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Original article

Phytochemical contents and biological evaluation of *Ruta chalepennsis* L. growing in Saudi Arabia

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ABSTRACT

Phytochemical screening of *Ruta chalepensis* L. exhibited the presence of different chemical groups. The dried aerial parts of the plant was total extracted by ethanol and successively using chloroform, ethyl acetate and Butanol, out of the successive extracts four compounds namely, scopletin, kaempferol, quercetin, quercetin 3-*O*-α-L-rhamno glucopyranosyl (Rutin) were isolated and biological evaluations. Total ethanol and successive extracts; chloroform, ethyl acetate and Butanol were produced excellent antimicrobial activities against gram negative bacteria, gram positive bacteria and fungi. Ethyl acetate extract was the best for inhibition of the microorganism's growth. All extracts (total ethanol, and successive extracts) showed DPPH radical scavenging activity in a concentration–dependent manner. The best antioxidant activity was obtained by ethyl acetate & *n*-butanol extract (94.28%, IC_{50} = 56.6 μg/ml). Also All extracts (total ethanol, and successive extracts) showed anticoagulant activity at higher concentration with prolonged clotting time 6:30 and 4:30 s at 10 mg/ml concentrations, respectively.

1. Introduction

Infectious diseases caused by bacteria, fungi, viruses are a critical challenge to health and they are believed to be one of the main causes of increasing the rates of morbidity and mortality worldwide (Drusano, 2004). Numerous infections and disorders caused by bacterial and fungal pathogens including *Salmonella*, *Staphylococcus*, *Bacillus*, *Klebsiella*, *Proteous*, *Pseudomonas Aspergillus*, *Candida*, *Cryptococcus* and *Trichophyton* (Bibi et al., 2011). For several decades, natural remedies and medicinal plants were the main, and in fact the only, resource for the physicians. Until the present, most of the people, especially in developing countries, depend on plants for medicines (Amabye and Shalkh, 2015).

The significance of plants to homeopathy and modern medicine is correlated to their chemical constituents such as such as terpenoids, phenolics, alkaloids, flavonoids, amino acids, saponins, glycosides, diterpenes, triterpenes and their compatibility with the human body. It is expected that more than 30% of the worldwide sales of drugs is based mainly on plant products (Patwardhan et al., 2004, De Fatima et al., 2002). Plants of the family Rutaceae 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, Di Stasi et al., 2002, Zeichen de et al., 2000, Atta and Alkofahi, 1998).

Ruta chalepensis (Rue) is an aromatic evergreen shrub which is originally from the Mediterranean region and is at present distributed worldwide (Akkaria et al., 2015). In many countries, it is cultivated for its pharmacological and biological activity and it is widely used for treatment of gastric, diuretic, inflammation, headache and rheumatism disorders. Analysis of the chemical constituents of *R. chalepensis* extracts revealed that the aerial parts contain alkaloids, phenols, flavonoids, amino acids, saponins and furocoumarins (Kacem et al., 2015). The present study was conducted for determination of the phytochemical composition and the antimicrobial, anticoagulant, and antioxidant activities of different of *Ruta chalepensis*.

2. Material and methods

2.1. Phytochemical contents

2.1.1. Plant material

Ruta chalepennsis L. was collected in March 2016 from Jizan province, KSA. The plant was identified by Dr. Ahmed Al-Farhan;

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Professor of Plant Taxonomy, College of Science, King Saud University. For phytochemical analysis and biological activities, the aerial parts of the plant were air-dried, grounded to powder, packed and stored in a clean, tightly, and closed container.

2.1.2. Phytochemical screening

The plant powder of *Ruta chalepennsis* L. was subjected to preliminary phytochemical screening for determination of its contents of biologically active phytochemical groups according to the method described by Ayoola et al. (2008).

2.1.3. Plant extraction

The powdered plant materials (2 kg) were percolated in 3 L of ethanol (95%) for 3 days. The obtained solvent was filtered by cotton piece and the marks lift was re-extracted for 4 times by the same way (Awaad et al., 2016). The total alcohol extracts were concentrated using rotatory evaporator at low temperature. The obtained alcohol free gummy residue (157 g) was dissolved in boiled-water and filtered using piece of cotton, the non-filtered part (non-polar materials chlorophyll and fatty matters) was removed.

The filtered (aqueous) was re-extracted successively till exhaustion using, ether, chloroform, ethyl acetate and *n*-butanol (water-saturated) respectively. Each extract was passed over an anhydrous sodium sulphate then concentrated using reduced pressure, at low temperature, and residues; 8.3, 10.6 and 30.5 g, were obtained from chloroform, ethyl acetate and *n*-butanol, respectively.

2.1.4. Isolation and purifications

The obtained successive extracts were chromatographically investigated on pre-coated silica gel GF plates using the following three solvent systems; (a) (Benzene- ethyl acetate 86:14 v/v), (b) (Chloroform- methanol 96:4 v/v), and (c) (Ethyl acetate-Methanol- water 30: 5: 4 v/v/v). Visualization of the spots was carried out under UV- light before and after spraying of TLC with $AlCl_3$ and $SbCl_3$.

Ether & Chloroform extract were collected together (8.3) and symbolized as **D-1**. Also ethyl acetate and *n*-butanol (41.19 g) are collected together and symbolized as **D-2** (Based on similarity of spots (colour, number and R_f).

The fractions D-1 and D-2 were subjected to further chromatographic investigation to isolate and identify their active compound (s) as following:

For isolation of compound(s) from D-1, five grams were applied onto the top of a glass column (120 × 2 cm) packed with 150 g silica gel G, eluted using system chloroform-methanol (95: 5), and 100 fractions (50 ml each) were obtained. All fractions were concentrated under reduced pressure, chromatographically screened on TLC, and reduced (according of number, colour and R_f of spots) to three sub-fractions; D-1-A (2.1 g), D-1-B (1.7 g), and D-1-C (0.5 g). The sub-fraction D-1-C (showed many spots with very pale colour) was ignored.

Sub-Fraction D-1-A (2.1 g) was applied onto top of a glass column (100 × 1.5 cm) packed with 60 g silica gel, eluted with chloroform-methanol (98: 2, v/v), ninety fractions (40 ml each) were collected, concentrated under reduced pressure. A semi-purified compound was obtained; purified using re-crystallization from methanol and compound **(R1)** was isolated.

Sub-Fraction D-1-B (1.7 g) was applied onto top of a glass column (80 × 1 cm) packed with 50 g silica gel, eluted with chloroform-methanol (97: 3, v/v), 30 fractions (30 ml each) were collected, dried from the solvent, re-crystallized (dissolved in methanol), and compound **(R2)** was isolated.

For isolation of compound(s) from D-2, twenty grams were dissolved in methanol, applied onto the top of a column (150 × 5 cm) packed with 200 g Sephadex LH-20, and eluted with methanol. A hundred fractions (100 ml each) were obtained and according of number, colour and R_f of spots were reduced to two sub-fractions; D-2-G (3.7 g) and D-2-H (2.7 g). For isolation of compound(s) from D-2-G, 3.5 g was applied onto top of a glass column (100 × 1.5 cm) packed with 90 g silica gel G, eluted with chloroform-methanol (92:8, v/v), 50 fractions (60 ml each) were obtained, dried from solvent, and compound **R3** was isolated.

For isolation of compound(s) from D-2-H, 2.5 g was applied onto top of a glass column (100 × 1.5 cm) packed with 90 g silica gel G, eluted with chloroform (polarity was gradually-increased with ethyl acetate and methanol), 40 fractions (60 ml each) were collected and chromatography examined using TLC and ethyl acetate- Methanol- water 30: 5: 4, v/v/v. A semi-purified compound was obtained; purified using recrystallization from methanol, dried from solvent, and compound **R4** was isolated.

2.2. Biological evaluations

2.2.1. Antimicrobial activity

2.2.1.1. Test organisms. Strains of microorganisms; namely, *Escherichia coli* (RCMB 010,052), *Klebsiella pneumonia* (RCMB 003-1), *Proteous vulgaris* (RCMB 004-1), *Pseudomonas aeruginosa* (RCMB 0,100,243-), *Salmonella typhimurium* (RCMB 006-1), *Bacillus substilis* (RCMB 015-1), *Staphylococcus aureus* (RCMB 010,010), *Staphylococcus epidermidis* (RCMB 009-2), *Streptococcus mutans* (RCMB 017-1), *Stroptococcus pyogenes* (RCMB 101,001,742), *Aspergillus fumigatus* (RCMB 002,008), *Aspergillus niger* (RCMB 002,005), *Candida albicans* (RCMB 005,003), *C. tropicalis* (RCMB 005,004), *Cryptococcus neoformans* (RCMB 0,049,001), *Geotricum candidum* (RCMB 05,097), *Penicillium expansum* (RCMB 001,001-2), and *Syncephalastrum racemosum* (RCMB 0,016,001-1) were provided from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt and used as test organisms.

2.2.1.2. Antimicrobial assay. The antimicrobial activity of ethanol and collected successive extracts **D1** and **D2** of *Ruta chalepennsis* was determined using well-diffusion method (Zain et al., 2012). Petri plates containing 20 ml of nutrient or malt extract agar medium were seeded with 1–3 day cultures of microbial inoculums. Wells (6 mm in diameter) were cut off from agar and 50 μl of the plant extracts were separately added, in a concentration of 100 mg/ml, and incubated at 37 °C for 24–48 h and 3–5 days for bacterial and fungal strains, respectively. The antimicrobial activity was determined by measurement of the diameter of the inhibition zone around the well.

2.2.1.3. Determination of minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) was determined by well-diffusion method (Zain et al., 2012). The MIC of *Ruta chalepennsis* extracts was determined using twofold dilutions for concentrations from 0.0 to 10 mg/ml. Wells (6 mm in diameter) were cut off from agar and 100 μl of each concentration of the plant extracts were separately added and incubated at 37 °C for 24–48 h and 3–5 days for bacterial and fungal strains, respectively. The lowest concentration (highest dilution) of the plant extract that produced no visible microbial growth (no turbidity) when compared with the control tubes were considered as MIC.

2.2.2. Antioxidant activity (DPPH (1-diphenyl-2-picrylhydrazyl) radical-scavenging assay)

The antioxidant activity of ethanol and collected successive extracts D1 and D2 of *Ruta chalepennsis* was determined using the DPPH free radical scavenging assay according to the method described by Yen and Duh (1994). Freshly prepared (0.004%w/v) methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

radical was prepared and stored at 10 °C in the dark. A methanol solution of the test compound was prepared. A 40 µl aliquot of the methanol solution was added to 3 ml of DPPH solution, under light protection. Absorbance measurements were recorded immediately with a UV–visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. The percentage inhibition (PI) (scavenging activity) of the DPPH radical was calculated according to the formula (Yen and Duh, 1994):

$$PI = (AC - AT)/AC \times 100$$

where *AC* = Absorbance of the control at t = 0 min and *AT* = absorbance of the sample + DPPH at t = 16 min.

2.2.3. Anticoagulant activity

Anticoagulant activity of ethanol and collected successive extracts **D1** and **D2** of *Ruta chalepennsis* was determined. Concentrations of each extract were tested on plasma using prothrombin time test. The time required for clotting was considered as the parameter for the anticoagulant action.

Blood samples were obtained in containers containing sodium citrate from healthy human volunteers. Centrifugation was carried out at 3000 rpm for 15 min. The freshly-prepared plasma was stored at 4 °C. Prothrombin time test, 0.2 ml test plasma, 0.1 ml of each extract of *Ruta chalepennsis* of different concentration 0.001, 0.01, 0.1, 1 and 10 mg/ml and 0.3 ml of calcium chloride were added and incubated at 37 °C. The coagulation time was recorded in seconds using a stopwatch. Normal saline was used in place of the extracts for the negative control and 50 mg/ml of heparin for the positive control.

3. Results and discussion

3.1. Phytochemical contents

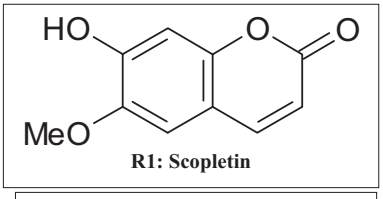
Phytochemical screening of *Ruta chalepensis* L. indicated the presence of carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, alkaloids, protein and/or amino acids, Resins, Lactones and/or esters and tannins. On the other hand, saponin, anthraquinones, cardinolides, and oxidase enzyme were absent. The presence of variations in phytochemical groups in any plant can be used as promising support of possible presence of biological activities (Mohammed et al., 2014, Lunga et al., 2014, Dahija et al., 2014).

Four phenolic compounds were isolated and identified as following; **Compound R1** was obtained as white needles (20 mg) with R_f = 0.77 (on TLC, system b); soluble in methanol and chloroform, m.p. (226–227 °C). UV λ_{max} (MeOH), 266, 367 nm; and 275, 420 nm in NaOAc. ^{1}H NMR (DMSO-d_6): δ 7.9 (1H, J = 9, H-4); δ 7.2 and 6.75 (2H, 2S, H-5 and H-8, respectively); δ 6.2 (1H, d, J = 9 Hz, H-3) and δ 3.8 (3H, S, OCH_3). ^{13}C NMR (DMSO-d_6) δ 161.23 (C2), 110.17(C3), 145.04(C4), 112.21(C5), 150.09(C6), 151.78(C7), 103.33(C8), 145.83(C9), 111.06(C10). R1 was identified as **7-hydroxy-6-methoxycoumarin** (scopletin) (Fig. 1).

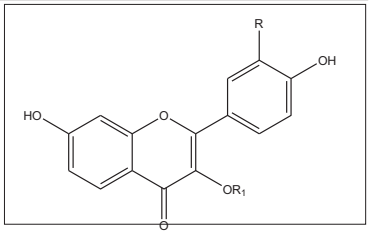
Compound R2 was obtained as light yellow crystals (10 mg) from methanol with R_f 0.55 (system b) and 0.79 (system c), m.p. (226–227 °C). UV, λ max in MeOH: nm 367, 268;($AlCl_3$): 265, 350, 420;($AlCl_3$/HCl): 265, 350, 420; (NaOA): 275, 300(sh), 380; ($NaOAc/H_3BO_3$): 267, 319(sh), 380; (NaOMe): 285, 322, 430. ^{1}H NMR (DMSO-d_6): δ 8.01 ppm (2H, td, J = 9.5 Hz, J = 2.8 Hz, H-2′,6′), δ 6.89 ppm (2H, td, J = 9.5 Hz, J = 2.8 Hz, H-3′,5′), 6.40 ppm (1H, d, J = 2.04 Hz, H-6), δ 6.15 ppm (1H, d, J = 2.04 Hz, H-8), δ 12.44 ppm (1H, s, for OH at C-5), δ 10.74 ppm (1H, s, OH at C-4′), δ 10.06 ppm (1H, s, OH at C-3), δ 9.34 ppm (1H, s, OH at C-7). ^{13}C NMR (DMSO-d_6); Signals at δ 176.48 ppm (C-4) ketonic carbon, δ 164.46 ppm (C-5), δ 161.28 ppm (C-7), δ 159.76 ppm (C-4′), δ 156.74 ppm (C-9), δ 147.39 ppm (C-2), δ 136.23 ppm (C-3), δ 130.07 ppm (C-2′,6′), δ 122.24 ppm (C-1′), 116.01 ppm (C-3′,5′), δ 103.61 ppm (C-10),δ 98.77 ppm (C-8), and δ 94.04 ppm (C-6). This compound identified as **Kaempferol** (Fig. 1).

Compound R3 was obtained as yellow crystals (45 mg) from methanol, m.p 316–317 °C, R_f 0.40 in (system a) & 0.67 in (system b). UV: λ_{max} (MeOH): (nm) 255, 301 and 371, (NaOMe) 245, 330, ($AlCl_3$) 272, 301, 454., ($AlCl_3$/HCl) 270,357, 426, (NaOAc) 275, 324,387, (NaOAc/H3BO3) 262,385. ^{1}HNMR(DMSO –d6) δ 7.64 (1H, d, J = 8.5H-2′), 7.49 (1H, q, J = 8.5, H-6′), 6.85 (1H, d, J = 8.5, H-5′), 6.37 (1H, d, J = 2.5, H-6) and 6.14 (1H, d, J = 2.5, H-8). ^{13}C NMR (DMSO-d_6): δ ppm 176.36 for ketonic carbon (C-4), 164.39 (C-7), 161.24 (C-5), 156.64 and 148.21 for (C-9) and (C-2), respectively, 147.31 (C-4′), 145.57 (C-3′), 136.25 (C-3), 122.46 (C-6′), 120.49 (C-1′), 116.11 (C-2′), 115.56 (C-5′) and 103.52 (C- 10). The lower affected aromatic carbons C-6 and C-8 appeared at 98.69 and 93.87 respectively. These data were confirmed with DQF-COSY, HMQC & HMBC. This compound identified as **Quercetin** (Fig. 1).

Compound R4 was obtained as yellow crystals (25 mg) from methanol, m.p. 190–191 °C, its R_f values was 0.48 (system d). It gave positive Molisch's test indicated to the presence of sugar moiety in it. UV λ_{max} λ_{max} (MeOH): 255, 355; (NaOMe), 272, 425., (NaOAc), 266, 393; ($NaOAc/H_3BO_3$), 261, 387; ($AlCl_3$), 275, 430.; ($AlCl_3$/HCl), 271, 335. ^{1}H NMR (DMSO-d $_6$): δ 8.10 (1H, d, J = 2 Hz, H2′); δ 7.86 (1H, d, J = 8 Hz, H-6′); δ 6.89 (1H, d, J = 8 Hz, H-5′); ^{1}H NMR (DMSO-d_6): δ 8.10 (1H, d, J = 2 Hz, H2′); δ 7.86 (1H, d, J = 8 Hz, H-6′); δ 6.89 (1H, d, J = 8 Hz, H-5′); δ 6.65 (1H, d, J = 2 Hz, H-8); δ 6.5 (1H, d, J = 2 Hz H6); δ5.13 (1H, d, J = 7.50 Hz H1″); δ 4.55 (1H, d, J = 1.3 Hz, H1‴); δ 3.82 (1H, dd, J = 10 Hz, J = 2 Hz H6″); δ 3.65 (1H, dd, J = 3.5, H2‴); δ 3.47–3.87 (6H, m, Sugar



R1: Scopletin



Symbole	Name	R	R_1
R2	kaempferol	H	H
R3	Quercetin	OH	H
R4	Quercetin 3-*O*-α-L-rhamno glucopyranosyl (Rutin).	H	Rhamnose glucose

Fig. 1. The isolated compounds of *Ruta chalepennsis* L.

protons) and δ 1.23 (3H, d, J = 6 CH_3). ^{13}C NMR (DMSO-d_6): δ 157.98 (C2), 134.28 (C3), 178.08 (C4), 161.66 (C5), 98.61 (C6), 164.75 (C7), 93.52 (C8), 157.18 (C9), 104.27 (C10), 121.77 (C1′), 116.33 (C2′), 145.51 (C3′), 148.48 (C4′), 114.70 (C5′), 122.19 (C6′), 103.37 (C1″), 74.38 (C2″), 76.83 (C3″), 70.00 (C4″), 75.87 (C5″), 67.19 (C6″). Acid hydrolysis to this compound produced rhamnose and glucose units upon testing on TLC comparing with authentic reference sample. This compound was identified as **quercetin 3-*O*-α-L-rhamno glucopyranosyl** (Rutin). (Fig. 1).

3.2. Biological evaluations

3.2.1. Antimicrobial activity

The antimicrobial activity and the minimum inhibitory concentration (MIC) of ethanol and collected successive extracts **D1** and **D2** (ether &chloroform, ethyl acetate & *n*-butanol respectively) of *Ruta chalepennsis* were determined using the well diffusion method (Table 1). With the exception of *Proteous vulgaris* and *Pseudomonas aeruginosa*, the obtained results revealed that all the extraction solvents of *R. chalepennsis* possessed antibacterial activity against all the investigated Gram-negative and Gram-positive test organisms and the activity varied according to the solvent.

The **D2** extract (ethyl acetate & Butanol) of *R. chalepennsis* best antibacterial activity was 21 mm (0.625 mg/ml); 20 mm (1.25 mg/ml); 20 mm (2.5 mg/ml); 19 mm (1.25 mg/ml); 19 mm (2.5 mg/ml) against *Staphylococcus aureus*; *Proteous vulgaris*; *Staphylococcus epidermidis*; *Klebsiella pneumonia* and *Escherichia coli*, *Bacillus substilis* and *Stroptococcus byogenes*, respectively. However, the antimicrobial activity of **D1** extract (ether & chloroform) was 22 mm (0.156 mg/ml); 21 mm (2.5 mg/ml); 19 mm (1.25 mg/ml) and 18 mm (2.5 mg/ml) against *Proteous vulgaris*; *Streptococcus mutans*; *Klebsiella pneumonia* and *Bacillus substilis* and *Stroptococcus pyogenes*, respectively (Table 1).

Interestingly, the **D1** and **D2** extracts of *R. chalepennsis* showed antimicrobial activity higher than ethanol extract against *Escherichia coli*, *Proteous vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 1). On the hand, the ethanol extract showed antibacterial activity against *Staphylococcus epidermidis* (29 mm, 1.25 mg/ml) was higher than **D2** extract (23 mm, mg/ml) and **D1** extract (19 mm, 2.5 mg/ml). The antimicrobial activity of **D2** extract was variable against microorganisms as following zone of inhibition; 24 mm (0.156 mg/ml) *Klebsiella pneumonia*, 23 mm (2.5 mg/ml) *Staphylococcus epidermidis*, 23 mm (5 mg/ml) *Staphylococcus aureus*, 22 mm (0.312) *Escherichia coli* and 21 mm (0.625 mg/ml) *Proteous vulgaris*. While the **D1** extract exhibited antibacterial activity (zone of inhibition) against *Proteous vulgaris* (24 mm, 0.156 mg/ml), *Escherichia coli* (23 mm, 0.312 mg/ml), *Klebsiella pneumonia* (21 mm, 0.625 mg/ml), *Staphylococcus aureus* (20 mm, 0.625 mg/ml) and *Salmonella typhimurium* (20 mm, 1.25 mg/ml) (Table 1).

The different extracts of *Ruta chalepennsis* ethanol and collected successive extracts **D1** and **D2** (ether &chloroform, ethyl acetate & *n*-butanol respectively) showed antifungal activity against *Candida albicans*, *C. tropicalis*, *Cryptococcus neoformans*, and *Geotrichum candidum* (Table 1). However, there was no activity against *Aspergillus fumigatus*, *A. niger*, *Microsporum canis*, *Penicillium expansum*, *Syncephalastrum racemosum* and *Trichophyton mentagrophytes*. The highest antifungal activity; (23 mm, 0.156 mg/ml) and (22 mm, 0.312 mg/ml) was obtained by collected successive extracts **D1** and **D2** against *Candida albicans* (Table 1).

3.2.2. Antioxidant activity

The obtained results of the antioxidant activity indicated that the ethanol and collected successive extracts **D1** and **D2** (ether &chloroform, ethyl acetate & *n*-butanol respectively) of *Ruta chalepennsis* showed DPPH radical scavenging activity in a

Table 1
Antimicrobial activity of ethanol, chloroform, ethyl acetate & *n*-butanol extracts of *Ruta chalepennsis*.

Test organism	Sample							
	Ruta chalepennsis						Standard antibiotic	
	Ethanol		Ether & chloroform		Ethyl acetate and *n*-butanol			
	Inhibition zone (mm)	MIC (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)
Bacteria G -ve							**(Gentamycin)**	
Escherichia coli	17	2.500	23	0.312	22	0.312	36	0.0005
Klebsiella pneumonia	22	0.625	21	0.625	24	0.156	23	0.0010
Proteous vulgaris	00	ND	24	0.156	21	0.625	31	0.0005
Pseudomonas aeruginosa	00	ND	18	1.250	18	2.500	25	0.0010
Salmonella typhimurium	19	2.500	20	1.250	18	2.500	27	0.0010
G + ve							**(Gentamycin)**	
Bacillus substilis	17	5.000	17	2.500	19	2.500	32	0.0005
Staphylococcus aureus	18	2.500	20	0.625	23	5.000	30	0.0005
Staphylococcus epidermidis.	29	1.250	19	2.500	23	2.500	34	0.0005
Streptococcus mutans	19	2.500	15	2.500	14	5.000	26	0.0010
Stroptococcus pyogenes	17	5.000	17	2.500	16	5.000	28	0.0010
Fungi							**(Ketaconazole)**	
Aspergillus fumigatus	00	ND	00	ND	00	ND	23	0.0005
Aspergillus niger	00	ND	00	ND	00	ND	24	0.0010
Candida albicans	16	5.000	14	10.00	14	5.000	26	0.0005
Candida tropicalis	14	10.00	23	0.156	22	0.312	27	0.0010
Cryptococcus neoformas	17	5.000	19	0.625	20	1.250	31	0.0010
Geotrichum candidum	16	5.000	17	5.000	23	1.250	30	0.0010
Microsporum canis	00	ND	00	ND	00	ND	30	0.0010
Penicillium expansum	00	ND	00	ND	00	ND	28	0.0020
Syncephalastrum racemosum	00	ND	00	ND	00	ND	24	0.0020
Trichophyton mentagrophytes	00	ND	00	ND	00	ND	29	0.0010

ND, not determined.

Table 2
The scavenging activity of DPPH radicals of ethanol, ethyl acetate & *n*-butanol and ether & chloroform extracts of *Ruta chalepennsis*.

Concentration (μg/ml)		DPPH scavenging (%)			
Extracts	Ascorbic acid	Ethanol	Ethyl acetate & *n*-butanol	Ether & chloroform	Ascorbic acid
000	00	00.00	00.00	00.00	00
001	05	09.34	30.64	13.79	12.98
002	10	17.96	42.39	23.64	16.38
004	15	28.73	51.87	31.25	62.98
008	20	37.80	57.25	40.82	76.81
016	25	54.97	63.18	49.07	78.72
032	30	65.14	70.94	57.81	78.94
064	35	79.02	78.63	71.29	80.21
128	40	87.51	92.38	80.37	86.36
IC_{50}		**320.7**	**84.70**	**414.9**	**11.20**

Table 3
Effect of ethanol, ethyl acetate & *n*-butanol and ether & chloroform extracts of *Ruta chalepennsis* on prothrombin time (PT) of normal human plasma.

Concentration (μg/ml)	DPPH scavenging (%)		
	Ethanol	Ethyl acetate & *n*-butanol	Ether & chloroform
0.001	2:40	2:30	00.00
0.010	3:20	2:50	00.00
0.100	4:00	3:20	00.00
0001	5:20	4:00	00.00
0010	6:30	4:30	00.00

Control: (Heparin 50 mg/ml): 2:10 min (PT); (saline): 1:10 min (PT).

concentration–dependent manner (Table 2). The best antioxidant activity of *R. chalepennsis* was obtained by **D2** (ethyl acetate & *n*-butanol) extract (94.28%, IC_{50} = 56.6 μg/ml). However, the ethanol and **D1** (ether & chloroform) extracts showed antioxidant activity (87.51%, IC_{50} = 320.7 μg/ml) and (80.37%, IC_{50} = 414.9 μg/ml), respectively (Table 2). All extracts possess very promising antioxidant activities which can be attributed to the presence of phenolic compounds in this plant (Carocho and Ferreira, 2013).

3.3. Anticoagulant activity

The anticoagulant activity of ethanol and collected successive extracts **D1** and **D2** (ether &chloroform, ethyl acetate & *n*-butanol respectively) of *Ruta chalepennsis* was determined. Different concentrations of each extract were tested on plasma using prothrombin time test. The required time for clotting was recorded as the parameter for the anticoagulant action.

All extracts (ethanol and **D1** and **D2**) of *Ruta chalepennsis* showed anticoagulant activity at higher concentration with prolonged clotting time 6:30 and 4:30 s at 10 mg/ml concentrations, respectively (Table 3). However, there was no anticoagulant activity for **D1** extract (ether & chloroform) (Table 3).

4. Conclusion

In the present study four phenolic compounds were isolated from the *Ruta chalepennsis* and those compounds might be responsible about the anticoagulant activity (Ferhat et al., 2014, Haddouchi et al., 2013). In addition, these compounds are synthesized during the secondary metabolism and their production and accumulation might vary according to the species and the environmental conditions (Da Silva et al., 2014, Ferhat et al., 2014, Conti et al., 2013).

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