Qualitative and Quantitative Phytochemical Analysis of Retama raetam (forssk) Leaves

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Abstract
Retama raetam is widespread in North Africa and considered to be a medicinal plant in folk treatment besides its importance as feed for livestock. It has been used as herbal remedy for healing several ailments like diarrhea, skin rash, sore throat, rheumatism, fever and injury sterilization. This study was conducted to estimate the medicinal and nutritional values of R. raetam plant by determining some essential contents, minerals, vitamin C and preliminary phytochemical compounds in its leaves. The primary analysis showed that the pH value of water extract was 7.18 ± 0.03 while the percentages of essential contents in leaves were 50.83 ± 1.04 crude fiber, 12.33 ± 0.76 crude lipids, 6.02 ± 0.01 crude protein, 5.00 ± 0.00 total ash content, 10.00 ± 0.00 ash soluble in water, 5.00 ± 0.00 ash insoluble in acid and 19.67 ± 0.58 moisture content.

Primary tests of the crude aqueous extract revealed the presence of tannins, saponines, alkaloids flavonoids, glycosides, resins, terpenoids and steroids, while the ethanolic extract showed positive results for tannins, alkaloids, flavonoids, glycosides, terpenoids and steroids, but negative results for saponines and resins. The percentages of phytochemical compounds in the air dried leaves powder were 5.67 ± 0.58 tannins, 10.42 ± 0.72 saponines, 8.33 ± 0.76 alkaloids and 9.80 ± 1.13 flavonoids. The amounts of minerals and vitamins (mg/100) that were determined in the R. raetam dried leaves powder were 333.75 ± 1.77 potassium, 501.25 ± 1.77 sodium, 235.50 ± 6.36 magnesium, 500.00 ± 0.00 calcium and 35.33 ± 0.29 vitamin C. The obtained results prove the traditional medicinal use of R. raetam in North and East Mediterranean regions. In addition, knowing the amounts of the essential components and bioactive compounds may help in the preparation of the exact dosages for the treatment of diseases or for livestock feeding.

Keywords: Phytochemical Analysis, R. raetam (forssk), Leaves.

Introduction
Plants have been used as natural resources for decades. People have been consuming plants as food due to their low cost, low risk and availability. Plants have also been used as medicines instead of using manufactory chemicals. Retama raetam (forssk) is one of the desert shrubs which grows in sandy and dry dune environment. It belongs to the fabaceae family, with needle leaves and white flowers. In North Africa, it is used in folk medicine as a healing plant with antibacterial, anti-inflammatory and anti-diarrheal effects. It is also used for skin rash, fever, hypertension and diabetes control as well as for livestock feeding.

The scientific evidence for different uses of Retama raetam in medicine and industry were illustrated in recent studies. For example, various medicine properties as anti-proliferative activates against breast cancer were reported by Najjaa et al. Awen et al provided a clue of using Retama raetam extract as antimicrobial activity. In addition, a recent study reported the R. raetam extract as a renewable source for the production of cellulose microfibers. Moreover, the antimicrobial and antioxidant properties of Retama raetam essential oils and their applications in food and pharmaceutical industries were demonstrated in previous works. However, not much research has been done on phytochemical analysis of Retama raetam (forssk) leaves. Utilization of crude herbs without knowing their composition or adequate doses may cause health problems or harmful side effects instead of curing diseases. Therefore, the aim of this work is to evaluate the minerals, nutrients and preliminary phytochemical compounds in the leaves of Retama raetam plant grown in Libya.

Material and Methods
Plant material: The Retama raetam leaves were collected from Sabratha city, Libya. They were cleaned, washed with distilled water and then dried at room temperature in the shade for a week. After that, the leaves were ground to fine powder and were kept for analysis.

Extraction: The extraction was prepared by the addition of 2 g of the dried leaves powder to 100 ml of the solvent (distilled water or ethanol). The solution was stirred for three hours and then filtered using a centrifuge. The filtrate was then dried in a water bath for 5 min. Brown, red or green precipitate proves the presence of glycosides.

Alternative test for Glycosides: Add 1 ml of dilute sulfuric acid to 1 ml of the Retama raetam leaves extract. Boil, filter

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and shake with the same volume of chloroform. Then, separate the lower layer of chloroform and shake it with half of its volume of dilute ammonia or ferric chloride. The formation of red or pink colour shows a positive result27.

**Alkaloid test:** Add 1 ml of Mayer’s reagent to 5 ml of the extract. The appearance of whitish creamy precipitate indicates the existence of alkaloids 23.

**Flavonoids test:** 5 ml of 1% ammonium hydroxide was added to 2 ml of the leaves extract in a test tube. The formation of yellow precipitate which disappears by adding few drops of dilute H₂SO₄ indicates the presence of flavonoids 23.

**Phenol test:** 3 ml of the Retama raetam leaves extract was added to 2 ml of 1% FeCl₃ solution. The formation of blue colour confirms the test22.

**Resins test:** 5 g of the leaves powder was dissolved in 25 ml of ethanol and then placed in a boiling water bath for 2 min followed by the addition of few drops of acidic water (HCl). Appearance of cloudy white colour indicates the presence of resins16.

**Steroids test:** 1 ml of the extract was dissolved in 2 ml of chloroform in test tube followed by the addition of 10 drops of acetic acid and 4 drops of con. H₂SO₄ with shaking. Changing the color from red to blue or green confirms the presence of steroids30.

**Terpenoids test:** 1 ml of the Retama raetam leaves extract was mixed with 2 ml of chloroform in test tube. 6 ml of con. H₂SO₄ was added carefully along the test tube wall. Formation of reddish brown ring (burnle) is an indication of the presence of terpenoids8.

**Saponins test:** 5 ml of the extract was vigorously shaken with 5 ml of distilled water. Appearance of stable froth indicates a positive result19.

**Test for tannins:** 2 ml of the leaves extract was treated with few drops of 10% lead acetate solution. Formation of white precipitate shows the presence of tannins19.

**Quantitative chemical analysis**

**pH value:** The pH of the water extract of Retama raetem leaves was measured by using a pH meter15.

**Protein:** The protein content in the Retama raetem leaves powder was calculated from the nitrogen content; 0.5 g of the plant sample was digested by the addition of 15 ml of 98% H₂SO₄ and 3–5 ml of 30% H₂O₂ (slowly) under the fume hood until clear solution was obtained.

The concentration of ammonium sulphate was then determined by Nessler's reagent method (spectrometer at 430 nm). Finally, the protein value was calculated by multiplying the nitrogen concentration by a special factor (6.25)25.

**Crude fats:** The total fat was measured according to the Soxhlet extraction method; 2 g of the powdered Retama raetem leaves was placed in a thimble using petroleum ether (60%) as a solvent. After 16 hours, the crude fat was obtained. It was then dried, weighed and the fat percentage was calculated7.

**Crude fiber:** This method depends on the removal of fats, organic and inorganic compounds. The sample was extracted using the Soxhlet method. Then, the residue was washed with H₂SO₄, boiled with NaOH solution, filtered through a filter cloth and burnt at 600°C. Finally, the percentage of the crude fiber was calculated30.

**Moisture:** Moisture was determined as loss in weight by heating 2 g of the Retama raetem leaves powder in a furnace at 105°C until a constant weight was obtained. The percentage of moisture content was then calculated30.

**Total ash:** 2 g of the sample was placed in a silica crucible and incinerated in furnace at 500⁰-600°C. The white ash was then cooled and weighed for calculation17.

**Insoluble acid ash:** 25 ml of 3M HCl was added to ash and boiled for 5 min. The remaining ash was filtered using an ashless filter paper, washed with hot water, collected in a crucible, dried in a desiccator and finally weighed. The insoluble acid ash percentage was then calculated17.

**Water-soluble ash:** 25 ml of distilled water was added to the ash, boiled for 5 min, filtered by an ashless filter paper, burnt at furnace at 500°C, cooled and dried in a desiccator. Then, the percentage of water-soluble ash was calculated6.

**Vitamin C:** The value of vitamin C was determined by the titration method (Retama leaves extract was titrated with 0.01 M iodine solution). The standard iodine solution was prepared and titrated with the standard ascorbic acid solution; 5 ml of the standard ascorbic acid solution was pipetted into 50 ml conical flask and 1 ml of the starch solution indicator was added. The solution was then titrated with iodine solution (0.01 M). Changing the color to dark blue indicates the endpoint of titration. The sample solution was prepared by mixing 2 g of the dried leaves powder of Retama raetem with 50 ml of distilled water. 5 ml of the resultant solution was poured into a 50 ml conical flask and titrated in the same way as the standard ascorbic acid. The concentration of vitamin C was calculated from the observed value of the iodine standard solution28.

**Mg and Ca values:** Determination of Mg and Ca concentrations in Retama raetem extract was carried out by the EDTA titration. It is based on the formation of a complex with Ca and Mg ions using a metallochromic indicator and the pH value needs to be 10 and 12. The estimation of Mg...
was done by subtracting Ca concentration from the Ca and Mg concentrations \(^{29,31}\).

**K and Na:** The Flame photometry was used for the measurement of K and Na. Briefly, 2g of the powdered leaves was ashed in a burning furnace at 500°C. The ash was dissolved in 5 ml of con. HNO\(_3\) and then diluted to 100 ml with distilled water to prepare a solution of *R. raetam* plant ash. After that, the sample was aspirated into a flame photometer using a filter to each selected element. The concentrations of elements were obtained using the flame photometer instrument \(^1\).

**Alkaloids:** 40 ml of 10% acetic acid in ethanol was added to 2 g of the powdered leaves of *R. raetam* plant sample in 250 ml beaker, left for four hours, filtered and concentrated on water bath. NH\(_4\)OH was then added drop wise until complete precipitation. The precipitate was filtered, washed with dilute NH\(_4\)OH, dried and weighed. Thus, the percentage of alkaloids was calculated\(^{13}\).

**Flavonoids:** 20 g of the powdered sample was extracted by using 200 ml of methanol (80%) at 25°C for 24 hours. The resultant mixture was filtered and residue was re-extracted with methanol. The whole solution was transferred to a crucible, evaporated the solvent over a water bath, cooled and dried in a desiccator. Then, the flavonoids content was calculated in percentage \(^{13}\).

**Tannins:** 0.5 g of the plant sample was extracted by 25 ml deionized water, placed in a water bath for 30 min, centrifuged at 2000 rpm for 20 min, transferred into a 100 ml conical flask and mixed with 20 ml of 4% lead acetate solution for one hour and then filled up to the mark. After that, the solution was filtered, dried, burned and weighed. The first weight (T1) was taken at 105°C while the second weight (T2) was measured after burning. Lastly, the percentage of tannins was calculated\(^{15}\).

**Saponins:** 10 g of the plant sample was extracted with 150 ml of 20% ethanol in a flask, heated in water bath at 55°C for four hours under reflux and then filtered. The filtrate was evaporated at 90°C until 20 ml of the solution remained. The remaining solution was then transferred into a separatory funnel followed by the addition of 10 ml of di ethyl ether with shaking. The solution was left for a while.

The ether layer was discharged while the water layer was kept. The process was repeated three times. Then, 30 ml of n-butanol was added to the water layer with shaking, washed with 5 ml of 5% NaCl three times, evaporated in a water bath, dried and finally, the percentage of saponins was calculated\(^{23}\).

**Results and Discussion**

The obtained results of the nutritive contents found in *R. raetam* leaves extract are shown in table 1 and table 2. As it can be seen in table 1, the percentages of contents were 6.02 ± 0.01, 50.83 ± 1.04, 12.33 ± 0.76, 5.00 ± 0.00, 10.00 ± 0.00, 5.00 ± 0.00 and 19.67 ± 0.58 for protein, crude fiber, crude fat, ash, total ash, ash soluble in water, ash insoluble in acid and moisture respectively. However, the concentrations of Na, K, Ca, Mg and vitamin C in mg/100g are given in table 2. They were 501.25 ± 1.77, 333.75 ± 1.77, 500 ± 0.00, 235.5 ± 6.36 and 35.33 ± 0.29 respectively.

Comparing our findings with previous works showed that the protein content value was lower than the one reported by Barakat et al\(^5\) for white broom *R. raetam* while the value of fiber content was higher. With respect to the ash content value, it was slightly different from the result demonstrated by Laudadio et al\(^{21}\) which was 8.5 % for white broom *R. raetam*. Also, the crude fat value was higher than that mentioned by Fadil et al\(^{14}\) for *Retama monosperma* (L.) Bois. For vitamin C, our value was lower than the value revealed by Saada et al\(^{26}\).

For the mineral contents, the amounts of K, Na, Mg and Ca were lower than that in the stem of *R. Retama plant* reported by Al-Onazi et al\(^3\). These variations are accepted since there are several factors that can affect the nutritive, minerals and vitamin C levels in *R. retama* plant such as climate, soil, light, temperature, harvesting time, analytical method and fresh or dried material used\(^5,26\). In addition, the part and type of the plant used may affect the previous mentioned levels.

Table 3 presents the preliminary phytochemical screening of *R. raetam* leaves extracts. The aqueous extract showed various active chemical compounds; tannins, saponins, alkaloids, flavonoids, glycosides, resins and steroids, whereas the ethanol extract was found to contain tannins, alkaloids, flavonoids, glycosides, terpenoids and steroids while saponin and resin were absent. These results show agreement with a previous study conducted by Alfalluos et al\(^2\) except for terpenoids.

Table 4 shows the quantitative analysis of the aqueous extract of *R. raetam* leaves. Tannins were 5.67 ± 0.58%, saponins were 10.42 ± 0.72%, alkaloid were 8.33 ± 0.76% and flavonoids were 9.80 ± 1.13 % which are in a good agreement with the results reported by Alfalluos et al\(^2\).

**Table 1**

<table>
<thead>
<tr>
<th>Protein (%)</th>
<th>Lipid (%)</th>
<th>Ash (%)</th>
<th>Moisture (%)</th>
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<tr>
<td>6.02 ± 0.01</td>
<td>12.33 ± 0.76</td>
<td>5 ± 0.00</td>
<td>19.67 ± 0.58</td>
</tr>
<tr>
<td>50.83 ± 1.04</td>
<td>19.67 ± 0.58</td>
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*W.S =10 ± 0.00, Ins.Acid = 5± 0.00*
These findings demonstrate the customary medication utilization of *R. raetam* in North and East Mediterranean areas due to containing various phytochemical components such as saponins, alkaloids, flavonoids, glycosides, resins, terpenoids, steroids and tannins.

Likewise, knowing the measures of the fundamental parts and bioactive mixtures might help in the arrangement of the specific doses for the treatment of sicknesses or for animals taking care of.

**Conclusion**

Based on the above findings, it is clear that the leaves of *R. raetam* plant can be used in medicinal and synthetic purposes as useful source of different phytochemical components such as saponins, alkaloids, flavonoids, glycosides, resins, terpenoids, steroids and tannins. It is also due to its richness in fiber and having good values of nutritional components. In addition, knowing the amounts of the essential components and bioactive compounds present in this plant may help in the preparation of the actual dosages for the treatment of diseases.

However, further research extended to other parts of *R. raetam* plant is suggested in order to get further information and validation for using this plant. Studying the effects of the plant growth stages and climate change on the amounts of nutrients and bio active compounds is also needed.

**References**


of Analgesic Activity of Oroxylum indicum, Indian J Pharm Sci., 76(6), 571 (2014)


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