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Research Article

Qualitative and Quantitative phytochemical Analysis and Antimicrobial Activity of "*Retama" E*xtract Grown in Zliten Libya

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ABSTRACT: Medicinal plants gain attention due to theirs extensive treatment and have fewer side effects. In actual fact, plants were the mere basis of healing till the initiation of manufactured pharmaceutical products during previous years. The aim of this study was to investigate the qualitative and quantitative phytochemical analysis and anti-bacterial activity for medicinal plant *"Retama", "raetem (forssk) Webb".* The plant "*Retama"* was extracted by using Soxhlet apparatus with appropriate solvents (aqueous and ethyl alcohol separately), The phytochemical screening (qualitatively) of leafs crude extracts showed a presence of Carbohydrates, Tannins, Alkaloids, Carbohydrates, Flavonoids, Cardiac Glycosides, Saponins, Fatty Acids, Protein, Amino Acid, Steroids, and Flavonoids. And absent of Fatty Acids, Coumarins, Anthraquinone and Terpenoids. In addition the phytochemical screening (quantitatively) showed the highest concentrations were saponins (10%), Flavonoids (9%) and then alkaloids (8%) were observed. The antibacterial activity of leafs crude extracts (Aqueous and Ethanolic) of the plant was confirmed by using two Gram-positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus epidermis*) and two Gram-negative bacterial strains (*Klebsiella pneumonia and Escherichia coli*). Where was determined by Disc Diffusion Method on nutrient agar medium. The treatments also included of used solvents and Antibiotics (Augmentin, Amoxicillin, Tetracycline, Ciprofloxacin, Ceftriaxone and Cefotaxime) attended as a standard controls. The late were incubated for 24 h at 37ºC and zone of inhibition if any around the discs were measured in mm (millimeter). The tested plant crude extracts have a good antibacterial activity against *E. coli* for aqueous extract with an inhibition zone of 11mm and with an inhibition zone of 12mm for ethanolic extract and *Staphylococcus Aureus* with an inhibition zone of 9mm for ethanolic extract and with an inhibition zone of 8mm for aqueous extract and *Staphylococcus Epidermis* with an inhibition zone of 8mm for ethanolic extract, and with an inhibition zone of 9mm for aqueous extract, while moderate antibacterial activity against *Klebsiella pneumonia* with an inhibition zone of 7mm for ethanolic extract and with an inhibition zone of 6mm for aqueous extract compared with appropriate antibiotics used.

Key Words: Phytochemicals, medicinal plants, bioactive compounds, Antibiotics, Anti-Bacterial.

Introduction:

Plants remain inactive considered by way of believable bases of novel therapeutic composites. All over the environment, plants exist dispensed with habitually to treat numerous illnesses. Various studies have proved that medicinal plants contain several bioactive constituents which are accountable for their beneficial wellbeing properties, "*Retama raetam (Forssk.) Webb"* **("***Raetam"***)** is one of the species of family "*Fabaceae"* and comprises four species spread in the Mediterranean and North Africa. "*Retama"* a spontaneous plant common in the North and East Mediterranean region, is a desert shrub common to several countries of North-Africa[1]. *Retama* in Libya, found in North and Sahara, and is used in traditional remedy with the public name "*Ratam*" with regard to skin disease and decrease the blood glucose[2], however in other Mediterranean countries used as traditional herbal medication against joint aches and skin inflammations[3, 4]. Moreover, this plant used as emetic and an abortifacient in the traditional medicine systems of the source countries[5]. And the earlier pharmacological revisions on this plant have exposed its diuretic and microbial and hypoglycemic properties and hepatic protective and cytotoxic effects[6-12]. The aim of this study was the phytochemical screening and to evaluate the antibacterial activity of Crude extracts of plant **("***Raetam"***)** species used in traditional herbal medicine.

Materials and Methods:

Plant Material:

The plant material was collected from Montareha area (Zliten region, Libya) during April-May (2016). Plant was authenticated at Botany Department, Science College El-Mergeb University Al-Khums Libya. A voucher specimen was kept in dark bottles until furthered use.

Preparation of plant extract:

Collected plant leaf was dried for two days at room temperature (in shade) then finishing drying was in oven at 50 ^{0}C. Dried plant was grinded in a blender to fine particles. Crude plant extract was prepared by Soxhlet extraction method. Water and Ethyl alcohol was selected for the study. 20 gm of dried fine grinded powder was uniformly packed into thimble and phytochemicals were extracted with 400 mL of mentioned solvents separately. The extraction was carried out for 6-8 hours. Later the extract was filtered using Whatmann no. 1, concentrated in vacuum under reduced

pressure and dried in the desiccator[13], and then stored in the refrigerator at 4 ° C until use.

Phytochemical screening:

Qualitative study:

Phytochemical qualitative study of plant extracts of Leafs was carried out for the given study.

Test for carbohydrates:

Molisch's test: To 4 mL of the test solution, 2mL of α-naphthol solution was added, concentrated Sulphuric acid (H_2SO_4) was poured through the sides of the test tube. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.

Braford's test: To 1mL of test solution, 6 mL of Braford's solution was added; the tubes were placed in a boiling water bath a rusty or brownish-red color indicated presence of monosaccharides.

Benedicts test: To 1 mL of test solution, 6 ml of Benedict's reagent was added. The test tubes were shaken to assure uniform mixing. The tubes were placed in a boiling water bath for 3 minutes. Red or green or yellow ppt. was obtained and showed presence of reducing sugar

Tannins test:

The extract of the sample was treated with 15% ferric chloride test solution. The resultant colour was noted. A blue colour indicated the presence of hydrolysable tannin or into 5 mL of freshly prepared potassium hydroxide (KOH) in a beaker; 1ml of the extract was added and shaken to dissolve. A dirty precipitate observed indicates the presence of tannin[14, 15].

Test for alkaloids:

The plant extracts was dissolved in chloroform and the solution was extracted with dil. H_2SO_4 and acid layer taken and tested for presence of alkaloids:

Dragendroff's test: To 5 mL of acid layer of test solution, 5 mL of Dragendroff's reagent (potassium bismuth iodide solution) and 5mL of dil. HCl were added. An orange-red precipitate indicated the presence of alkaloids.

Mayer's test: To the 5mL of acid layer of test solution, 5mL of Mayer's reagent (potassium mercuric iodide solution) was added. Whitish or cream colored precipitate indicated the presence of alkaloids.

Wagner's test: To the 5mL of acid layer of test solution, 5mL of Wagner's reagent (iodine in potassium iodide) was added. Reddish-brown colored precipitate indicated the presence of alkaloids[16].

Cardiac glycosides:

Keller-Kilian's test: 0.5 mL of glacial acetic acid was dissolved in 50 ml of test solution containing one drop of ferric chloride solution. This was then under layer with 0.5 mL of concentrated Sulphuric acid. A brown ring obtained at the interface indicated the presence of Cardiac glycosides[17].

Test for Saponins:

1mL of the plant extract was shaken with water in a test tube. Frothing, which persist on warming was taking as a preliminary evidence for the presence of saponins. Few drops of olive oil was added to 1ml of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicated the presence of Saponin[18].

Coumarins test:

In a test tube, 2 ml of each of the test solution were placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated on water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indicated the presence of coumarins[19].

Fatty acids test:

10 mL of test solution was mixed with 10 mL of ether. This extract was allowed to evaporate on filter paper and dried the filter paper. The transparency on filter paper indicates the presence of fatty acids[20].

Test for Flavonoids:

Shonda's test: About 2 ml of each of the extracts was dissolved with 10 ml of ethanol (98 %). To this a small piece of magnesium foil metal was added, this was followed by drop wise addition of concentrated hydrochloric acid. Intense cherry red colour indicated the presence of flavonones. Orange red colour indicated the presence of flavonols[21].

Lead acetate test: Few drops of lead acetate solution were added to each of the extracts in test tubes. Formation of yellow colored precipitate indicated the presence of flavonoids[22].

Alkaline reagent test: About 2 ml test solution was treated with few drops of sodium hydroxide solution and observed for intense yellow coloration which disappeared on the addition of dilute HCl[23].

Gum and Mucilage test:

The plant extract was dissolved in 20 mL of distilled water and to this; 50 mL of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage's[24].

Protein and Amino Acid:

Ninhydrin test: 2-3 drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) was added to the small quantity of extract solution and heat it. Development of blue color reveals the presence of proteins, peptides, or amino acids[25].

Test for Phenols:

Ferric chloride test: To 5 mL of alcoholic solution of extract, 1 mL of distilled water followed by few drops of 10% aqueous Ferric chloride ($FeCl_3$) solution was added. Formation of blue colour indicates the presence of phenols[26].

Test for Steroids:

Liebermann Burchard Test: To 2mL of extract, 2mL of glacial acetic acid and 2mL of acetic anhydride and 3-4 drops of concentrated Sulphuric acid were added. The solution becomes red, then blue and finally bluish green, indicates the presence of steroids[27].

Anthraquinone:

Borntrager's test: About 1ml of the test solution was taken into a dry test tube and 10 mL of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer)

indicated the presence of Anthraquinone[28].

Test for terpenoids:

Salkowski's test: 1 mL of each the extract was added to 4 mL of chloroform. 6 mL of concentrated Sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids[29].

Phytochemical quantitative test:

The extracts were subjected to quantitative phytochemical tests for plant secondary metabolites such as alkaloids, flavonoids, saponins.

Alkaloid determination:

In 500 mL conical flask, 10g of the dried fine powdered plant leaf sample was taken and 400 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed[30].

Flavonoid determination:

5g of the plant sample was extracted repeatedly with 50 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was evaporated into dryness over a water bath and weighed[31].

Saponin determination:

In conical flask, 10 g of dried fine particles plant sample was taken and 50 mL of 20% aqueous ethanol was added. This mixture was heated (55˚C) on water bath for 4 h with continuous stirring. Later the mixture was filtered and the residue re-extracted with another 100 mL of 20% ethanol. This extract was further reduced to 20 mL over hot water bath (90˚C). The concentrated extract was transferred into a 250 mL separating funnel and 10 mL of diethyl ether was added and shaken vigorously. Ether layer was discarded and aqueous layer was collected. This step of purification was repeated. 30 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 5 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven and weighed[32].

Anti-bacterial activity assay:

The antibacterial activity of leafs extracts (Aqueous and Ethanolic) of the plant was confirmed using Gram-positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus epidermis*) and Gram-negative bacterial strains (*Klebsiella pneumonia and Escherichia coli*). Where was determined by Disc Diffusion Method on nutrient agar medium[33]. Sterile Whatmann filter discs (6 mm diameter) were placed on nutrient agar plates and inoculum containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all aqueous and solvent extracts were placed in the discs made in inoculated plates. The treatments also included 50 µl of solvents served as control and Antibiotics (Augmentin, Amoxicillin, Tetracycline, Ciprofloxacin, Ceftriaxone and Cefotaxime) as a standard control. Then were incubated for 24 h at 37ºC and zone of inhibition if any around the wells were measured in mm (millimetre). Each treatment was repeated least twice replicates **.**

Results and Discussion:

Table 1 preliminary phytochemical screening of "*Retama"*:

Phytochemicals	**Plant Name / Tests**	***Retama***	
		Water Extract	**Ethanolic Extract**
Carbohydrates	**Molisch's test**	+	+
	Braford's test	+	+
	Benedicts test	+	+
Tannins test	**Using $FeCl_3$**	+	+
Alkaloids	**Dragendroff's**	+	+
	Mayer's test	+	+
	Wagner's test	+	+
Cardiac Glycosides	**Keller- Killani test**	+	+
Saponins	**Frothing test**	+	-
Fatty acids test	**Paper test**	-	-
Flavonoids	**Shinoda test**	+	+
	Alkaline reagent test	+	+
	Lead acetate test	+	+
Gum and Mucilage		+	-
Protein and Amino Acid	**Ninhydrin test**	+	+
Phenols	**Ferric chloride test**	+	+
Coumarins	**Alkali test (Using NaOH)**	-	-

Steroids	**Liebermann, Burchard reaction**	+	+
Anthraquinons	**Borntrager's test**	-	-
Terpenoids	**Salkowski's test**	-	-

+ = Present, - = Absent.

Obtainable of the some sorts available in Libya, the *"Retama (raetem (forssk) webb)"* were used in indigenous medicine and traded under the name *"Ratam".* Where the preliminary qualitative analysis (table 1) showed its leafs contained Carbohydrates, Tannins, Alkaloids, Carbohydrates, Flavonoids, Cardiac Glycosides, Saponnins, Fatty Acids, Protein, Amino Acid, Steroids, and Flavonoids. And absent of Fatty Acids, Coumarins, Anthraquinone and Terpenoids

The steroidal saponins were reported to have, anti-inflammatory, immune-stimulant, and immune-adjuvant, antitumor and antibacterial effects[34]. Furthermore extensively used as hemostatic and diuretics[35]. "*Retama"* generally produce several secondary metabolites like alkaloids, flavonoids, phenols, tannins, saponins and quinines which are important sources of biocides and many other pharmaceutical drugs[36-43]. These significant secondary plant metabolites have typical biological activity[44]. Moreover, traditionally, the powdered leafs is used to heal circumcision wounds and as an antiseptic for wounds, pruritus, skin rashes, and in the supervisory control of microbial infections[45].

Table 2 The quantitative analysis of "*Retama"*:

Plant Name	**Percentage Yield %**			
	Chemical Constituents	**Alkaloids**	**Flavonoids**	**Saponins**
Retama	**17**	**8**	**9**	**10**

Table 2 showed the results obtained from the quantitative analysis of "*Retama"* extracts of selected medicinal plant whereas the presence of phytochemicals percentage yield (17%) and the clearly indicated that the highest amount of was the saponnins (10%), flavonoids (9%) and alkaloids (8%).

Table 3 results of the *Retama* extracts against *Escherichia coli, Staphylococcus aureus* and *Enterococcus sp.*:

Bacteria spp. / **Plant Extracts & Antibiotics**		**Klebsiella pneumoniae**	**Escherichia Coli**	**Staphylococcus aureus**	**Staphylococcus Epidermis**
"Retama"	**Aqueous Extract**	**6 (mm)**	**11 (mm)**	**9 (mm)**	**8 (mm)**
	Ethanolic Extract	**7 (mm)**	**12 (mm)**	**8 (mm)**	**9 (mm)**
Augmentin		**R**	**R**	**R**	**R**
Amoxicillin		**R**	**R**	**R**	**R**
Penicillin		**S**	**S**	**R**	**R**
Tetracycline		**S**	**S**	**R**	**R**
Ciprofloxacin		**R**	**S**	**S**	**S**
Ceftriaxone		**R**	**S**	**S**	**S**
Cefotaxime		**R**	**S**	**S**	**S**

R = Resistant, S = Sensitive, (mm) = millimeters

Table 3 showed the results of Antibiotics were used as reference drugs for definition of antimicrobial activities and compared with the activities of the plant crude extracts (aqueous and ethanolic) such as standard drugs (Augmentin, Amoxicillin, and Penicillin etc.…). The tested plant crude extracts have a good antibacterial activity against *E. coli* for aqueous extract with an inhibition zone of 11mm and with an inhibition zone of 12mm for ethanolic extract and *Staphylococcus Aureus* with an inhibition zone of 9mm for ethanolic extract with an inhibition zone of 8mm for aqueous extract and *Staphylococcus Epidermis* with an inhibition zone of 8mm for ethanolic extract, with an inhibition zone of 9mm for aqueous extract, while moderate antibacterial activity against *Klebsiella pneumonia* with an inhibition zone of 7mm for ethanolic extract with an inhibition zone of 6mm for aqueous extract compared with appropriate antibiotics used. This may be deals with the fact that on the site of flavonoids compounds the number of hydroxyl groups determines the toxicity against the microorganisms[45]. And also, related to the antimicrobial effects of flavonoids to their capacity to form complexes with extracellular and soluble proteins and with the cell wall. The antibacterial activity against strains was achieved by *"Retama"* conceivably owing to their high content of chemical constituents such as Flavonoids and Phenolics; also, its activity may be to presence of its high content of the Alkaloids.

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

All authors took part in experimental project, gaining of data, interpretation and writing of the manuscript. All authors read and approved the final version of manuscript.

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