JJBS

Volume 13, Supplementary Issue, December 2020
ISSN 1995-6673
Pages 675 - 680

Jordan Journal of Biological Sciences

Total Phenolic Content, Antioxidant and Antimicrobial Activity of *Ruta chalepensis* L. Leaf Extract in Al-Baha Area, Saudi Arabia

Abdulaziz Y. Al-Ghamdi[1], Abdelfattah A. Fadlelmula[2], and Mohamed O.M. Abdalla[3,*]

[1] *Department of Biology, Faculty of Science, Al-Baha 61008,* [2] *Department of Chemistry,* [3] *Department of Biology, Faculty of Science and Arts, Al-Makhwah 65931, Al-Baha University, Saudi Arabia*

Received: February 7 2020; Revised: February 28, 2020; Accepted: April 16, 2020

Abstract

This study was conducted to evaluate the phenolic content, antioxidant activity and antimicrobial activity of *Ruta chalepensis* L. leaf extract grown in Al-Baha area, Saudi Arabia. The collected fresh leaves were air-dried, powdered, and extracted with ethanol, petroleum ether, chloroform, ethyl acetate and n-butanol. The total phenolic content, antioxidant activity and antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus* and *Candida albicans* were determined. The results indicated the maximum total phenolic content in n-butanol extract (2580.47 mg/gm) and the minimum in chloroform extract (18.93 mg/gm), while the antioxidant activity ranged between 34.83% in ethanol extract and 88.13% in n-butanol extract. The ethanolic extract showed antibacterial activity against *P. aeruginosa* and *S. aureus* (10 mm inhibition zone), while *B. cereus* was resistant (5 mm inhibition zone), and the petroleum ether, chloroform and ethyl acetate extracts showed no antibacterial activity, whereas n-butanol extract was active against *B. cereus* (10 mm inhibition zone). All extracts except ethanol showed antifungal activity against *Candida albicans* (10 mm inhibition zone). There is a large number of phenolic compounds that exhibit antibacterial effect, which are synthesized by various plants including many medicinal plant species employed in traditional medicine. The biological activity of the plant depends on factors such as plant part, geographical source, soil conditions, harvest time, moisture and post-harvest methods. The study concluded that the leaf extract of *Ruta chalepensis* has high total phenolic content and antioxidant activity in addition to moderate antimicrobial activity, therefore this plant can be useful as a medicinal plant.

Keywords: *Ruta chalepensis*, leaf extract, phenolic content, antioxidant activity, antimicrobial activity

1. Introduction

Herbs attracted the industries of biotechnology, cosmetics, pharmaceutical, and food as an ancient source of medicine, flavouring, beverages, dyeing, fragrances, and cosmetics (Zaidi and Dahiya, 2015). The use of plants and plant products as medicinal products could be traced back to the beginning of human civilization (Bharathi *et al*., 2014). Natural substances with antimicrobial and antioxidant properties from essential oils and plant extracts supplied as food components or specialized pharmaceuticals to human are increasingly of interest (Shafique *et al.*, 2011). Most natural plants containing a wide variety of phytochemical constituents are a major source of antioxidants that affect the decrease in the possible stress caused by reactive oxygen species. Natural antioxidants may have free-radical scavengers, reduction agents, possible pro-oxidant metal complexes, and single-single-oxygen quenches (Basoudan *et al.*, 2019). Medicinal plants and plant-based products that contain a wide variety of free radical scavenging molecules such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites are rich in antioxidant activity (Zheng and Wang, 2001; Cai *et al.*, 2003). The frequent usage of the common antibiotics and therapeutic compounds for the prevention of disease-causing pathogens lead to the emergence of microbial resistance to antibiotics, which caused side effects such as immune suppression, allergic reactions and hypersensitivity reactions respectively, therefore, there is an urgent need for the invention of novel molecules with fewer side effects (Almalki, 2017).

Ruta chalepensis, as a member of the family *Rutaceae*, is used for treating a variety of diseases in traditional medicine in many countries (Pollio *et al.*, 2008). It is an important medicinal plant well recognized in the Mediterranean area as well as in some temperate and tropical countries, and well known for its strong biological activities (Kacem *et al*., 2015). This perennial herb was found to possess potent antimicrobial activities against Gram-positive and Gram-negative bacteria, in conjunction with the presence of phenolic compounds (Kacem *et al.*, 2015; Amdouni *et al.*, 2016). The leaves and young stems of *R. chalepensis* have been reported to contain alkaloids, flavonoids, phenols, amino acids, furanocoumarins, and

* Corresponding author e-mail: abutahany@yahoo.com.

saponins (Cowan, 1999; Alemayehu *et al.*, 2019). The biological activities of *R. chalepensis* are frequently utilized in herbal therapy and the plant is used as a promoter of menstruation, treatment of hypertension, a topical treatment for ear aches and headaches, and an as external treatment in the form of a skin antiseptic and insect repellent (Guarrera, 1999; Steenkamp, 2003), and these plants are a source of huge variety of natural products with antibacterial, antifungal, antioxidant, spasmolytic, antihelmintic, emmenagogue, antitumoral, analgesic, anti-inflammatory, and antidepressant activities (Raghav *et al*., 2006). Rue's active ingredients have antifungal and insecticidal properties that could prove beneficial to agriculture as well (Emam et al., 2010). In Saudi Arabia, a decoction of its aerial parts is used as an analgesic and antipyretic and for the treatment of rheumatism and mental disorders (Iauk *et al*., 2004).

The Kingdom of Saudi Arabia is gifted with a wide range of flora consisting of a large number of medicinal herbs, shrubs, and trees (El-shabasy, 2016). The study was conducted to evaluate the phenolic content and antioxidant activity of different extracts of the leaves of *R. chalepensis* and to ensure its pharmaceutical value as it was used as a medicinal plant traditionally.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh leaves of *R. chalepensis* were collected from Al-Baha area, Kingdom of Saudi Arabia. The leaves were washed with fresh water to remove the soil and dust particles, and subjected to air-drying under shade for three weeks until they were completely dried, then ground into fine particles using an electric grinder.

2.2. Preparation of Ethanolic Extract of Samples

Two hundred grams (200 gm) of powdered sample were weighed into a clean dried flask or beaker (2-3 L size). Two-thousand milliliters (2000 ml) of 80% ethanol (800 ml ethanol+200 ml distilled water) were added. The mixture was well mixed and soaked for 2 days at room temperature. The mixture was then filtered with filter paper (Whatman no. 4). This procedure was repeated three times to ensure that all contents were extracted with ethanol. The filtrates were collected and allowed to air dry for 10 days, and the extract was stored in a coloured bottle at 4-6°C till analysis.

2.3. Fractionation of the ethanolic extract by liquid–liquid extraction

The ethyl alcohol extract was soaked in 200 mL distilled water and extracted consecutively using different solvents (petroleum ether, chloroform, ethyl acetate, and *n*-butanol, respectively) for 7 days at room temperature to produce extracts. The solvents are chosen depending on their polarity difference. The filtrate was dried by using a rotary evaporator at room temperature, and stored at 4-6°C till used.

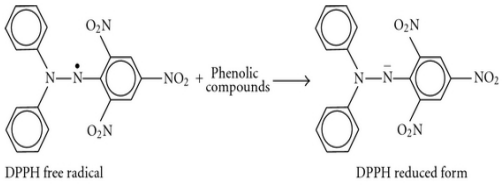
2.4. Total Phenolic Content (TPC)

The concentration of phenolics in the leaves extracts was determined using a spectrophotometric method (Singleton *et al*., 1999), with some modifications. Sample solutions of the ethanolic extracts in the concentration of 1 mg/ml were used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of sample solutions of fractions, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated at 30°C for 90 min. The absorbance was determined using a spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/gm of extract).

2.5. Total Antioxidant Activity

2.5.1. Free Radical Scavenging Assay

The antioxidant assay used was based on the scavenging ability of the antioxidant (s) in plant extracts towards the stable free radical 1,1-diphenyl-2-picrylhydrazile (DPPH), which is deep purple, to form the corresponding hydrazine with the accompanying change of colour to light purple or golden yellow.



2.5.2. Free radical Scavenging Procedure

This method was carried out according to Shyur *et al.* (2005) with some modifications. Stock solution was prepared by dissolving 1mg of the sample in 1ml of absolute ethanol (98%). The stock solution was diluted to final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 µg/ml in ethanol. 0.9ml of Tris-HCl and 1ml of 0.1 mM DPPH in methanol solution were added to each concentration and incubated at room temperature in the dark for 30 minutes. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity using the formula below:-

Scavenging activity (DPPH scavenged) (%) = $\frac{(Ac - As)}{Ac} \times 100$

Where: Ac= Absorbance of control; As = Absorbance in the presence of the sample of extract

A solution of 0.9 ml Tris-HCl+ 0.1ml absolute ethanol+ 1ml absolute ethanol was used as a blank, while solution of 0.9 ml tris-HCl+0.1ml absolute ethanol+1ml DPPH was used as a control. Freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance at 517 nm. The purple colour disappears when an antioxidant is present in the medium. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of compound to act as a free radical scavenger.

2.6. Antimicrobial activity test

2.6.1. 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 a fungal strain *Candida albicans* (ATCC 7596) were used in this study for antimicrobial activity test.

2.6.2. Preparation of bacterial suspensions

Aliquots (1 ml) of a 24 hr broth culture of the bacteria were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 h. The bacterial growth was harvested and washed off with 100 ml sterile normal saline to produce a suspension containing about 10^8- 10^9cfu/ ml, which was stored at 4°C till used. The average number of viable organisms/ml of the stock suspension was determined employing the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made 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 h at room temperature for the drops to dry and then incubated at 37°C for 24 h. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and the dilution factor to get the viable count of the stock suspension, expressed as colony forming units (cfu)/ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.6.3. Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 ml sterile normal saline and stored at 4°C until used.

2.6.4. Agar disc diffusion

The disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA). The experiment was carried out according to Boudjema *et al.* (2018). Bacterial and fungal suspensions were diluted with a sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial and fungal suspensions were swabbed uniformly on the surface of MHA and SDA and the inoculum was allowed to dry for 5 min. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of MHA and SDA and soaked with 20 µl of a solution of each plant extract. The inoculated plates were incubated at 37°C for 24 h in an inverted position. After incubation, the antimicrobial activity was determined by measuring the diameter of the inhibition zone surrounding each disc. The antimicrobial activity results were expressed in terms of the diameter of the 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).

2.7. 2.7. Statistical Analysis

The statistical analyses were performed using Statistical Analysis Systems (SAS, Ver. 9, SAS Institute Inc., Cary, NC, USA) 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 $P \leq 0.05$.

3. Results and Discussion

3.1. Total phenolic content (TPC)

From results in Table 1, TPC of *R. chalepensis* leaf extract is significantly ($P<0.001$) higher in n-butanol extract (2580.47mg/gm) followed by ethanol (944.63 mg/gm), petroleum ether (720.93 mg/gm), ethyl acetate (717.13 mg/gm) and lower in chloroform extract (18.93 mg/gm). Total phenolic content is affected by the polarity of the solvent used for extraction which is being maximum in polar solvents and minimum in non-polar solvents, therefore TPC of *R. chalepensis* is polar and extracted well with polar solvents. Ereifej *et al*. (2015) extracted TPC using methanol at 60°C and reported that the results depict that all plants contain appreciable amounts of phenolic compounds and that TPC of *R. chalepensis* leaves/flowers extracted with methanol was 1328.8 mg GAE/100gm extract. Amdouni *et al*. (2016) reported that the pattern and degree of total phenolic concentration (TPC) differed with the organ type and treatment. In all circumstances, TPC was much higher in leaves and stems than in roots. These results are in agreement with the findings of Defa *et al.* (2017) who reported that TPC in *R. chalepensis* is polar and highly extracted by the polar solvents (like dissolves like). Ouerghemmi *et al*. (2017) informed that the total phenolic content was more organ- than provenance-dependent and varied 0.2-168.91 mg of GAE/gm dry weight. Pavi´c *et al*. (2019) established that the highest TPC (38.0±0.11 mg GAE/gm dry matter) was recorded in the rue extract obtained at 30°C, 20% water for 90 min, whereas the lowest content (28.84±1.96 mg GAE/gm dry matter) was found in rue extract obtained at 30°C, 20% water for 30 minutes.

Table1. The percentage of total phenolic content of *Ruta chalepensis* leaf extracted by different solvents

Extract	Total phenolic content (mg/gm)
Ethanol	944.63 ± 71.42^{c}
Petroleum ether	720.93 ± 88.31^{b}
Chloroform	18.93 ± 10.84^{d}
Ethyl acetate	717.13 ± 258.57^{b}
n-butanol	2580.47 ± 175.59^{a}

The values are presented as the mean ± the standard deviation (n = 3). Mean values with different letters within a column are significantly different ($p<0.001$).

3.2. Total antioxidant activity

The degree of discolouration of the deep purple colour of DPPH radical, as it is reduced, indicates the radical scavenging potential of the antioxidant (Singh *et al*., 2002). The DPPH radical scavenging effects of extracts of *R. chalepensis* in different solvent systems are shown in Table 2. The scavenging effect ranged between 34.83±11.98% in ethanol extract and 88.13±2.46% in n-

butanol extract (P<0.001), with the scavenging effect decreasing in the order of n-butanol>ethyl acetate>petroleum ether>chloroform>ethanol. Amdouni *et al*. (2016) outlined that the total antioxidant capacity of *R. chalepensis* was higher in leaves (13 mg GAE g-1 DW) than in stems (11 mg GAE g-1 DW) and roots (10 mg GAE / gm DW) and reached its maximum under salt stress (24.26 mg GAE / gm DW) in leaves of plants treated with 100 mM NaCl, followed by stems (17 mg GAE / gm DW) and roots (16 mg GAE/gm DW). The IC_{50} values showed that the ethyl acetate IC_{50} was 0.00165 µg/ml, whereas the n-butanol IC_{50} was 0.000299 µg/ml, while for the rest of solvents the test was not carried out, suggesting that the higher the antioxidant activity value, the lower the IC_{50} value (Table 2). Such findings are lower than those reported by Amdouni *et al*. (2016) for *R. chalepensis* leaf extract (IC_{50} = 0.097 µg/mL) and Ben Sghaier *et al*. (2018) for which IC_{50} was 54.1±1.5 µg/ml for methanol extract compared to aqueous extract (IC_{50}=73.6±2.6 µg/ml). Ouerghemmi *et al.* (2017) recorded 189.61 mg GAE/gm DW and 172.63 61 mg GAE/gm DW for spontaneous and cultivated *Ruta* leaves, respectively, and 169.6 mg GAE / gm DW and 161.36 mg GAE / gm DW for spontaneous and cultivated *Ruta* flowers, respectively. Alotaibi *et al*. (2018) recorded the best antioxidant activity of *R. chalepensis* to be obtained by D2 (ethyl acetate and n-butanol) extract (94.28%, IC_{50}=56.6 mg/ml), while ethanol and D1 (ether and chloroform) extracts displayed an antioxidant activity of 87.51%, IC_{50}=320.7 mg/ml, and 80.37%, IC_{50}=414.9 mg/ml, respectively, and all extracts had very good antioxidant activity. Similar results of 68.23±0.89 were reported by Basoudan *et al*. (2019). These results are consistent with the findings of Pavi´c *et al*. (2019) who reported that 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 in rutin extracted at 70°C, 27% water for 52 min; and in disagreement with Alemayehu *et al*. (2019) who reported that at the concentration of 1000 µg/ml used, the scavenging effect of *R. chalepensis* extracts on the DPPH radical decreased in the order of methanol>acetone>ethyl acetate>n-hexane (93.851±0.148%>78.937±0.961% > 76.590±0.131% > 64.499±0.679, respectively).

Table 2. DPPH radical scavenging activity (%) of leaves extracts of *Ruta chalepensis* leaf extracted with different solvents

Extract	Radical scavenging concentration (%)	IC_{50}
Ethanol	34.83±11.98^{c}	ND
Petroleum ether	43.53±2.64b^{c}	ND
Chloroform	43.10±1.51b^{c}	ND
Ethyl acetate	52.17±4.54^{b}	0.00165
n-butanol	88.13±2.46^{a}	0.000299

The values are presented as the mean ± the standard deviation (n = 3). Mean values with different letters within a column are significantly different (p<0.001).

ND = Not determined

3.3. Antimicrobial activity

Medicinal plants have become part of alternative medicines worldwide because of their potential health benefits and can be consumed or directly used to treat infections (Gloria-Garza *et al*., 2013). The biological activity of the plant depends on many factors, such as plant part, geographical source, soil conditions, harvest time, moisture and post-harvest methods (Gloria-Garza *et al*., 2013). High temperatures during tissue grinding may denature certain chemical constituents. For the maximum recovery of bioactive compounds, different concentrations of solvents or different solvents alone or combinations are used, because different plants constitute different compositions of active compounds (Amabye and Shalkh, 2015). The leaves of *R. chalepensis* extracted with different solvents were tested for antimicrobial activity using agar disc diffusion method. The findings reported in Table 3 showed that the ethanolic extract demonstrated antibacterial activity against *P. aeruginosa* and *S. aureus* with inhibition zones of 10 mm, while *B. cereus* was resistant (5 mm inhibition zone). Petroleum ether, chloroform, and ethyl acetate extracts displayed no antibacterial activity against all of the bacteria studied, and only n-butanol extract was active against *B. cereus* (inhibition zone of 10 mm). Our results are consistent with the findings of Merghache *et al*. (2008) who reported the essential oil in the aerial part of *R. chalepensis* to be unsuccessful in the inactivation of *E. coli*, *P. aeruginosa*, *S. aureus* and *L. monocytogens*; and in disagreement with the findings of Alemayehu *et al*. (2019) who reported that methanol, acetone, hexane, and ethyl acetate extracts possess antimicrobial activity against *B. cereus*, *S. aureus*, *S. typhi* and *E. coli* with ≥ 11 mm mean zone of inhibition, and not in line with Ouerghemmi *et al*. (2017) who found that wild and cultivated *Ruta* organ extracts exhibited marked antibacterial activity, with spontaneous *Ruta* stem extracts being effective against *S. aureus* and *P. aeruginosa* (16.3 mm and 17.7 mm inhibition zone, respectively) and the cultivated *Ruta* stem extracts showed the highest antibacterial activity against *E. coli*. The methanol extract of *R. chalepensis* leaf at 5 mg/disk displayed a potent inhibiting activity against *C. perfringens* and *E. coli*, and no or poor activity against *Bifidobacterium bifidum*, *Bifidobacterium longum*, *L. acidophilus*, and *L. casei*, while the chloroform extract exhibited a potent activity against *C. perfringens* and *E. coli*, whereas butanol and water extracts showed no activity (Jang-Hee *et al*., 2005). *R. chalepensis* methanol extract showed MICs of 250 µg/ml and 3.9 µg/ml, and induced a maximum of 63% and 94% growth inhibition against *S. mutans*, as measured by the MTT reduction and CFU methods, respectively, whereas the vehicle control and medium alone (both free from plant extract) did not alter bacterial growth (Gloria-Garza *et al*., 2013). Babu Kasimala *et al*. (2014) found that the acetone extract of *R. chalepensis* showed maximum inhibition (8.5 mm) against Gram negative bacteria, while ethanol extract showed maximum inhibition against Gram positive bacteria (8 mm). The inhibitory effect of plant extracts against bacterial pathogens was due to their phenolic content and the inhibitory effect of phenolic compounds could be explained by cell membrane adsorption, enzyme interaction, substrate and metal ion deprivation (Ouerghemmi *et al*., 2017). Alotaibi *et al*. (2018) reported that except for *Proteus vulgaris* and *P. aeruginosa*, the obtained results revealed that all *R. chalepensis* solvent extracts (ethanol and collected successive extracts of ether and chloroform, ethyl acetate and n-butanol, respectively) possessed antibacterial activity against all Gram-negative

and Gram-positive bacteria under investigation, with the activity being variable according to the solvent. Tedila *et al*. (2019) reported that among the solvents used to extract the biologically active substances from *R. chalepensis*, ethanol and methanol were the best solvents; followed by acetone and the least were diethyl ether and hexane, and they concluded that the extraction of medicinal plants with different solvents could produce different results depending on the ability of the solvent. Al-Majmaie *et al*. (2019) stated that rutin (1) and its two derivatives (2 and 3) from *R. chalepensis* flowers showed varying degrees of activity against all tested organisms in the resazurin microtitre assay with compound 3 being the most potent, and the order of antimicrobial potency among these compounds was 3>2>1. All extracts except ethanol significantly (P<0.001) showed antifungal activity against *Candida albicans* with a 10 mm inhibition zone (Table 3). The essential oil of the aerial portions of *R. chalepensis* demonstrated antifungal activities against *Aspergillus niger*, *Aspergillus flavus*, *Alternaria sp*., *Trichoderma sp*. and *Candida albicans* (Merghache *et al*., 2008). Such results are consistent with those of Emam *et al*. (2010) who reported that the ethanolic extract (80%) of *R. chalepensis* leaves exhibited antifungal activity against the three phytopathogenic fungi *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium solani*, which cause root-rot and wilt diseases in a number of economically important food crops such as potato, sugar beet, and tomato, when examined with the disk diffusion technique, with inhibition zone diameters being 10, 18, and 12 mm, respectively. Sepahvand *et al*. (2018) reviewed the most common dermatophytosis medicinal plants in traditional medicine and concluded that the medicinal plants including *R. chalepensis* are the most effective plants on dermatophytes that have been identified to date.

Table 3. Antimicrobial activity (inhibition zone in mm) of *Ruta chalepensis* leaf extract determined by agar disc diffusion assay (100 mg/ml)

Microorganisms	Extract					S.L.
	Ethanol	Petroleum ether	Chloroform	Ethyl acetate	n-butanol	
Gram-positive						
E. coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
P. aeruginosa	10.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	***
Gram-negative						
S. aureus	10.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	***
B. cereus	5.00±0.07^{ab}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	10.00±0.00^{a}	N.S.
Fungus						
C. albicans	0.00±0.00^{b}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	***

The data are presented as mean ±standard deviation (n=3) Means in each row bearing similar superscripts are not significantly different (P>0.05) ,*** = P<0.001

N.S. = Not significant, S.L. = Significance level

4. Conclusion

In this study, *R. chalepensis* leaf extracted with ethanol, petroleum ether, chloroform, ethyl acetate, and n-butanol were evaluated for total phenolic content in addition to antioxidant and antimicrobial activity evaluation. The results indicated that the overall phenolic content decreased by n-butanol extract (2580.47mg/gm) > ethanol (944.63 mg/gm)>petroleum ether (720.93 mg/gm) > ethyl acetate (717.13 mg/gm) > chloroform extract (18.93 mg/gm). The plant has a high antioxidant activity and mild antimicrobial activity, although the essential oil had antimicrobial activity against the organism in the study in a previous study of the same authors (unpublished data). Since the area of Al-Baha is in rich *R. chalepensis*, the plant could be a potential source of natural bioactive molecules that could substitute for synthetic antioxidants and function as a source of antibacterial agents in the food industry and can serve as a medicinal plant

Acknowledgments

The authors would like to acknowledge the financial support provided by Al-Baha University for this project.

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