Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/10886
DOI URL: http://dx.doi.org/10.21474/IJAR01/10886

*RESEARCH ARTICLE*

# COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *RUTA CHALEPENSIS* L. IN AL-BAHA AREA, SAUDI ARABIA

**Abdulaziz Yahya Al-Ghamdi**
Department of Biology, Faculty of Science, Al-Baha University, Al-Baha, Saudi Arabia.

## Manuscript Info

***Manuscript History***
Received: 20 February 2020
Final Accepted: 22 March 2020
Published: April 2020

***Key words:-***
Antimicrobial Activity, Essential Oil, GC/MS, *Ruta chalepensis*

## Abstract

*Ruta chalepensis* L. is an aromatic plant belonging to the family Rutaceae, widely distributed in Saudi Arabia. The study focuses on the extraction by steam distillation, phytochemical analysis, GC/MS analysis and antimicrobial activity of essential oil from the leaves of the plant. The results revealed the presence of flavonoids, terpenoids, steroids, and sugars at reasonable concentrations in ethanol, chloroform, ethyl acetate, petroleum ether and n-butanol extract of *R. chalepensis* leaves. GC/MS analysis identified twenty-eight compounds with the major compounds being 2-Tridecanone (38.88%), 2-Nonanone (29.56%), 2-Acetoxytridecane (18.58%), while 2-Pentadecanol acetate (3.42%), 2-Decanone (1.67%) and 2-Dodecanone (1.25%) were found in small concentrations and the other compounds were found in trace amounts of less than 1% for each. The antimicrobial activity determined by the disc diffusion method showed that the highest activity was observed against *Staphylococcus aureus* (inhibition zone of 20.33±0.151 mm), followed by *Escherichia coli* (inhibition zone of 16.67±0.158 mm), *Bacillus cereus* and *Candida albicans* (inhibition zones of 15.67±0.254 mm and 15.33±2.52 mm, respectively) and the lowest activity was against *Pseudomonas aeruginosa* (inhibition zone of 14.67±1.64 mm). The study, therefore, highlighted on the fact that extracted essential oil could be considered as a potential antimicrobial agent in therapeutic and pharmaceutical applications.



## Introduction:-

A large number of aromatic and medicinal plants have biological properties of potential applications in the pharmaceutical, medical, cosmetic and agribusiness industries (Boughendjioua, 2019). There is an increasing interest in the use of herbal and spices extracts of natural compounds in food preservation for their characteristic flavour and intermittent exhibition of antioxidant and antimicrobial activities (Ouerghemmi et al., 2017). Infectious diseases caused by bacteria, fungi, and viruses are a critical health hazard and are considered as one of the main causes of rising morbidity and mortality rates worldwide (Drusano, 2004). Medicinal plants have been the sole tool for physicians for several decades, and up to the present, most people, especially in developing countries, rely on medicinal plants (Amabye and Shalkh, 2015).

Plants of the family Rutaceae are a source of a wide variety of natural products with antibacterial, antifungal, antioxidant, spasmolytic, antihelmintic, emmenagogue, antitumor, analgesic, anti-inflammatory and antidepressant

**Corresponding Author:- Abdulaziz Yahya Al-Ghamdi**
Address:- Department of Biology, Faculty of Science, Al-Baha University, Al-Baha, Saudi Arabia.

activities (Raghav et al., 2006; Alotaibi et al., 2018). *R. chalepensis* L., commonly known as Rue or Finged rue, is a member of the family Rutaceae, a wild, perennial herbaceous shrub, widely distributed in the Mediterranean Sea regions, that grow on the rocky slopes of the mountains and has glabrous, alternately bipinnate leaves which have narrow oblong lanceolate segments and cymose inflorescences (Iauk et al., 2004; Jaradat et al., 2017). The herb is widely used for the treatment of rheumatism, fever, mental disorders, dropsy, neuralgia, menstrual issues, convulsions, and other bleeding and nervous disorders (Pollio et al., 2008; Ouerghemmi et al., 2017). The aerial parts of *R. chalepensis* contain alkaloids, phenols, flavonoids, amino acids and furocoumarins (Kacem et al., 2015).

Essential oils are the most important herb and plant compounds which are rich in hydrocarbon compounds with oxygenated, hydrogenated and dehydrogenated functional groups (Jaradat et al., 2017). Most of these chemicals are odorous mono-or sesquiterpenoids, being found in different parts of the plant and evaporate at normal temperatures (Jaradat et al., 2017). The biological activities of *R. chalepensis* may be associated with the presence of natural products such as alkaloids, coumarins, phenols, saponins, flavonoids, triterpenes and essential oils (Boudjema et al., 2018). These compounds are complex natural volatile liquids characterized by a strong odour, rarely coloured, soluble in lipid and organic solvents, synthesized by buds, bulbs, leaves, stems, twigs, seeds, fruits, roots, and wood or bark and deposited in secretary cell cavities, canals, epidermal cells or granular trichomes (Boudjema et al., 2018). Numerous studies tested the antimicrobial activity of *R. chalepensis* essential oil against Gram-negative, Gram-positive bacteria and fungi (Ben Bnina et al., 2010; Abdelwahab et al., 2011; Bouratoua et al., 2013; Orlanda and Nascimento, 2015; Jaradat et al., 2017; Boudjema et al., 2018; Oughendjioua, 2019). In this study, the antimicrobial activity and total phenolic content of *R. chalepensis* essential oil were investigated and the chemical composition was determined using GC/MS method.

## Materials and Methods:-

### Sample Collection:

*R. chalepensis* L**.** leaves were collected from Al-Baha area, Kingdom of Saudi Arabia and used to prepare the oil extracts. The plant was taxonomically identified and authenticated by Dr. Haidar Abd Algadir, Department of Biology, Faculty of Science, Al-Baha University, where the voucher specimen was deposited for future reference. The leaves were rinsed with freshwater to remove soil and dust particles and sliced into small pieces.

### Essential Oil Extraction:

The essential oil was extracted from fresh leaves (300 gm) by hydro-distillation using a Clevenger apparatus at 350°C for 4 hr (Majdoub et al., 2014). The extracted essential oil was dried over anhydrous sodium sulphate ($Na_2SO_4$) and further preserved at 4°C in sealed opaque bottles until analyzed (Boudjema et al., 2018).

### Preparation of Ethanolic Extract:

The fresh leaves of the plant were cleaned, dried under shade for 20 days and subsequently powdered. The ground material (500 g) was extracted with 80% ethanol and left for three days. The sample was then filtered and the collected filtrate was left to dry at room temperature for 10 days (Amabye and Shalkh, 2015). The extract was stored in a coloured bottle at 4-6°C till analysis.

### Fractionation of the Ethanolic Extract by Liquid–Liquid Extraction:

The ethanol extract was dissolved in 200 mL distilled water and further extracted with petroleum ether, chloroform, ethyl acetate, and n-butanol, respectively. After evaporation of the solvents, the solid extracts were collected and used for the purposes of phytochemical analysis and antimicrobial activity test (Amabye and Shalkh, 2015).

### Phytochemical Analysis:

Phytochemical analysis was carried out to determine the flavonoids, alkaloids, tannins, terpenoids, steroids, saponins and sugars contents of leaf extracts of ethanol, chloroform, ethyl acetate, petroleum ether, and n-butanol (Abioye et al., 2013).

### Analysis of Essential Oil by GC/MS:

Various essential oil components were identified and quantified by a gas chromatography/mass spectrophotometer (GC/MS-QP2010 ultra, Shimadzu Company, Japan). The sample (0.1 µL) was injected in split mode (a ratio of 1:10) into the capillary column (30 m x 0.25 mm x 0.25 µL film thickness). The instrument was operating in EI mode at 70eV. The procedure was carried out using helium as a carrier gas with a flow rate of 1.69 ml/min, and the oven temperature was programmed from 50°C at a rate of 7°C/min to 180°C and from 10°C/min to 280°C and then

kept for 28 min. The injection port temperature was 300°C. The temperature of the ion origin was 200°C, while the interface temperature was 250°C. An electron ionization device with a detector volt of 1.7 kV was used for GC/MS detection. The sample was analyzed using scan mode in the range of 40-500 m/z. Chemical constituents of the essential oils were identified by contrasting their MS with the reference spectra in the mass spectrometry data center of the National Institute of Standards and Technology (NIST) and their retention and Kovats indices were compared with the literature. Quantitative data were collected digitally from area percentages and combined peaks without the use of a correction factor (Boudjema et al., 2018).

**Antimicrobial Activity Test:**

**Microbial Organisms:**

Bacterial strains of *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), in addition to *Candida albicans* (ATCC 7596) were used in this study for antimicrobial activity test.

**Preparation of Bacterial Suspensions:**

Aliquots (1 ml) of a 24 hr bacterial broth culture were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hr. The bacterial growth was harvested and rinsed off with 100 ml sterile normal saline to produce a suspension containing about $10^8$-$10^9$cfu/ ml, which was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by a surface viable quantification technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were prepared in a sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates, which were allowed to stand for 2 hr at room temperature for the drops to evaporate and then incubated at 37°C for 24 hrs. After incubation, the number of developed colonies in each drop was counted, and the average number of colonies per drop (0.02 ml) was multiplied by 50 and the dilution factor to obtain the viable count of the stock suspension, expressed as cfu/ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that the suspensions with very close and accurate viable counts would be obtained.

**Preparation of Fungal Suspension:**

The culture of *C. albican* was grown on Sabouraud dextrose agar and incubated at 25°C for 4 days, then harvested, washed with sterile normal saline solution and stored in 100 ml sterile normal saline solution at 4°C till used.

**Agar Disc Diffusion Method:**

The disc diffusion method was utilized to test the antibacterial activity of the extracts on Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA) media (Boudjema et al., 2018). Bacterial and fungal suspensions were diluted with sterile physiological solution to $10^8$cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters (100 µL) of bacterial and fungal suspensions were swabbed uniformly on the surface of MHA and SDA and the inoculum was allowed to dry for 5 minutes. Sterile filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of MHA and SDA and soaked in 20 µl extracts. The inoculated plates were incubated at 37°C for 24 hr in an inverted position. After the incubation period, the antimicrobial activity was determined as diameter of inhibition zone as follows: <9 mm zone (resistant strain); 9-12 mm (partially sensitive strain);13-18 mm (sensitive strain); >18 mm (very sensitive strain).

**Statistical Analysis:**

The statistical analyses were performed using Statistical Analysis Systems (SAS, Ver. 9, SAS Institute Inc., Cary, NC, US) and the results were presented as the mean ± standard deviation (SD) of three replicates. All the data were statistically assessed using the General Linear Model (GLM) and the significant difference was performed using Duncan multiple range test at $P \leq 0.05$.

## Results and Discussion:-

**Phytochemical Analysis:**

The current study showed that *R. chalepensis* leaves extracted with ethanol, chloroform, ethyl acetate, petroleum ether, and n-butanol contain flavonoids, terpenoids, steroids and sugars at appropriate concentrations with the exception of tannins in petroleum ether extract and saponins in chloroform and petroleum ether extracts. Alkaloids are present in ethanol, chloroform, and petroleum ether extracts, while ethyl acetate and n-butanol extracts were not tested (Table 1). After screening, the crude extract of *R. chalepensis* leaves was reported to contain phytosterols,

steroids and tannins with green fluorescence, blue-green and yellow precipitate, respectively, while flavonoids were absent (Mohammed and Ahimad, 2015). Qualitative analysis of *R. chalepensis* leaves demonstrated the presence of alkaloids, flavonoids, coumarins, tannins and triterpenoids (Beatriz et al., 2010). Other studies conducted on the aerial parts of the plant reported the isolation of flavonoids, routin, furanocoumarins furoquinoline, quinolone, and acridone alkaloids (Shehadeh et al., 2007; Emam et al., 2009; Emam et al., 2010). Mejri et al. (2010) confirmed the existence of monoterpenes and ketone undecanone as major components of the essential oil extracted from the aerial parts of the plant.

**Chemical Composition:**

The essential oil was obtained by steam distillation of fresh leaves, and the chemical composition was analyzed by GC/MS, and the results are presented in Table 2 and Figure 1. Twenty-eight (28) components were reported with the main component being 2-Tridecanone (38.88%), 2-Nonanone (29.56%), 2-Acetoxytridecane (18.58%), 2-Pentadecanol acetate (3.42%), 2-Decanone (1.67%), and 2-Dodecanone (1.25%), while other components were detected in trace amounts of less than 1% each, and the components with lowest concentrations were Gamma-Terpinen (0.02%) and o-Cymene (0.01%). Literature surveys showed great variability which could be due to factors such as geographical location, season and environmental factors, and the method of extraction (Boughendjioua, 2019). The presence of 2-Undecanone as a dominant component in *R. chalepensis* leaf oil extract was also reported in Ethiopia (31.74%), Algeria (35.51%), Tunisia (72%) and Brazil (47.21%) (Ben Bnina et al., 2010; França Orlando and Nascimento, 2015; Defa et al., 2017; Boudjema et al., 2018), while 2-Nonanone was the substantial component in *R. chalepensis* oil extract from Algeria (32.79%), Chile (41.71%) and Jordan (37.13%) (Haddouchi et al., 2013; Al-Shuneigat et al., 2015; Tampe et al., 2016), and the components of Octylacetate (33.16%), 2-Dodecanone (40.15%) and Methanol (43.92%) were identified as major components of *R. chalepensis* oil extract from Tunisia and Algeria, respectively (Fakhfakh et al., 2012; Ghazghazi et al., 2015; Boughendjioua, 2019). The components of 2-Tridecanone (38.88%) and Acetoxytridecane (18.58%) which are detected in this study, were not identified in previous studies. Also, components of 2-Decanone (1.67%), Dodecanone (1.25%), Undecanone (0.26%) were identified as minor components.

**Antimicrobial Activity:**

The antimicrobial activity of *R. chalepensis* L. essential oil against Gram-negative (*E. coli, P. aeruginosa*), Gram-positive (*S. aureus, B. cereus*) bacteria and fungi (*C. albicans*) is presented in Table 3. The disc diffusion method is based on the measurement of the clear zone surrounding colonies due to inhibition of growth produced by a film disc containing the antimicrobial agent when in direct contact with a bacterial culture. There is a significant variation ($P<0.01$) in the antimicrobial activity of the essential oil against microorganisms tested, with the highest activity being observed against *S. aureus* (inhibition zone of 20.33±0.151 mm), *E. coli* (inhibition zone of 16.67±0.158 mm), *B. cereus* and *C. albicans* (inhibition zones of 15.67±0.254 mm and 15.33±2.52 mm, respectively), and the lowest activity was against *P. aeruginosa* (inhibition zone of 14.67±1.64 mm). These results are in agreement with Ben Bnina et al. (2010), who reported that *R. chalepensis* essential oil had very powerful antibacterial activity against all Gram-positive cocci and Gram-negative rods and that the activity against Gram-positive bacteria exceeded Gram-negative ones, and Chibani et al. (2013) who reported that the essential oil of *R. chalepensis* subsp. *angustifolia* inhibited the growth of *Klebsiella pneumoniae* ATCC, *Klebsiella pneumoniae* (HS), *E. coli* ATCC, *E. coli* (HS), *Streptococcus enterococcus* (HS) and *P. aeruginosa* ATCC strains with inhibition zones of 21, 21, 20, 20, 20 and 20 mm, respectively. These results, however, contradicted the findings of Haddouchi et al. (2013), who concluded that *R. chalepensis* var. *bracteosa* essential oils had low in vitro antibacterial activity against all 12 bacteria tested, and Ghazghazi et al. (2015) who stated that all the bacterial strains demonstrated some degree of resistance to the tested essential oil. Moreover, Boughendjioua (2019) indicated that *R. chalepensis* L. essential oil exhibited a moderate antimicrobial activity against all bacterial strains tested. Boudjema et al. (2018) reported that *R. chalepensis* essential oil reacts differently against all strains studied, and both yeast strains (*S. cerevisiae* and *C. albicans*) tended to be more sensitive than others, with inhibition zones ranging from 27 mm to 28 mm. Abdelwahab et al. (2011) reported that the essential oil extracted from *R. montana* (Clus.) L. prevented the growth of the tested microorganisms with the inhibition zone increasing proportionally with the concentrations of the tested samples. The inhibition zone varied from 9.33 to 24 mm with the highest inhibition activity being recorded against *S. aureus* at 2 mg/ml, and a considerable inhibition activity with the same concentration against *Klebsiela pneumoniae*. Franca Orlanda and Nascimento (2015) reported a wide variation in the antibacterial properties of essential oil against different bacteria, and the highest activity was observed against *B. cereus* and *S. aureus* (25.60±0.03 and 22.00±0.06 mm, respectively), moderate activity was against *M. flavus, E. coli*, *M. luteus*, *E. aerogenes* and *S. typhi*, and the lowest activity was against *P. aeruginosa* (8.30±0.05 mm). Jaradat et al. (2017)

reported that the essential oil of *R. chalepensis* from Jerusalem, Hebron, and Jenin regions of Palestine extracted by microwave-ultrasonic method demonstrated activity against the growth of all microbes studied. The highest antibacterial activity (lowest MIC) was for *R. chalepensis* essential oil from Jerusalem against *E. coli*, *P. aeruginosa* and *S. aureus*, while the highest antifungal activity was for *R. chalepensis* essential oil from Jenin region against *C. albicans* (Jaradat et al., 2017).

**Table 1:-** Phytochemical analysis of *Ruta chalepensis* leaf extracts.

| **Phytochemicals** | **Extract** | | | | |
|---|---|---|---|---|---|
| | **Ethanol** | **Chloroform** | **Ethyl acetate** | **n-butanol** | **Petroleum ether** |
| **Flavonoids** | + | + | + | ++ | + |
| **Alkaloids** | + | + | ND | ND | + |
| **Tannins** | ++ | + | +++ | + | - |
| **Terpenoids** | + | ++ | + | + | + |
| **Steroids** | + | ++ | + | + | + |
| **Saponins** | ++ | - | + | + | - |
| **Sugars** | + | + | ++ | + | + |

Key: +: Present; ++: Present; +++: Present; -: Absent; ND: Not determined

**Table 2:-** Essential oil components of *Ruta chalepennsis* leaves separated by GC/MS.

| **Compound No.** | **Compound name** | **Retention Time (min)** | **Peak area** | **Peak area (%)** |
|---|---|---|---|---|
| 1. | o-Cymene | 5.999 | 39145 | 0.01 |
| 2. | D-Limonene | 6.061 | 121976 | 0.05 |
| 3. | Acetic acid, 5-methylhex-2-yl ester | 6.278 | 242531 | 0.09 |
| 4. | Gamma -Terpinene | 6.640 | 43861 | 0.02 |
| 5. | 2-Nonanone | 7.308 | 78131930 | 29.56 |
| 6. | 2-Nonanol | 7.504 | 1407461 | 0.53 |
| 7. | 1-Methoxy-3-hydroxymethylheptane | 7.534 | 2258045 | 0.85 |
| 8. | 2-Octanol, acetate | 8.188 | 1696532 | 0.64 |
| 9. | Cyclohexene, 3,4-diethenyl-3-methyl- | 8.294 | 608746 | 0.23 |
| 10. | Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- | 8.404 | 223106 | 0.08 |
| 11. | 2-Decanone | 9.300 | 4410040 | 1.67 |
| 12. | 2-Acetoxytridecane | 10.128 | 49120944 | 18.58 |
| 13. | 2-Undecanone | 10.573 | 699461 | 0.26 |
| 14. | 2-Tridecanone | 11.305 | 102716213 | 38.88 |
| 15. | 2-Undecanol | 11.458 | 1741475 | 0.66 |
| 16. | Acetic acid, nonyl ester | 11.579 | 952519 | 0.36 |
| 17. | 2-Acetoxytetradecane | 12.013 | 524954 | 0.20 |
| 18. | 2-Dodecanone | 12.627 | 3307981 | 1.25 |
| 19. | 2-Pentadecanone | 13.196 | 797573 | 0.30 |
| 20. | 2-Pentadecanol acetate | 13.812 | 9037180 | 3.42 |
| 21. | 2-Tridecanone | 15.033 | 761625 | 0.29 |
| 22. | Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]- | 16.019 | 846901 | 0.32 |
| 23. | 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.(R*),5.alpha.]]- | 16.595 | 354274 | 0.13 |
| 24. | 3-Heptenoic acid, 7-phenyl-, ethyl ester, (E)- | 18.115 | 596389 | 0.23 |
| 25. | 5-[3,4-Methylenedioxybenzyl]hydantoin | 20.107 | 1447874 | 0.55 |
| 26. | 1,6-Anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose | 22.898 | 682936 | 0.26 |
| 27. | Phytol | 23.447 | 80228 | 0.03 |
| 28. | 1H-Xanthen-1-one, 2,3,4,9-tetrahydro-9-(2- | 24.269 | 1461361 | 0.55 |

| | | | | |
|---|---|---|---|---|
| | hydroxy-6-oxo-1-cyclohexenyl)- | | | |
| | Total | - | - | 100 |

**Table 3:-** Antimicrobial activity of *Ruta chalepensis* leaf oil extract by agar disc diffusion assay at concentration of 100 mg/ml.

| **Microorganisms** | **Inhibition zone (mm)** |
|---|---|
| A. **Gram negative** | |
| *Escherichia coli* | 16.67±0.158[b] |
| *Pseudomonas aeruginosa* | 14.67±0.164[b] |
| B. **Gram positive** | |
| *Staphylococcus aureus* | 20.33±0.151[a] |
| *Bacillus cereus* | 15.67±0.254[b] |
| C. **Fungus** | |
| *Candida albicans* | 15.33±2.52[b] |
| **SL** | ** |

The data are presented as mean ±standard deviation (n=3)
Means in each column bearing similar superscripts are not significantly different (P>0.05)
**= P<0.01
SL = Significance level

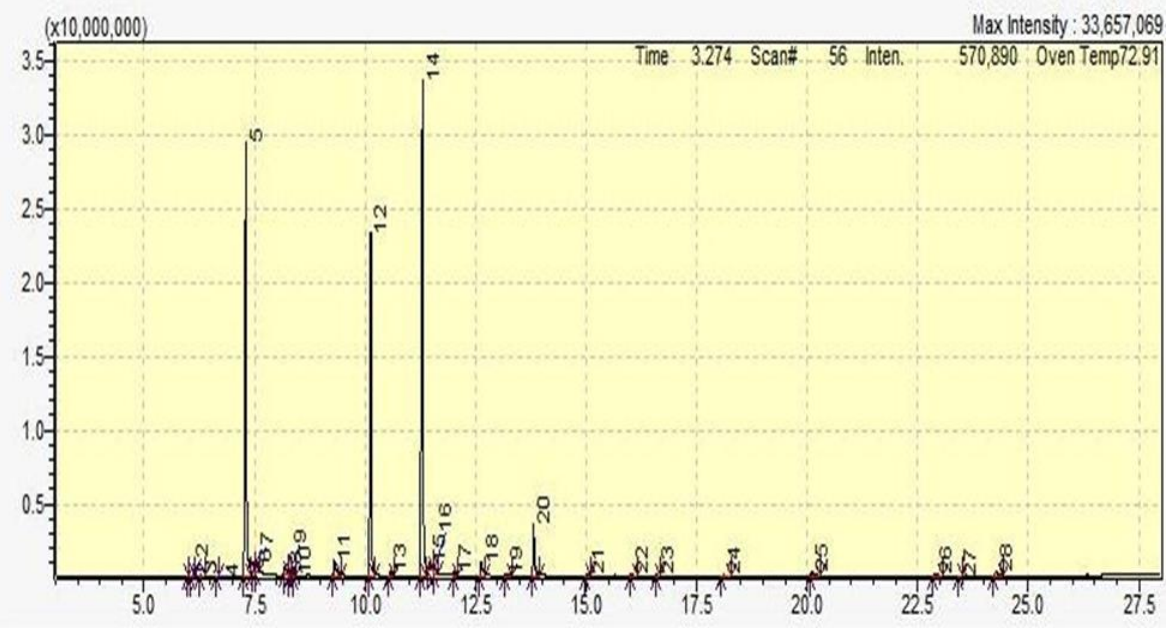


**Figure 1:-** GC/MS chromatogram of essential oil obtained from *Ruta chalepennsis* leaves.

## Conclusion:-

*R. chalepensis* leaves have been shown to possess reasonable concentrations of flavonoids, terpenoids, steroids, and sugars when extracted with ethanol, chloroform, ethyl acetate, petroleum ether, and n-butanol. The qualitative and quantitative analysis of GC/MS oil extract identified twenty-eight (28) compounds with an abundance of 2-Tridecanone, 2-Nonanone, and 2-Acetoxytridecane. The essential oil had an antimicrobial effect against *S. aureus*, *E. coli*, *B. cereus*, *C. albicans*, and *P. aeruginosa*. To the best of my knowledge, this investigation was the first endeavour to study this species in Al-Baha area, and the findings of both the chemical constituents and the antimicrobial activity varied substantially from those of the earlier studies.

## Acknowledgment:-

The author would like to acknowledge University of Al-Baha in providing financial support.

**Disclosure Statement:**
The author declares that there is no conflict of interest related to this article.

## References:-


1. Abdelwahab, B., Amar, Z., Noureddine, G., Mesbah, L. and Salah, R. (2011): Essential oil composition of Algerian Ruta montana (Clus.) L. and its antibacterial effects on microorganisms responsible for respiratory infections. Adv. Nat. Appl. Sci., 5(3): 264-268.
2. Abioye, E.O., Akinpelu, D.A., Aiyegoro, O.A., Adegboye, M.F., Oni, M.O. and Okoh, A.I. (2013): Preliminary phytochemical screening and antibacterial properties of crude stem bark extracts and fractions of Parkia biglobosa (Jacq.). Molecules, 18: 8485-8499.
3. Alotaibi, S.M., Saleem, M.S. and Al-humaidi, J.G. (2018): Phytochemical contents and biological evaluation of Ruta chalepennsis L. growing in Saudi Arabia. Saudi Pharm. J., 26: 504–508.
4. Al-Shuneigat, J.M., Al-Tarawneh, I.N., Al-Qudah, M.A., Al-Sarayreh, S.A., Al-Saraireh, Y.M. and Alsharafa, K.Y. (2015): The chemical composition and the antibacterial properties of Ruta graveolens L. essential oil grown in northern Jordan. Jordan J. Biol. Sci., 8(2):139-143.
5. Amabye, T.G. and Shalkh, T.M. (2015): Phytochemical screening and evaluation of antibacterial activity of Ruta graveolens L. a medicinal plant grown around Mekelle, Tigray, Ethiopia. Nat. Prod. Chem. Res., 3 (6):1-4.
6. Beatriz, V.C., Natalia, S.L., Ariel, R.B. and David, S.C. (2010): Antihypertensive activity of Ruta chalepensis L. leaves. Pharmacologyonline, 3: 354-363.
7. Ben Bnina, E., Hammami, S., Daamii-remadi, M., Ben Jannet, H. and Mighri, Z. (2010): Chemical composition and antimicrobial effects of Tunisian Ruta chalepensis L. essential pols. J. Soc. Chim. Tunisie, 12: 1-9.
8. Boudjema, K., Mouhouche, A., Guerdouba, A. and Hali, L. (2018): Composition, phytochemical analysis, antimicrobial and anti-inflammatory activities of the essential oils obtained from Ruta chalepensis L. growing wild in northern of Algeria. J. Chem. Soc. Pak., 40:1054-1062.
9. Boughendjioua, H. (2019): Yield, chemical composition and antibacterial activity of Ruta chalepensis L. essential oil growing spontaneously in Algeria. Pharm. Pharmacol. Int. J., 7(1):33-36.
10. Chibani, S., Bouratoua, A., Kabouche, A., Laggoune, S., Semra, Z., Smati, F. and Kabouche, Z. (2013): Composition and antibacterial activity of the essential oil of Ruta chalepensis subsp.angustifolia from Algeria. Der. Pharmacia. Lettre., 5 (5):252-255.
11. Defa, K., Shiferaw, G. and Feleke, S. (2007): Total phenolic compound, antioxidant activity of cultivated Ethiopian Ruta chalepensis crude extract and its essential oils. Int. J. Basic Appl. Sci., 6 (3): 83-91.
12. Drusano, G.L. (2004): Antimicrobial pharmacodynamics: critical interactions of ‘‘bug and drug”. Nat. Rev. Microbiol., 2: 289–300.
13. Emam, A.M., Swelam, E.S. and Megally, N.Y. (2009): Furocoumarin and quinolone alkaloid with larvicidal and antifeedant activities isolated from Ruta chalepensis leaves. J. Nat. Prod., 2: 10-22.
14. Emam, A., Eweis, M. and Elbadry, M. (2010): A new furoquinoline alkaloid with antifungal activity from the leaves of Ruta chalepensis L. Drug Discov. Ther., 4: 399-404.
15. Fakhfakh, N., Zouari, S., Zouari, M., Loussayef, C. and Zouari, N. (2012): Chemical composition of volatile compounds and antioxidant activities of essential oil, aqueous and ethanol extracts of wild Tunisian Ruta chalepensis L. (Rutaceae). J. Med. Plants Res., 6(4): 593-600.
16. França Orlanda, J.F. and Nascimento, A.R. (2015): Chemical composition and antibacterial activity of Ruta graveolens L. (Rutaceae) volatile oils, from São Luís, Maranhão, Brazil. South Afr. J. Bot., 99: 103–106.
17. Ghazghazi, H., Aouadhi, C., Weslati, M., Trakhna, F., Maaroufi, A. and Hasnaoui, B. (2015): Chemical composition of Ruta chalepensis leaves essential oil and variation in biological activities between leaves, stems and roots methanolic extracts. TEOP, 18(3): 570-581.
18. Haddouchi, F., Chaouche, T.M., Zaouali, Y., Ksouri, R., Attou, A. and Benmansour, A. (2013): Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria. Food Chem., 141: 253–258.
19. Iauk, L., Mangano, K., Rapisarda, A., Ragusa, S, Maiolino, L., Musumeci, R., Costanzo, R., Serra, A. and Speciale, A.(2004): Protection against murine endotoxemia by treatment with Ruta chalepensis L., a plant with anti-inflammatory properties,”. J. Ethnopharmacol., 90 (2-3): 267–272.
20. Jaradat, N., Adwan, L., Kaibni, S., Zaid, A.N., Shtaya, M.J.Y., Shraim, N. and Assali, M. (2017): Variability of chemical compositions and antimicrobial and antioxidant activities of Ruta chalepensis leaf essential oils from three Palestinian regions. BioMed. Res. Int., Volume 2017, Article ID 2672689, 9 pages.

21. Kacem, M., Kacem, I., Simon, G., Ben Mansour, A., Chaabouni, S., Elfeki, A. and Bouaziz, M. (2015): Phytochemicals and biological activities of Ruta chalepensis L. growing in Tunisia. Food Biosci., 12(1):73-83.
22. Majdoub, O., Dhen, N. and Souguir, S. (2014): Chemical Composition of Ruta chalepensis Essential Oils and their Insecticidal Activity against Tribolium castaneum. Tunis. J. Plant Prot., 9(1): 83-90.
23. Mejri, J., Abderrabba, M. and Mejri, M. (2010): Chemical composition of the essential oil of Ruta chalepensis L: Influence of drying, hydro-distillation duration and plant parts. Ind. Crops Prod., 32:671-673.
24. Miles, A.A. and Misra, S.S. (1938): The estimation of the bactericidal power of the
25. blood. J. Hyg. (London), 38(6): 732–749.
26. Mohammed, S. and Ahimad, A. (2015): Extraction and phytochemical detection of some selected traditional medicinal plants for antimicrobial susceptibility test, in Adama, Ethiopia. Int. J. Sci. Eng. Technol., 3 (5): 1290-1297.
27. Ouerghemmi, I., Rebey, I.B., Rahali, F.Z., Bourgou, S., Pistelli, L., Ksouri, R., Marzouk, B. and Tounsi, M.S. (2017): Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian Ruta chalepensis. J. Food Drug Anal., 25:350-359.
28. Pollio, A., De Natale, A., Appetiti, E., Aliotta, G. and Touwaide, A. (2008): Continuity and change in the Mediterranean medical tradition: Ruta spp. (Rutaceae) in Hippocratic medicine and present practices. J. Ethnopharmacol., 116:469–482.
29. Raghav, S.K., Gupta, B., Agrawal, C., Goswami, K. and Das, H.R. (2006): Anti-inflammatory effect of Ruta graveolensL. in murine macrophage cells. J. Ethnopharmacol., 104(1-2): 234–239.
30. Shehadeh, M.B., Afifi, F.U. and Abu-Hamdah, S.M.(2007): Platelet aggregation inhibition from aerial parts of Ruta chalepensis grown in Jordan. Integr. Med. Ins., 2: 35-39.
31. Tampe, J., Parra, L., Huaiquil, K. and Quiroz, A. (2016): Potential repellent activity of the essential oil of Ruta chalepensis (Linnaeus) from Chile against Aegorhinus superciliosus (Guérin) (Coleoptera: Curculionidae). J. Soil Sci. Plant Nutr.,16 (1): 48-59.