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Ruta tuberculata Forsk: Phytochemical screening, total phenolic contents and antioxidant activity of crude coumarins and alkaloids extracts

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ABSTRACT

The present work focuses essentially on the phytochemical screening, total phenolic compounds content, and in vitro antioxidant activity of two crude extracts: coumarins and alkaloids from Ruta tuberculata Forsk, an Algerian saharien species. The results revealed the presence of some chemical groups such as volatile oils, flavonoids, sterols or triterpenes, coumarins, alkaloids, tannins, carotenoids and saponins. The quantification of total phenolic content was done using the folin-ciocalteu method and Aluminum chloride colorimetric method for flavonoids and the amounts are 20.36±0.48and 19.67 ±0.34 polyphenols a nd flavonoids respectively. The crude alkaloids extract and the crude coumarins extract were screened for their potential antioxidant activity in vitro by DPPH free radical-scavenging test. The findings showed that percentage of reduction are 46.65 % and 87.37 % at 10^{-1}M (crude alkaloids and coumarins respectively).

Keywords: *Ruta tuberculata* Forsk, phenolic and flavonoids contents, antioxidant activity, coumarins, alkaloids.

INTRODUCTION

The genus *Ruta*. (Rutaceae) is represented in Algeria by three species: *R. montana* (Clus.) L., *R. chalpensis* L. [1], *R. Tuberculata* [2]. *R. Tuberculata* is characterised by its leaves lanceolate or often elongated and small flowers with four petals yellow. *R. tuberculata* treats bone and joint pain, dysmenorrhea, infertility in women, anaemia and headache [3].

Ruta species are sources of diverse classes of natural products such as flavonoids, alkaloids, essential oils, coumarins, phenols, saponins lignans, and triterpenes, with biological activities including antifungal, antioxidant, phytotoxic, abortive, depressant, antidotal and anti-inflammatory [4,5,6,7,8,9].

In our continuing investigation carried out on the *genus Ruta* [10, 11], the present work focuses on on *R. tuberculata* growing in El oued (the east of the Algerian sahara) and the main objectives of this study were:
(i) Phytochemical sceening
(ii) Dosage of polyphenols and flavonoids
(iii) Extraction of alkaloids and coumarins
(iv) Assessing the scavenging activity *in vitro* using DPPH test of the two crude extracts (alkaloids and coumarins).

MATERIALS AND METHODS

Plant material

Ruta tuberculata Forsk. was collected in April 2014 (flowering stage) in El-oued, (East Algerian sahara) Algeria. The plant was identified by Dr. Hallis youcef (Centre for Arid Areas (CRSTRA), Biophysical Station, Touggourt,

Algeria, A voucher specimen was deposited at the life sciences and nature Department, University Larbi Ben M'hidi, Oum el Bouaghi, Algeria under the code number (N° ZA 133).

Extraction and detection of chemical groups [12]
25g of each powdered dried plant were extracted with petroleum-ether in a continuous extraction apparatus soxhlet. The ether extracts were combined, filtered and concentrated up to 40 mL. The remaining dry vegetable product was extracted three times with methanol for 20 minutes. The vegetable product residue was then extracted with warm water for 20 minutes. The constituents were identified as follows:

•Identification of volatile oils
The ether extract was evaporated to dryness. The residue had a characteristic pleasant odour, thus the plant product contains volatile oils. The vegetable product was distilled with water in a Neo-Clevenger apparatus to extract the volatile oils.

•Identification of sterols and triterpenes
The residue of ether extract was dissolved in 0.5 mL acetic anhydride and then in 0.5 mL of chloroform. Then 1 mL of concentrated sulphuric acid is added (Libermann- Burchards reaction). At the contact zone of the two liquids a brownish red ring was formed denoting the presence of sterols and triterpenes.

•Identification of carotenoids
The ether extract was evaporated to dryness and 3 drops of saturated solution of antimony trichloride in chloroform were added (Carprice's reaction). The pigments are firstly blue and later became red, denoting the presence of carotenoids.

•Identification of flavonoids
The residue of methanolic extract was dissolved in 2 mL of methanol at 50 °C. Metallic magnesium and 5 drops of concentrated HCl were added. A red or orange colour indicates the presence of flavones aglycones (Shibata's reaction).

•Identification of coumarins
The residue of methanolic extract or alcohol extract is dissolved after dryness in hot water. The solution is divided into two equal volumes: one of which contains the reference, and the second is made alkaline with 0.5 mL of 10 % ammonia solution. The appearance of an intense fluorescence under UV light indicates the presence of coumarins and derivatives.

•Identification of tannins:
The water extract (1mL) was diluted with water (2 mL) and a diluted solution of ferric chloride (3 drops) was added. The appearance of a blackish blue or blackish green colour indicates the presence of tannins.

•Identification of saponins
The water extract (1mL) was diluted with water (2 mL) and the mixture was vigorously shaken for 2 minutsthe formation of froth which persists for 10 minuts indicate the presence of sapnins.

Extraction procedure of Alkaloids:
Air dried aerial parts of *Ruta tuberculata* Forsk. (100g) were extracted in a soxhlet apparatus with MeOH (80%). The concentrated MeOH extract was dissolved in HCl 2% aqueous solution at pH=2, and extracted with CH2Cl2 (twice). The acidic solution(mother liquor) was brought to PH= 9 with NH4OH 25%, and extracted again with CH2Cl2 (thrice). The CH2Cl2 extracts were evaporated to give the crude alkaloids[13].

Extraction procedure of coumarins:
After drying and powdering, the crude material was extracted with MeOH in soxhlet apparatus. The solvent was evaporated until dryness, the crude residue was taken up in a NH_4OH 25%. The aqueous basic phase was extracted with methiline chloride. The aqueous extract was brought to pH=2 with HCl 2% then extracted again with CH_2Cl_2 (Thrice). The CH_2Cl_2 extracts were evaporated giving the crude coumarins [12].

Dosage of total polyphenols
The dosage of polyphenols was performed through the Folin-Ciocalteau photometric method [14] which was slightly modified. It comprises mixing 20µl of the methanol extract with : 250 µl of Folin-Ciocalteu reagent (1N), 1.25 ml sodium carbonate (2%) and 480 µl distilled water. The solutions were homogenized, capped and protected from light and kept at room temperature for 30mn. The absorbance was measured at 750 nm using water as a blank.

The test was performed in triplicate. The standard curve was obtained using standard solutions of gallic acid at the same concentration of the sample. The content of total polyphenols was expressed as microgram of gallic acid equivalent per milligram of dried plant.

Dosage of flavonoids

Aluminum chloride colorimetric method was used for flavonoids [15]. 1 ml of plant extract and standard (dissolved in methanol) were added to an equal volume of a solution of aluminum chloride (2%).It remained at room temperature for 30 min. The solutions were homogenized, capped and protected from light and kept at room temperature. The absorbance was measured at 430 nm using the methanol as a blank. The test was performed in triplicate and the standard curve was obtained using a serial concentration of quercetin solutions. The flavonoids content was expressed as microgram of quercetin equivalent per milligram of dried plant.

DPPH radical-scavenging activity

The capacity of two extracts from *Ruta tuberculata* Forsk. to reduce the radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) was assessed using the method of Masuda *et al* [16]. 15 µl of the extract at different concentrations was added to 1.5 ml L of a DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The normal purple color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. The scavenging activity of two extracts were evaluated according to the formula:

DPPH scavenging effect (%) = [$(A_0 - A_1)/A_0$]×100, where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in three replications.

RESULTS AND DISCUSSION

Phytochemical screening

R. tuberculata Forsk has been screened for several chemical groups. It is interesting to note the presence of volatile oils, alkaloids, coumarins, flavonoids, sterols or triterpenes, saponins, tannins and carotenoids, which have not already been reported in the literature (Table 1).

Dosage of total polyphenols and flavonoids

The total polyphenols contents of methanolic extract, determinated by Folin-Ciocalteu method, are reported in micrograms equivalent gallic acid by micrograms of the powder dry mass of the plant material, where the content of flavonoids exposed as quercetine equivalents. The concentration of polyphenols and flavonoids in this extract was 20.36±0.48 and 19.67 ±0.34 respectively.

RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of volatil oils, sterols or triterpenes, carotenoids, coumarins, alkaloids, flavonoids, tanins and saponins.

Table 1. Phytochemical screening from aerial parts of *R. tuberculata* Forsk

Extract	Chemical groups	tests
Petroleum ether	Volatil oils	++
	Sterols or triterpenes	++
	Carotenoids	+
Methanol	Coumarins	++
	Alkaloids	++
	Flavonoids	+
Aqueous	Tanins	+
	Saponins	+

The total phenolic content of methanolic extract was determinated using Folin –ciocalteu reagent and aluminum trichloride reagent and there amounts were 20.36±0.48and 19.67 ±0.34 polyphenols and flavonoids respectively. *Ruta chalepensis* showed significantly higher concentrations of total phenolics (1328.8 mg GAE/100g) compared to *R. tuberculata* Forsk [17] a total phenolic content varying from 120.57 to 178.00 mg Gallic acid equivalents per gram of dry extract of R. chalpensis growing in Tunisia [18].

Table 2: Polyphenols and flavonoids contents of *Ruta tuberculata* Forsk

Methanolic extract of	Polyphenols contents	Flavonoids contents
Ruta tuberculata Forsk.	20.36±0.48	19.67 ±0.34

DPPH radical-scavenging activity

Free radical scavenging activity was performed using DPPH to the crude alkaloids extract (yield 0.05 %) and the crude coumarins extract (yield 0.05 %) at the following concentrations: 10^{-1}M,10^{-2}M,10^{-3}M,10^{-4}M. Vitamin C was used as standard at the final concentration of 1 mg/ml. the crude alkaloids extract has an antioxidant power (46.65%) higher than that of vitamin C (26%), while the crude coumarin extract present the highest antioxidant power (87.37%) and more than vitamin C.Wansi JD *et al* (2006); (Saamita *et al*., 2013) [19,20] showed that alkaloids Rutaceae family has antioxidant activity.

Also, it was showed that the methanol extract of *Ruta graveolens* had the strong antioxidant activity [21,22,23] Kacem *et al*.2015 [24]determined that the antioxidant activities of Ruta chalepensis L. growing in Tunisia were examined using various in vitro assays: DPPH radical scavenging, β-carotene bleaching method, reducing power and quantification of the total antioxidant capacity and the obtained results have confirmed that the ethanol extract exhibits the most potent antioxidant effect, followed by methanol and methanol/water (1/1) extracts.

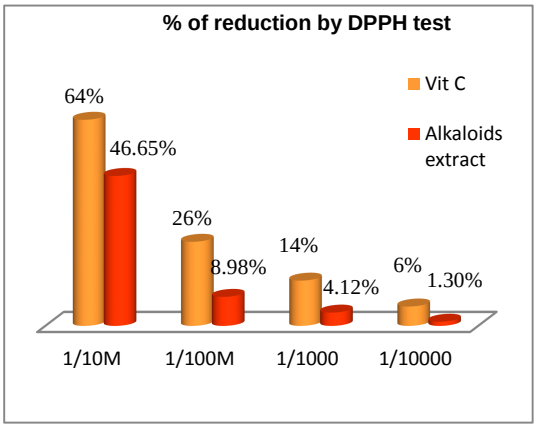

Fig. 1: Antioxydant activity of crude alkaloids extract from *Ruta tuberculata* Forsk by DPPH at different concentrations

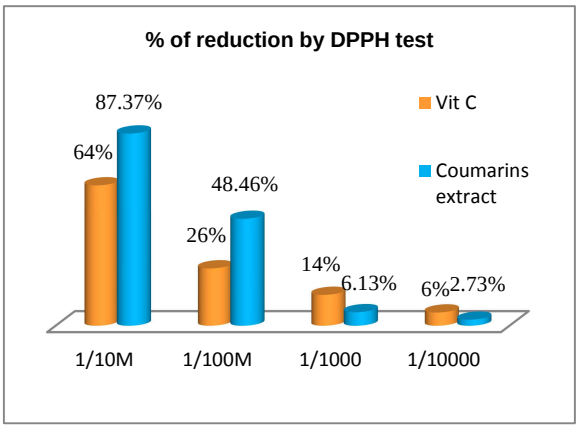

Fig. 2: Antioxydant activity of crude coumarins extract from *Ruta tuberculata* Forsk by DPPH at different concentrations

Our results demonstrate a high antioxidant activity in the coumarins extract of *Ruta tuberculata* Forsk. and stress the importance of investigations dealing with the chemical compounds.

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