflammatory, anti-atherosclerotic, and anticarcinogenic activities (14).

The investigation is based on *Ruta chalepensis* .L which describes the anti-bacterial and antioxidant activities, this study provides further information in documenting knowledge for potential researchers in the area of Phytochemistry. The present study was conducted for the determination of the phytochemical constituents, antioxidant properties, GC-MS analysis and the antimicrobial activity against food borne pathogens using the extract of *Ruta chalepensis*. L.

2.Materials and methods

2.1.Phytochemical content

2.1.1.Collection of plant sample

The plant *Ruta chalepensis* L was collected from Udhagamandalam , Longitude : 76.69 and situates at an altitude of 2,240Mt above sea level within the Nilgiri hills, TamilNadu-643215, India. The identity of the plant was confirmed by the Botanical Survey of India. The gene sequences were submitted to NCBI and the gene bank accession number were granted (**MK828113**).

2.1.2.Preparation of plant extract

The collected plant leaves were dried under the shade of sunlight for 2-3 days and powered using mortar and pestle. The powdered plant leaves were soaked (10g/100ml) in different solvents (aqueous, methanol, chloroform, acetone and n-hexane) for 24hrs at 40°C for 60-70 rpm in orbital shaker. After incubating the extracts were carried to filter using Whatman no. 1 filter paper and used for further study.

2.1.3.Phytochemical analysis (15)

2.1.3.1. Qualitative Phytochemical analysis

The extracted sample was screened qualitatively for the analysis of alkaloids, terpenoids, phenols, reducing sugar, saponins, flavonoids, quinines, protein and steroids.

Test for Alkaloids

The 1 ml of plant extract was mixed with 1ml of Mayer's reagent. To these 2 drops of Iodine solution was added. Formation of a yellow color precipitate indicates the presence of alkaloids.

Test for Terpenoids

1 ml of concentrated sulphuric acid was added to the 1ml of extract. It was later incubated for 2-4 minutes in a water bath. Greyish color indicates the presence of terpenoids.

Test for Phenol

Two drops of ferric chloride were added to the tubes containing 1ml of the extracts. Greenish color indicates the presence of phenols.

Test for reducing Sugar

0.1 ml of Fehling's A and B was added to the tubes containing 1ml of the extract. It was later incubated for about 2-4 minutes in a boiling water bath. Red precipitate confirmed the presence of sugars.

Test for Saponins

Saponins were tested using distilled water (3ml) with 1ml of extract. Formation of foam was indicating the presence of saponins.

Test for Flavonoids

Flavonoids were tested using few fragments of magnesium ribbon in 2ml of the plant extract followed by add 2 -5 drops of concentrated hydrochloric acid. Appearance of pink scarlet color confirmed the presence of flavonoids.

Test for Quinines

Quinines were tested by adding 1ml of the 0.1% of sodium hydroxide reagent in 2ml of the plant extract. Appearance of blue, green or red indicates presence of quinines.

Test for Protein

Few drops of concentrated nitric acid were added to 1ml of the extract. Yellow coloration was indicated the presence of proteins.

Test for Steroids

1 ml of extract was mixed with 1 ml of chloroform and 1ml of concentrated H₂SO₄ sidewise. A red color ring at the lower chloroform layer indicates the presence of steroids.

2.1.3.2. Quantitative Assay

2.1.3.2.1. Anti-Oxidant Activity

2.1.3.2.2. DPPH radical scavenging activity

The method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolorizes the DPPH solution. 0.2 ml, 0.4ml, 0.6ml, 0.8ml and 1.0 ml of (aqueous, methanol, chloroform, acetone, n-hexane) leaf extracts were mixed with 1ml of 0.1 M DPPH. To all the tubes added to 0.4 ml of 50 mM Tris-HCl. Incubated the reaction mixture at room temperature for 30 minutes. The absorbance of the reaction mixture can be read at 517 nm. The percentage of radical scavenging was calculated using the formula mentioned below (15). The rutin (10mg/ml, Hi Media) was used to compared with the sample as standard.

$$\% \text{ of DPPH activity} = \frac{\text{Absorbance of Control OD} - \text{Absorbance of sample OD}}{\text{Absorbance of Control OD}} \times 100$$

2.1.3.2.3. FRAP

Ferric Reducing Antioxidant Power Assay was carried out for the sample by mixing 0.5ml and 1ml of the filtered extract with 2.5ml of the phosphate buffer solution and 2.5ml of the 1% of K₃Fe (CN)₆ solution. The reaction mixture was vortexed for 2-5 minutes and incubated 50°C for 20 minutes. After incubation of 2.5ml of 10% TCA solution added and centrifuged the reaction mixture at 3000 rpm for 5 minutes and 2.5ml of the supernatant was transferred to a fresh tube and added an equal volume of de-ionized water and 0.5ml of the 0.1% FeCl₃ solution. Incubated the mixture for 15 minutes and was read at 700nm using a spectrophotometer (Microprocessor spectrophotometer LT 291). Ascorbic acid was used as a standard to calculate the mg/g of FRAP content (16). The rutin (10mg/ml, Hi Media) was used to compared with the sample as standard.

2.1.3.2.4. SOD

When sample mixed with the reaction mixture I containing 1ml of 50mM PBS solution, 0.075ml of 20mM of L-Methionine, 0.04ml of 10mM hydroxylamine hydrochloride and 0.1ml of 50mM EDTA. After adding the tubes were incubated at 30°C for 5 minutes, added 80µl of 50µM riboflavin and exposed under 200W fluorescent light and added reaction mixture II, containing 1% of sulphanilamide in 5% phosphoric acid, mixed well and incubated for a few minutes and the OD was taken at 543nm using a UV visible spectrophotometer (Labtronics LT 291). Percentage of inhibition of nitrite formation was determined by the formula (17).

$$\% \text{ inhibition of nitrite formation} = 1 - \frac{\text{Absorbance of sample OD}}{\text{Absorbance of Control OD}} \times 100$$

2.1.3.2.5. Total phenolic content

TPC in the sample extract was determined by Folin-Ciocalteu's spectrophotometric method. The sample (0.5 ml and 1ml) was mixed with 0.5ml of the 10% Folin- Ciocalteu's reagent and incubated at room temperature for 5minutes, followed by added 2ml of 20% Na₂CO₃ solution and incubated at 45⁰C in shaking incubator for 15-20 minutes. The OD was taken at 765nm using UV a visible spectrophotometer (Labtronics LT 291). Gallic acid was used as a standard to calculate the mg/g of total phenolic content (15). The rutin (10mg/ml, Hi Media) was used to compared with the sample as standard.

2.1.3.4. Characterization of the compounds

2.1.3.4.1. TLC (Thin Layer Chromatography)(18)

TLC is a chromatography technique used to separate non-volatile mixtures using a solvent system (Methanol: Acetic acid: Formic acid: Water - 3:1:1:1). The procedure was done by the protocol and the R_f value was calculated by using the formula;

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}} \times 100$$

2.1.3.4.2. GC-MS Analysis

Bioactive compounds were studied using Agilent CH-GCMSMS-02. The temperature was programmed from 11⁰C (isothermal for 2 min) with an increase of 10⁰C/min to 200⁰C, then 5⁰C/min to 280⁰C, ending with a 9 min isothermal at 280⁰C. Mainly the Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative percentage of each component was calculated by comparing its average peak area to the total areas (19).

2.1.3.5. Antimicrobial activity (20)

2.1.3.5.1. Antibacterial activity

The antibacterial activity of the sample was studied using the well diffusion method against the pathogens. Mueller Hinton agar (Suspend 39g in 1000ml of distilled water and sterilized 121⁰ C for 15 minutes) was poured into the petri plate and allowed for solidification. After solidification 70µl of the bacterial culture (Bacillus subtilis, Escherichia coli, Straphylococcus aureus, and Pseudomonas aeruginosa) was spread using a cotton swab and well were made with a cork borer followed by the sample was added (20µl). Antibiotic disc (Cefazolin 30 mcg) was placed as positive control distilled water was used as negative control; the plate was incubated at 37⁰C for 24 hrs. After incubation anti -bacterial activity of the sample was confirmed based on the zone of inhibition in mm.

3. Results

3.1. Phytochemical screening

The phytochemical analysis of Ruta chalepensis L extracts with different solvents (aqueous, methanol, chloroform, acetone, n-hexane) is shown in Table 1 with different types of active constituents like alkaloids, flavonoids, terpenoids, tannins, saponins, and quinines .The result shown that the presence of alkaloids, terpenoids, saponin, flavonoids, protein in aqueous extract.

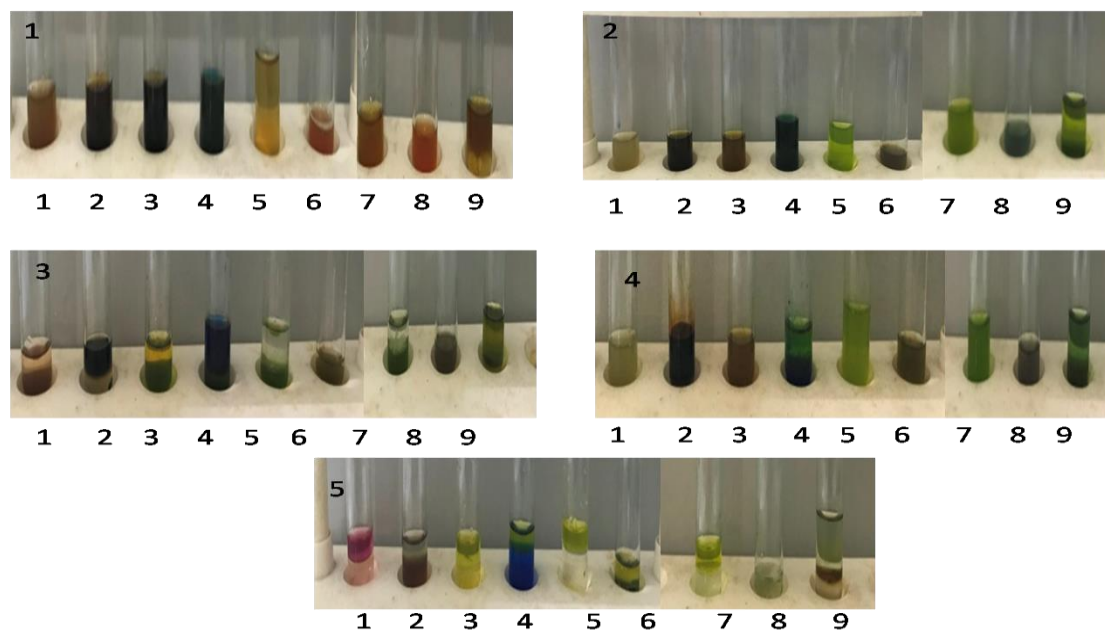


Fig. 1. Phytochemical analysis of *Ruta chalepensis* L using different solvents (1-aqueous, 2-methanol,3-chloroform,4- acetone, 5-n-hexane).

Table 1 Qualitative phytochemical analysis of leaves extracts of *Ruta chalepensis*.L

TESTS	Aqueous	Methanol	Chloroform	Acetone	n-hexane
Alkaloids	+	+	+	+	+
Terpenoids	+	+	+	+	-
Phenols& Tannins	-	+	+	+	-
Sugar	-	-	+	+	-
Saponin	+	+	+	-	+
Flavonoids	+	+	-	-	-
Quinines	-	+	+	+	+
Proteins	+	-	-	-	-
Sterols	-	-	-	-	+

(+) symbol indicates presence and (-) indicates absence with respect to extractive solvent

3.2. Anti-oxidant activity

3.2.1DPPH radical scavenging activity

Ruta chalepensis shows DPPH activity for the extract rutin 84.52 ± 0.458 , n-hexane $60 \mu\text{g/ml} \pm 0.112$, for methanol $10 \mu\text{g}/0.8\text{ml} \pm 0.01$, chloroform $24 \mu\text{g}/0.6\text{ml} \pm 0.011$, Acetone $15 \mu\text{g}/0.8\text{ml} \pm 0.015$ and finally the aqueous with $55.17/0.8\text{ml} 0.025$ were obtained. The result of DPPH radical scavenging effects of extracts of *Ruta chalepensis*. L in different solvent showed in Figure 2 , whereas rutin, aqueous and n-hexane extracts showed the highest antioxidant activity that identified using the percentage of DPPH activity which was carried on different concentrations of the sample with the given extract.

DPPH activity of the plant *Ruta chalepensis*.L in different extract

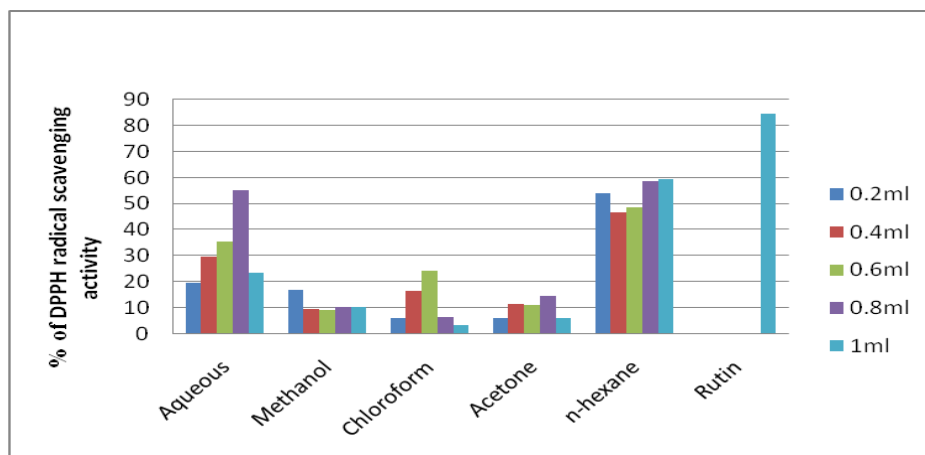


Fig. 2. Total DPPH radical scavenging activity of *Ruta chalepensis*.L

3.2.2. FRAP

FRAP assay of *Ruta chalepensis* L shown in the figure 3, the higher ferric reducing power indicates higher FRAP content in aqueous and methanol (43% to 97%). Moreover the chloroform and acetone have shown comparatively lower, reducing powder with 17% to 36% when used with the concentrated sample of 0.5ml and 1ml.

FRAP content of *Ruta chalepensis* L

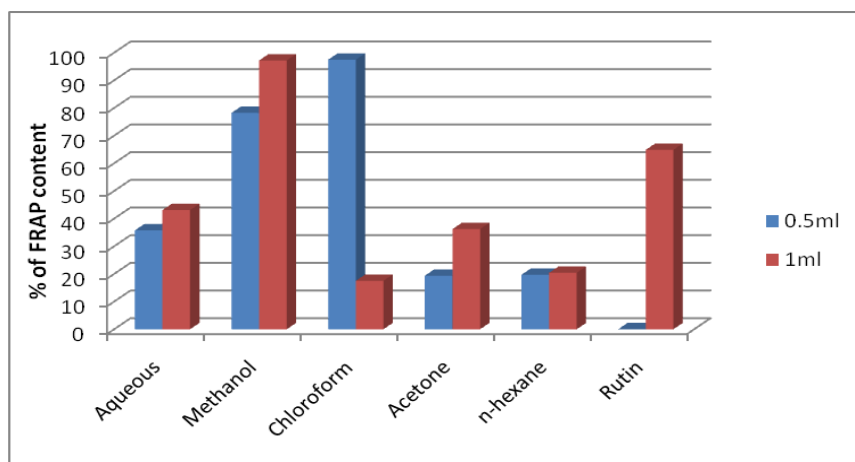


Fig 3 .FRAP of *Ruta chalepensis*.L

3.2.3. Total phenolic content

From the Fig.4, the plant *R.chalepensis* L have the sizable phenol content in all the extracts of rutin shows 204 ± 1.527 , methanol with $190 \pm 2 \mu\text{g/ml}$ followed by the aqueous with $148.6 \pm 4.611 \mu\text{g/ml}$, Whereas the chloroform is $72 \pm 3.60 \mu\text{g/ml}$, acetone is $75.3 \pm 0.571 \mu\text{g/ml}$ and n-hexane is $74.6 \pm 3.051 \mu\text{g/ml}$ were obtained.

Total phenolic content of *Ruta chalepensis* L

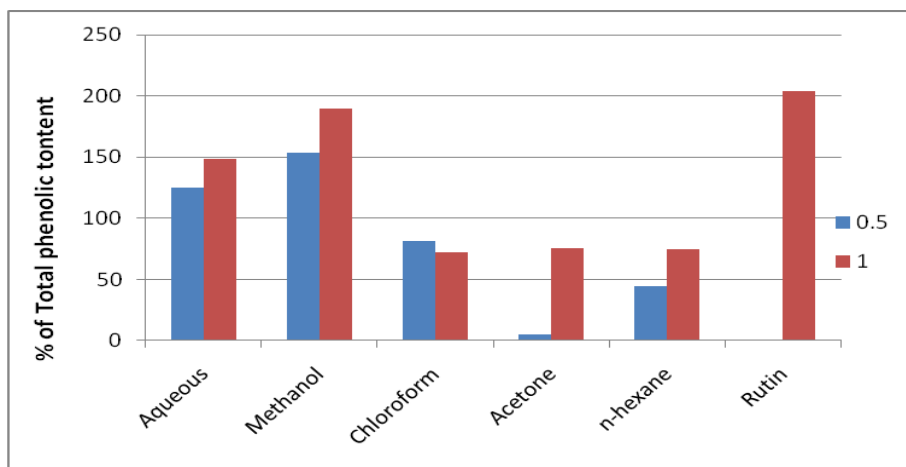


Fig 4 .Total phenolic content of Ruta chalepensis.L

3.2.4. SOD

Superoxide dismutase is the main enzyme that forms a defense against oxygen derived free radicals and catalyzes the removal of superoxide free radicals. The percentage of nitrite inhibition for the different extracts of Rutin was 103.6% acetone was 70%, water and n-hexane 63%, chloroform was 31% and the lower inhibition shows 23% in methanol was observed the results were given below in figure 5.

SOD of Ruta chalepensis L

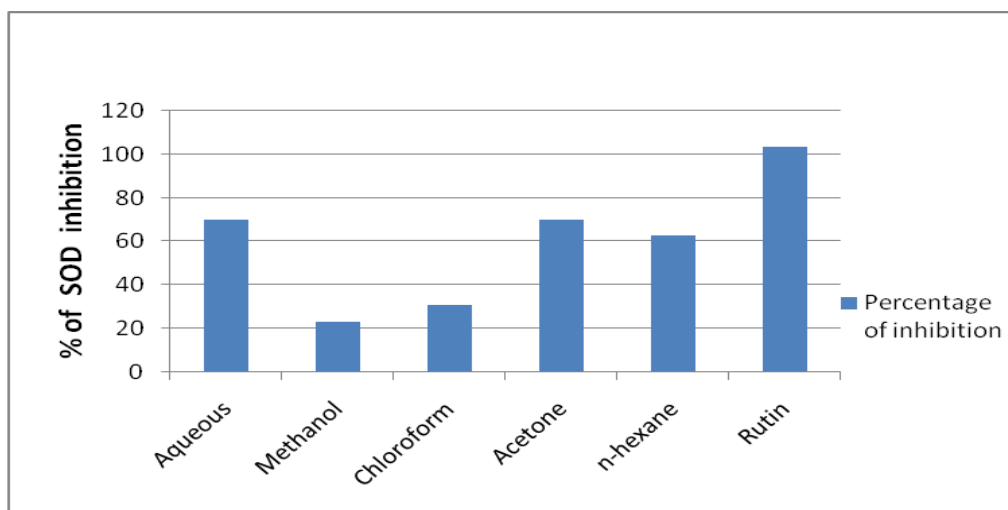


Fig 5. SOD of Ruta chalepensis.L

3.2.5. TLC (Thin Layer Chromatography)

TLC study was conducted to identify the most versatile and specific compound in the plant sample using the solvent system and calculated the R_f values. The result was shown in Table 2.

Table 2 . TLC

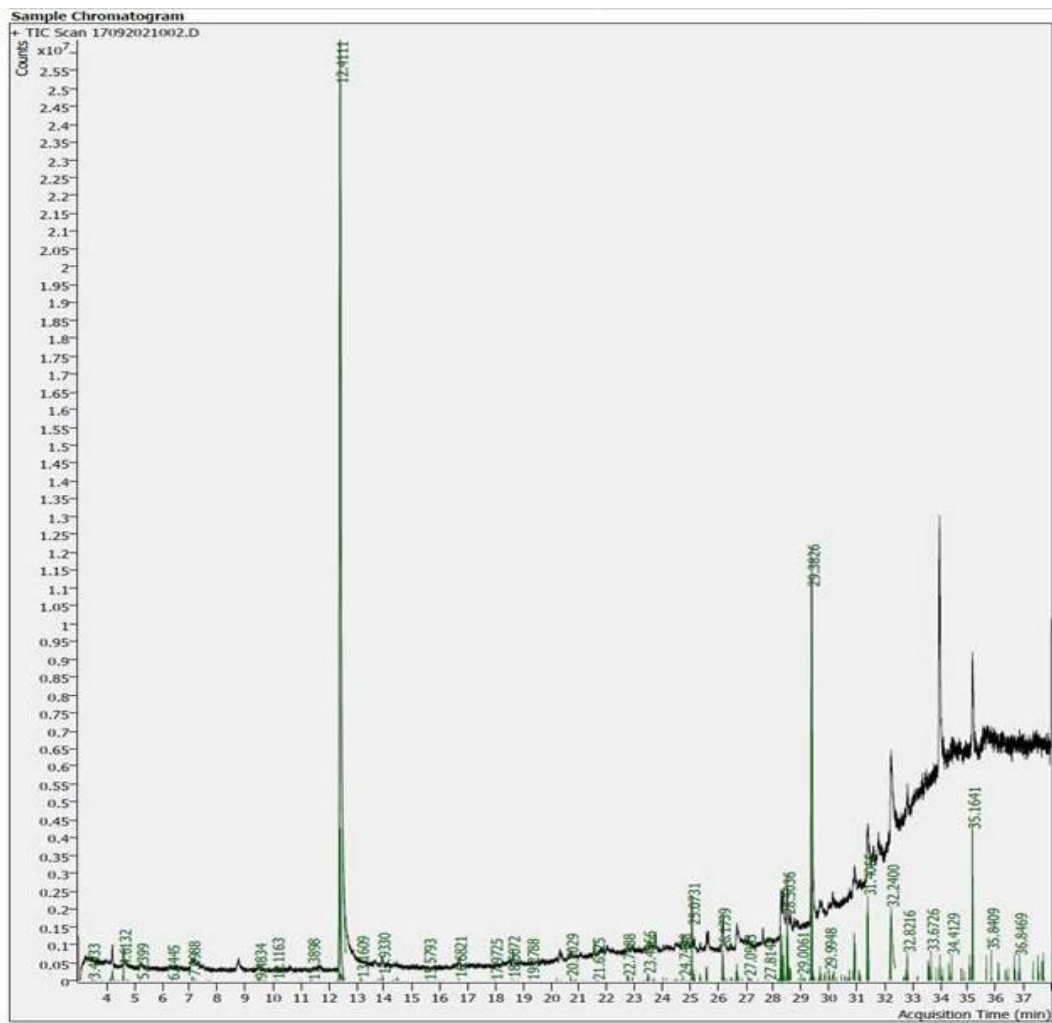
Sample	Spot formed	R _f value	Compounds
Plant extract	1	0.47	Alkaloids, phenolic compounds
	2	0.52	
	3	0.56	



Fig 6. TLC study of the methanol extract

3.2.6.GC-MS Analysis

The presence of different bioactive compounds in the *Ruta chalepensis* was further identified by GC-MS analysis. Totally 32 (Figure 8 and Table 7) compounds were identified in the plant extract which proven that the medicinal importance of the plant sample.



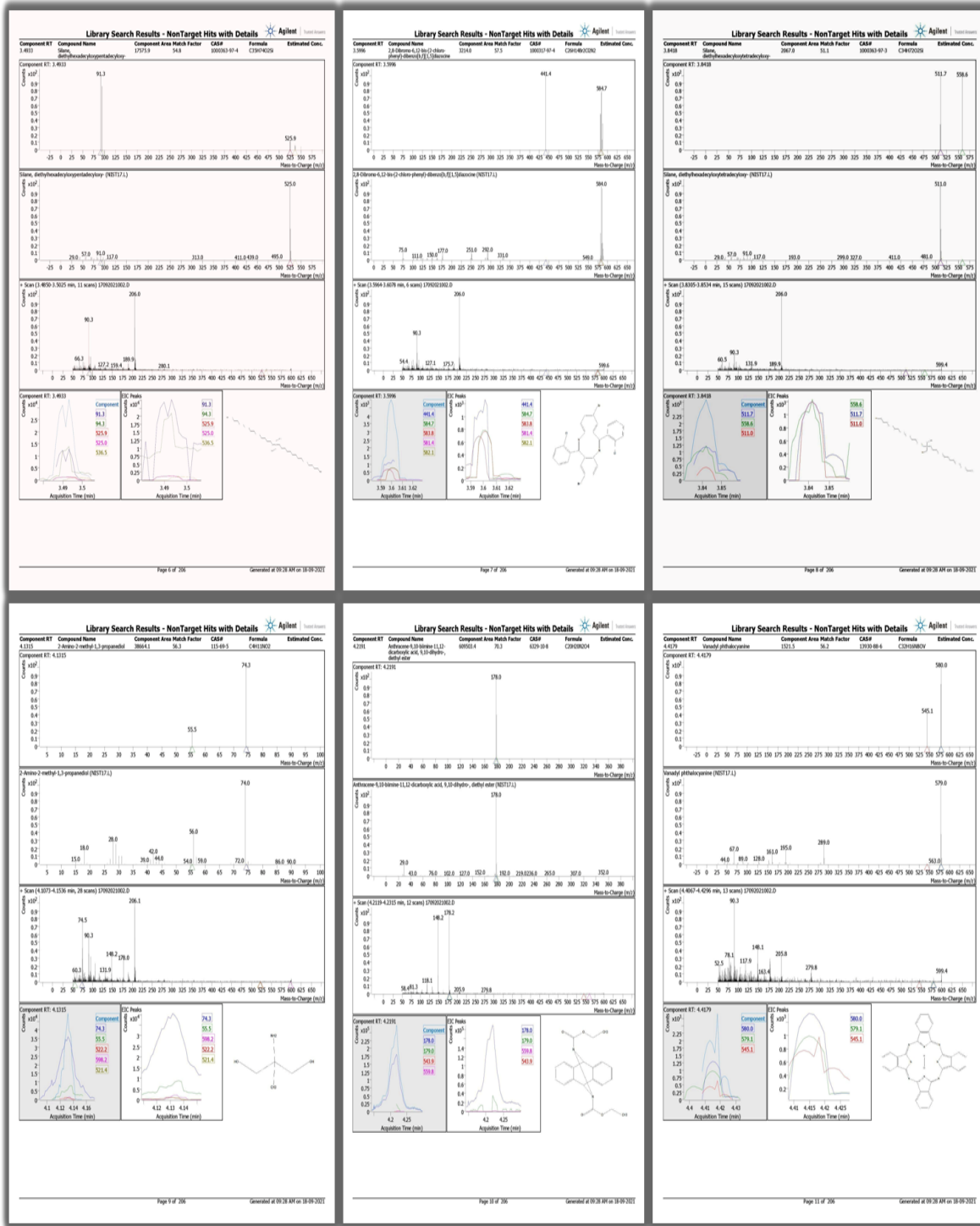


Fig. 7. GC-MS analysis of *Ruta chalepensis.L*

Table 3 Determination of chemical composition of *Ruta chalepensis* L using GC-MS analysis.

S.No	Compound Name	Molecular Formula	Application
1.	2-Amino-2-methyl-1,3-propanediol	C ₄ H ₁₁ NO ₂	Bio surfactant
2.	Vanadyl phthalocyanine	C ₃₂ H ₁₆ N ₈ OV	Biofilm applications
3.	2-Hydrazino-5-cyanopyridine	C ₆ H ₆ N ₄	Antoprolorative
4.	Silane, dimethyl(pentafluorobenzyloxy)docosyloxy-	C ₃₁ H ₅₃ F ₅ O ₂ Si	Skin application
5.	4-Methoxycarbonyl-2-methoxyphenyl isothiocyanate 1	C ₁₀ H ₉ NO ₃ S	Amino acid derivative
6.	Cholest-2-eno[3,2-a]naphthalene, 4'- (dimethylamino)-	C ₃₇ H ₅₅ N	Anti cancer
7.	Aziridine, 2-ethyl-	C ₄ H ₉ N	alkaloids potent <u>antitumor</u> , antibacterial, antimicrobial, and other activities.
8.	Pyridine-3-carboxamide, 4-methoxy-N-(3,5-dichlorophenyl)-	C ₁₃ H ₁₀ Cl ₂ N ₂ O ₂	Alkaloids
9.	9,12,15,18,21,24- Hexaoxa(2,16)[30]metacyclophan-1-ene, 1,2- diphenyl	C ₃₆ H ₃₈ O ₆	Anti microbial
10	2,6-Difluorobenzyl bromide	C ₇ H ₅ BrF ₂	reduce anxiety, cause sedation and reduce the dose of <u>anesthetic</u>
11	2,4-Diamino-6-methyl-1,3,5-triazine	C ₄ H ₇ N ₅	Anti inflammatory and pain killer
12	1,3,4-Thiadiazol-2-amine	C ₂ H ₃ N ₃ S	Anti microbial
13	o-Ethylhydroxylamine	C ₂ H ₇ NO	Anti cancer
14	1-Ethyl-1H-pyrazole-3,4-diamine	C ₅ H ₁₀ N ₄	Anti microbial , anti cancer and anti viral
15	Danthron, 2TMS derivative	C ₂₀ H ₂₄ O ₄ Si ₂	acne, psoriasis, calluses, corns, keratosis pilaris, and warts
16	quinoline, 1,2,3,4-tetrahydro-2,2,4,7-tetramethyl-	C ₁₃ H ₁₉ N	anti-cancer, anti-diabetic, anti-parasitic, anti-inflammatory, anti-oxidant, adrenergic, anti-HIV, anti-alzheimer, anti-hyperlipidemic
17	Diocetadecyl disulfide	C ₃₆ H ₇₄ S ₂	Anti flammatory
18	Mytiloxanthinone	C ₄₀ H ₅₂ O ₄	treatment of asthma
19	Cobalt phthalocyanine	C ₃₂ H ₁₆ CoN ₈	Anti oxidant and reducing agent
20	4-Phenylisoquinoline	C ₁₅ H ₁₁ N	antiprotozoal activity
21	2,5-di-tert-Butylaniline	C ₁₄ H ₂₃ N	Anti microbial
22	Hydrazinecarbothioamide	CH ₅ N ₃ S	Anti microbial, anti oxidant
23	Bambuterol, N-trifluoroacetyl-O-trimethylsilyl derive	C ₂₃ H ₃₆ F ₃ N ₃ O ₆ Si	treatment of asthma
24	Heptane, 4-azido-	C ₇ H ₁₅ N ₃	Anti bacterial
25	Dimethyl trisulfide	C ₂ H ₆ S ₃	Fragrance
26	Arachidamide, N-methyl-	C ₂₁ H ₄₃ NO	Treatment of skin diseases
27	Pipecolic acid, N-isobutoxycarbonyl-, isobutyl ester	C ₁₅ H ₂₇ NO ₄	Anti cancer
28	Octanenitrile, 2-methylene-	C ₉ H ₁₅ N	Anti microbial

29	Sarcosine, n-propargyloxycarbonyl-, propargyl ester	C ₁₀ H ₁₁ NO ₄	Surfactant
30	Vitamin K5, N,N,O-triethyl-	C ₁₇ H ₂₃ NO	Vitamin
31	Brayelin	C ₁₅ H ₁₄ O ₄	Antiplosmidial
32	Yohimbane	C ₁₉ H ₂₄ N ₂	Asthama

3.2.7. Antimicrobial

3.2.7.1 Antibacterial activity

The antibacterial activity was carried out against four pathogenic bacteria. Among the extracts tried water was found to be higher zone of inhibition (7mm) in E.coli.

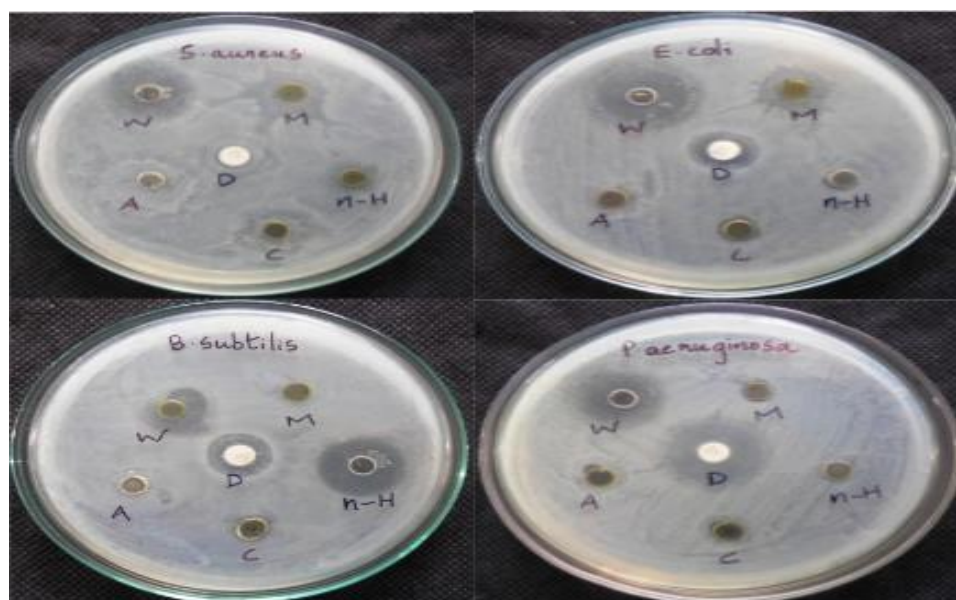


Fig.8. Antibacterial activity of *Ruta chalepensis.L* using extract

Table 4 Microorganisms used to study the antibacterial activity of *Ruta chalepensis.L*

<i>Ruta chalepensis</i> extracts	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
Aqueous	6	7	3	4
Methanol	4	4	NIL	1
n-Hexane	2	1	4	NIL
Chloroform	1	1	1	1
Acetone	NIL	1	1	NIL

Disc (Amoxicillin)	NIL	3	3	5
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4. Discussion

The phytochemical screening test helps in isolating and characterizing the chemical constituents present in the plant extracts and also gives us knowledge of the chemical constituents of plants. It is clear that the herbal drugs and their preparations are finally in discovering the actual value of folkloric remedies (21). In the present study, the phytochemical screening of *Ruta chalepensis* L. indicated the presence of glycosides, flavonoids, sterols and/or triterpenes, alkaloids, amino acids, resins, esters, and tannins (22). The variations between phytochemical groups in any plant can be used as promising support for the possible presence of biological activities were shown (12). Most of the alkaloids were obtained from leaves and stem extracts of Rue. Alkaloids are common constituents of other Rutaceae plants (23). The abundance of flavonoids, steroids, terpenoids, and saponins is similar to the components identified in the *Ruta chalepensis* were reported by (24).

In the present study, the DPPH test reveals that an increase in extract concentration results in an increase in radical scavenging activity in a dose-dependent manner. It was studied that extracts of *R. chalepensis* L reduce the radical DPPH when it reacts with the hydrogen ions released from the samples containing antioxidants were demonstrated (13). Moreover, rue extract obtained at 300C, 20% water, and for 90min exhibits the highest antioxidant activity among the invested extracts, whereas the lowest antioxidant activity was found in the case of rue extract obtained which has been previously determined. Meanwhile, rue extract obtained at 30°C, 20% water for 90 min, exhibited the highest antioxidant activity (72.53±0.31%) among the extracts studied, while the lowest antioxidant activity (57.54±0.15%) was extracted at 70°C, 27% water for 52 min; and in disagreement with who reported that at the concentration of 1000 µg/ml been used (25)

In the recent study, the reducing power assay of *Ruta* leaf and flower extracts demonstrates the highest FRAP. The result demonstrated the various *R. chalepensis* extracts exhibited a variable activity to reduce iron as a function of the plant part proven (26). It was found that the reducing power of aqueous and ethanol extracts increased with an increase in their concentrations. The aqueous extract, which contained the highest amount of total phenolics, showed a higher reducing power than the ethanol extract studied by (27). Whereas the Hexane, Chloroform, and aqueous extract showed a weak capacity to reduce iron with N200µg/ml were tested (28) . In another, study the methanol extract shows a total phenolic content of 1328.8 mg GAE/100gm been supported (29). Mostly the aqueous extract of *R. chalepensis* showed the highest phenolic content, whereas the ethanol with the highest flavonoid content. Unlike the ethanol and the aqueous extracts, the essential oil of *R. chalepensis* did not contain phenolic components after chemical analysis.

The highest phenolic content of *R. buxbaumii* were 54.10 ± 0.13 mg GAE/g extract in the EtOH extract and the lowest phenolic content of *R. buxbaumii* was 34.12 ± 1.2 mg GAE/g extract in the PE. The phenolic substance content of the EtOAc extract was 44. 25±0. 06 mg GAE/g extract and was statistically significant report given (27). The same author previously studied that the ethanol, aqueous and methanol extracts of *Ruta chalepensis* have 347.33 mg QE/g, 87.12 mg QE/g and 323.12 mg QE/g of total flavonoid respectively when Quercetin was taken as standard. Due to this, the before result reveals that the aqueous extract of *Ruta Chalepensis* has a less strong antioxidant. A recent study reveals that significant highest amounts (p <0.05) of phenols, flavonoids, and tannins were obtained from the methanol extracts, which explained that the use of methanol, as an organic solvent, is more efficient for the extraction of high amounts of phenolic compounds (30). Rutin, one of the flavonoid constituents of Rue, is well known to have nitric oxide scavenging activity were observed (31)

In the present study, the plant materials exhibit good antioxidant activity and reported that the presence of phenolic compounds in the medicinal plant enhances the various activity of antioxidant enzymes such as glutathione

peroxidase, superoxide dismutase, and catalyzes respectively. SOD is the defense mechanism in the antioxidant system against oxidative stress by catalyzing the dismutation of 2 superoxide radicals (O₂⁻) into molecular oxygen (O₂) and hydrogen peroxide (H₂O₂)(32). In such cases, extracts which are obtained from the medicinal plants could be useful for the protection of oxidative stress-related diseases. The percentage of inhibition of nitrite formation was determined using the SOD values have been determined (33) Moreover,(34) have clearly shown that *R. chalepensis* L extracts inhibit the LPS –induced nitric oxide production in BALB/c mice.

The TLC result of *Ruta chalepensis* revealed that the presence of various compounds such as alkaloids and phenolic compounds. In another study (35),the TLC analysis of methanol extract showed 6 bands under UV corresponding to various compounds. The methanolic extract of medicinal plants showed that the constituents that is responsible for anti- microbial activity reside more in methanolic extract, when compared to the other extracts were reported (36).

Recent studies of *R. chalepensis* L essential oils using GC-MS analysis revealed that ketones are the main class of constituents which accounts for 65.49 % of total compositions. Most of the extraction and analysis of the composition of *R. chalepensis* essential oils showed that the qualitative and quantitative differences based on the duration of extraction, the part of the plant, and the drying effect were revealed (37). Essential oils of aerial parts of *Ruta chalepensis* plants grown at different locations showed variations even in their major constituents. In another study, the *Ruta chalepensis* shows twenty constituents that represent more than 95.93% of the overall compositions of the essential oil have been identified, among which, six compounds were reported (38). The chromatography profiles of the essential oil from aerial parts of the plant revealed 20 different constituents, which represented 93.99-98.58 % of the total GC area for the essential oil were determined (39) Major essential oils obtained from *R. chalepensis* growing in different countries have shown very large differences in their major components (40).

Mostly the antibacterial studies of medicinal plants represent a rich source of antimicrobial agents. As synthetic and semi- synthetic antimicrobial drugs are abandoned in markets, there is a need for a continuous search for new ones to cope with the increased evolution of multiple antimicrobial- resistant strains of organisms were stated (41). The results suggest (42) that the presence of flavonoids, cardiac glycosides and tannins/phenolic compounds of these plants may contribute to their claimed antibacterial property in aqueous extracts.

Antimicrobial activity of the methanolic extract, stem of leaves, and roots was analyzed against *Pseudomonas* sp and *E.coli*. When the maximum zone of inhibition was observed for *P.aeruginosa*. Specific plant compounds such as anthraquinone and saponins have been proposed to have direct antibacterial activity and also reported in the case of *Aloe vera* were investigated (43). (44) showed that the tested medicinal plant has a less inhibiting effect against bacteria. The strains of microorganisms tested were resistant to the sample at 300µ/disc. A weekly activity was observed for the *S. typhi* and *B. subtilis* at 3mg/disc, while *B.cereus* was sensitive to all samples (3mg/disc) of species cultured in Tunisia, including *L.multifida* and *T.algeriensis* was described (45). Recently the methanolic extracts of different *R.chalepensis* parts have been tested against drug-resistant bacteria and pathogenic yeast, *E.coli*, *S.aureus*, and *P.aeruginosa*. From all *Ruta*, stem extracts were particularly more effective against *S.aureus* and *P.aeruginosa* exhibiting the highest antibacterial activity against *E.coli* (27) .

5.Conclusion

In this study the phytochemical analysis, antioxidant, TLC, GC-MS and antimicrobial activity of the leaves extract revealing the medicinal importance of *Ruta chalepensis*. *Ruta chalepensis* are rich in phytoconstituents such as alkaloids, flavonoids, tannins, steroids, quinines, phenols and saponin. Among the different extract aqueous extract was shown higher antioxidant activity. The bioactive compounds are characterized using TLC and GC-MS studies. Mainly presence of alkaloids and various bioactive compounds have potential antimicrobial, antifungal and antioxidant properties. The aqueous extract of the leaves of *Ruta chalepensis* possessed comparatively highest

antibacterial activity for *E.coli* and *B.subtilis*. Further study is required to quantify, isolate and characterize the biological activity of the particular plant.

Data Availability

The first author and corresponding author can supply all data used in the study.

Conflicts of interest

The authors declare that they have no competing interest.

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