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

Shorok M. Alotaibi *, Monerah S. Saleem, Jehan G. Al-humaidi

Chemistry Department, College of Science, Princess Nourah Bint Abdurrahman University, Riyadh 11671, Saudi Arabia

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**A B S T R A C T**

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 3-0-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%, IC50 = 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.

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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*, *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, spasmyloytic, antihelminthic, emmenagogue, antitumoral, analgesic, anti-inflammatory, and antidepressant activities (Raghav et al., 2006, Di Stasi et al., 2002). Plants of the family Rutaceae are a source of huge variety of natural products with antibacterial, antifungal, antioxidant, spasmyloytic, antihelminthic, emmenagogue, antitumoral, analgesic, anti-inflammatory, and antidepressant activities (Raghav et al., 2006, Di Stasi et al., 2002). Plants of the family Rutaceae are a source of huge variety of natural products with antibacterial, antifungal, antioxidant, spasmyloytic, antihelminthic, emmenagogue, antitumoral, analgesic, anti-inflammatory, and antidepressant activities (Raghav et al., 2006).

**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 chalepensis* L. was collected in March 2016 from Jizan province, KSA. The plant was identified by Dr. Ahmed Al-Farhan;
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, ground to powder, packed and stored in a clean, tightly, and closed container.

2.1.2. Phytochemical screening

The plant powder of *Ruta chalepensis* 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 methanol, applied onto the top of a glass column (150 mm, 1.5 cm) packed with 60 g silica gel G, eluted with methanol. A 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 AICl3 and SbCl5.

Ether & Chloroform extract were collected together (8.3) and symbolized as D-1. Also 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 AICl3 and SbCl5.

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 Rf)).

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 (accord of number, colour and Rf 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), thirty 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 Rf 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 chromatographed 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), *Proteus vulgaris* (RCMB 004-1), *Pseudomonas aeruginosa* (RCMB 01,100,243-3), *Salmonella typhimurium* (RCMB 006-1), *Bacillus subtilis* (RCMB 015-1), *Staphylococcus aureus* (RCMB 010,010), *Staphylococcus epidermidis* (RCMB 009-2), *Streptococcus mutans* (RCMB 017-1), *Streptococcus 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 *Syncephalstrum 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 chalepensis* 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 chalepensis* 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 chalepensis* was determined using the DPPH free radical scavenging assay according to the method described by Yen and Duh (1994). Freshly prepared (0.0045w/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 ascobic acid were also measured. The percentage inhibition (PI) (scavenging activity) of the DPPH radical was calculated according to the formula (Yen and Duh, 1994):

\[ PI = \frac{(AC - AT)}{AC} \times 100 \]

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 chalepensis 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 chalepensis of different concentration 0.001, 0.01, 0.1, 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 Rf = 0.77 (on TLC, system b); soluble in methanol and chloroform, m.p. (226–227 °C). UV \( \lambda_{max} \) (MeOH): 266, 367 nm; and 275, 420 nm in NaOAc. \(^1\)H NMR (DMSO-d6): \( \delta = 7.9 \) (1H, J = 9, H-4); \( \delta = 7.2 \) and 6.75 (2H, 2S, H-5 and H-8, respectively); \( \delta = 6.2 \) (1H, J = 9 Hz, H-3) and \( \delta = 3.8 \) (3H, S, OCH\(_3\)). \(^13\)C NMR (DMSO-d6): \( \delta = 161.23 \) (C-1), 110.17(C3), 120.49(C-1), 116.11(C-2), 115.56(C-3') 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, HMOC & HMBD. This compound identified as Quercetin (Fig. 1).

Compound R4 was obtained as yellow crystals (25 mg) from methanol, m.p. 190–191 °C, its Rf values was 0.48 (system d). It gave positive Molisch’s test indicated to the presence of sugar moiety in it. UV \( \lambda_{max} \) (MeOH): 255, 355; (NaOMe), 272, 425.. The reference compounds identified as Kaempferol (Fig. 1).

**Fig. 1.** The isolated compounds of Ruta chalepensis L.
protons) and δ 1.23 (3H, d, J = 6 CH₃). ¹³C NMR (DMso-d₆): δ 157.98 (C₂), 134.28 (C₃), 178.08 (C₄), 98.61 (C₅), 164.75 (C₇), 93.52 (C₈), 157.18 (C₉), 104.27 (C₁₀), 121.77 (C₁¹), 116.33 (C₂'), 145.51 (C₃'), 148.48 (C₄'), 114.70 (C₅'), 122.19 (C₆'), 103.37 (C₁²), 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 refer-

3.2. Biological evaluations

3.2.1. Antimicrobial activity

The antimicrobial activity and the minimum inhibitory concent-
tration (MIC) of ethanol and collected successive extracts D1 and D2 (ether & chloroform, ethyl acetate & n-butanol respectively) of Ruta chalepennis were determined using the well diffusion method (Table 1). With the exception of Proteus vulgaris and Pseudomonas aeruginosa, the obtained results revealed that all the extraction sol-

Table 1

| Test organism             | Sample                  | Inhibition zone (mg/ml) | MIC (mg/ml) | Inhibition zone (mg/ml) | MIC (mg/ml) | Inhibition zone (mg/ml) | MIC (mg/ml) | Standard antibiotic |
|---------------------------|-------------------------|-------------------------|-------------|-------------------------|-------------|-------------------------|-------------|---------------------|
| **Bacteria**              | Ruta chalepennis        |                         |             |                         |             |                         |             | Gentamycin          |
| G + ve                    | Ethanol                 | 17                      | 2.500       | 23                      | 0.312       | 22                      | 0.312       | 36                  |
|                           | Ethanol                 | 22                      | 0.625       | 24                      | 0.156       | 21                      | 0.625       | 31                  |
|                           | Chloroform              | 00                      | ND          | 18                      | 1.250       | 18                      | 2.500       | 25                  |
|                           | Ethanol                 | 19                      | 2.500       | 20                      | 1.250       | 18                      | 2.500       | 27                  |
| **G + ve**                | Ethanol                 | 17                      | 5.000       | 17                      | 2.500       | 19                      | 2.500       | 32                  |
|                           | Ethanol                 | 18                      | 2.500       | 20                      | 0.625       | 23                      | 5.000       | 30                  |
|                           | Chloroform              | 29                      | 1.250       | 23                      | 2.500       | 23                      | 2.500       | 34                  |
|                           | Chloroform              | 19                      | 2.500       | 15                      | 2.500       | 14                      | 5.000       | 26                  |
|                           | Chloroform              | 17                      | 5.000       | 17                      | 2.500       | 16                      | 5.000       | 28                  |
| **Fungi**                 | Ethanol                 | 00                      | ND          | 00                      | ND          | 00                      | ND          | 23                  |
|                           | Ethanol                 | 00                      | ND          | 00                      | ND          | 00                      | ND          | 24                  |
|                           | Chloroform              | 16                      | 5.000       | 14                      | 10.00       | 14                      | 5.000       | 26                  |
|                           | Ethanol                 | 14                      | 10.00       | 23                      | 0.156       | 22                      | 0.312       | 27                  |
|                           | Chloroform              | 17                      | 5.000       | 19                      | 0.625       | 20                      | 1.250       | 31                  |
|                           | Chloroform              | 16                      | 5.000       | 17                      | 5.000       | 23                      | 1.250       | 30                  |
|                           | Chloroform              | 00                      | ND          | 00                      | ND          | 00                      | ND          | 30                  |
|                           | Chloroform              | 00                      | ND          | 00                      | ND          | 00                      | ND          | 28                  |
|                           | Chloroform              | 00                      | ND          | 00                      | ND          | 00                      | ND          | 24                  |
|                           | Chloroform              | 00                      | ND          | 00                      | ND          | 00                      | ND          | 29                  |
|                           | Chloroform              | 00                      | ND          | 00                      | ND          | 00                      | ND          | 29                  |

Interestingly, the D1 and D2 extracts of R. chalepennis showed antimicrobial activity higher than ethanol extract against Escheria coli, Proteus 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) Proteus vulgaris. While the D1 extract exhibited antibiotic activity (zone of inhibition) against Proteus 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 chalepennis 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, Microsorum canis, Penicillium expansum, Syn- cephalus racemosus 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 chalepennis showed DPPH radical scavenging activity in a
The scavenging activity of DPPH radicals of ethanol, ethyl acetate \& n-butanol and ether \& chloroform extracts of Ruta chalepensis.

| Concentration (µg/ml) | DPPH scavenging (%) |
|-----------------------|----------------------|
| Extracts | Ascorbic acid | Ethanol | Ethyl acetate \& n-butanol | Ether \& chloroform | Ascorbic acid |
| 0.000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.001 | 0.05 | 0.94 | 30.64 | 13.79 | 12.98 |
| 0.002 | 0.10 | 17.96 | 42.39 | 23.64 | 16.38 |
| 0.004 | 0.15 | 28.73 | 51.87 | 31.25 | 62.98 |
| 0.008 | 0.20 | 37.80 | 57.25 | 40.82 | 76.81 |
| 0.016 | 0.25 | 54.97 | 63.18 | 49.07 | 78.72 |
| 0.032 | 0.30 | 65.14 | 70.94 | 57.81 | 78.94 |
| 0.064 | 0.35 | 79.02 | 78.63 | 71.29 | 80.21 |
| 0.128 | 0.40 | 87.51 | 92.38 | 80.37 | 86.36 |
| IC₅₀ | 320.7 | 84.70 | |

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

Ethanol, ethyl acetate \& n-butanol) extract (94.28%, IC₅₀ = 56.6 mg/ml) showed antioxidant activity (87.51%, IC₅₀ = 320.7 µg/ml) and (80.37%, IC₅₀ = 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 chalepensis 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 chalepensis 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 chalepensis and those compounds might be responsible about the anticoagulant activity (Ferhat et al., 2014, Haddouche 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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Table 2

| Concentration (µg/ml) | Ascorbic acid | Ethanol | Ethyl acetate \& n-butanol | Ether \& chloroform | Ascorbic acid |
|-----------------------|----------------|---------|--------------------------|-------------------|----------------|
| 0.000 | 2:40 | 2:30 | 0.00 |
| 0.010 | 3:20 | 2:50 | 0.00 |
| 0.020 | 4:00 | 3:20 | 0.00 |
| 0.0001 | 5:20 | 4:00 | 0.00 |
| 0.0002 | 6:30 | 4:30 | 0.00 |

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

Table 3

| Concentration (µg/ml) | DPPH scavenging (%) |
|-----------------------|----------------------|
| Control | 0.0001 | 0.010 | 0.0001 | 0.0001 | 0.0001 |
| Ether & chloroform | 0.0001 | 0.010 | 0.0001 | 0.0001 | 0.0001 |
| Ascorbic acid | 0.0001 | 0.010 | 0.0001 | 0.0001 | 0.0001 |

Concentration–dependent manner (Table 2). The best antioxidant activity of R. chalepensis was obtained by D2 (ethanol and n-butanol) extract (94.28%, IC₅₀ = 56.6 µg/ml). However, the ethanol and D1 (ether \& chloroform) extracts showed antioxidant activity (87.51%, IC₅₀ = 320.7 µg/ml) and (80.37%, IC₅₀ = 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).