

Study of Phytochemistry, Biological, activity, total phenol And Antioxidant Activities Of L
.shawi . f and C Citratus Plants in Libya

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ABSTRACT :This study involves phytochemical screening, Antibacterial Activity, antioxidant, total phenolic content, for the medicinal indigenous plants from Libya (Lycium shawi ,Cymbopogan Citratus i). Leaves extracts of the selected medicinal plants Phytochemical screening: The results of phytochemical screening of methanolic and chloroform extracts obviously show that presence of each tannin, phenols, flavonoids, alkaloids, saponins, cardiac glycosides and carbohydrates. Antibacterial Activity: Through Disk Diffusion Method, extracts were examined against the activity of Gram-negative pathogenic bacteria Escherichia coli, where, the methanolic extracts revealed a good inhibition effect result, Whereas chloroform extracts revealed a vulnerable inhibition effect result against Escherichia coli. Antioxidant Activity: Antioxidant potential of the extracts was analyzed as contents of total phenols and free radical scavenging activity extract were assessed by the declaration of a methanolic solution of 2, 2- diphenyl-1-picrylhydrazyl DPPH. Results of antioxidant activities were determined in three concentrations: 50mg/ml, 100 and 200 mg/ml of each extract. And showed that at increasing the concentration, the susceptibility extracts tending to increase also for DPPH.

Keywords: Medicinal Plants, HPLC, Phytochemical screening, Antibacterial Activity, Antioxidant Activity

Introduction

Libya possesses many natural resources, as Libya is considered among the countries rich in various herbs and plants, especially medicinal plants, although it is a country that has a very large area (Watanabe K., et al . 2007). Lycium shawii, desert thorn or Arabian boxthorn is a species of thorny shrub adapted to desert environments. The thin leaved, rigid bush grows up to three meters high with lots of branches and alternating spines that vary in size and grow along the branches and on their tips. The leaves narrow towards their base. It produces small pink or purple (Fukuda T., et al., 2001. Cymbopogan Citratus, are commonly cultivated as culinary and medicinal herbs because of their scent, resembling that of lemons). It is often used as a tea in African(Market et. al .,2006). Hence, presence of flavonoids widely in photosynthesizing cells in the plants, and therefore making that photosynthesizing becomes occurred considerably in it(Manthey J,etal .2001) .Besides, flavonoids have many and varied benefits, particularly against bacteria, as they act as a blocker to manufacture DNA, energy metabolism inhibitor, cytoplasmic membrane function or perhaps prevent the growth of bacteria and thus reduce the increase in the number of bacteria colonies as reported in several earlier

studies(Cushnie.T.p.T,etal .2005) . Also, existence of hydroxyl groups in flavonoids compounds and which own a potent antimicrobial agent against microorganisms (Suzgec .S ,etal.2011) Terpenes possess a powerfully effective toward fungi and bacteria (Trapp. S. C,etal 2001) Additional, the terpenoids considered as the most various classes and the most inclusive of plant. this plant was used in old and nowadays for diseases therapeutics. Its leaves and young stems contain phenols, alkaloids, flavonoids, saponins and amino acids, also, known to be used as anthelmintic, as spasmolytic, and abortion-inducing drug (Zeichen. R, etal. 2000). As for a plant *L. shawii*, is a native plant of many Arabian countries (Akindele, A.),eta.2007). Were several reported that generally used in conventional remedies to heal coughs, stomach, jaundice and mouth sores, Moreover, as hypotensive, antidiuretic and antioxidant potential . The chemical ingredients occur in this plant play a vital role in the identification and production of raw medicines which leads to the remedy of human ailments. While *C. Citratus* plant is the medicinal and pleasant plant where that is frequently utilised of past time until now by Libyan folks to healing many ailments such as headache, stomach and high fever. In addition, the *C. citratus* has a great property, which makes it a broadly used, for their pleasing flavour and healthful characteristics, and for its conventional aromatic usage and its therapeutic value, perhaps use in the nutrition manufacturing, also, in pharmaceutical and cosmetics production (Mirghani, M.E ,etal,2012) . The environment of plant living in, seasons, dates of the collection, plant chosen parts and methodology of extraction, will be affecting the presence and quantity of the chemical constituents in it. Phytochemicals ingredients in *E. lathyris* are perhaps present as free and bound Compositions, were produced in diverse metabolites, such as Diterpene (Hou, X.R;etal 2011), (Huang, J; etal 2014), flavones, and flavonol glycosides' (Surveswaran. S,etal.2007], and were considered as biological importance this reagent and with the Folin reagent which is used to recognise compounds containing sulfur and amines also. Whereas, the reagent measures the total reducing capacitance of a specimen, not merely the scale of phenolic composites, likewise, it is a portion of the Lowry protein examination, and will similarly react with some compounds containing, such as guanidine and hydroxylamine. Tri-hydroxy benzoic acid or 3,4,5-trihydroxy benzoic acid or as known by Gallic acid is one of the phenolic acid types, which is present in many plants such as tea leaves, gallnuts, sumac, oak bark, and witch hazel. The gallic acid has a chemical formula $C_6H_2(OH)_3COOH$. Gallic acid's ..

Material and Methods:-

Preparation of plant extract :- (Collection)

The Fresh plant of *L. shawii*, *C. Citratus*, were collected from the wild nearby Al-khums region .. The leaves of the freshly collected plants were separated and washed by tap water then by distilled water, after that at room temperature 25 ± 2 °C were air-dried in shadow for 72 hours, followed then drying was ended in the oven for 43 °C also for 72 hours, after which was grounded sieved obtain a fine uniform powder, and to obtained a mean particle size $d = 0.388$ mm, it was determined by knowing of the size of the sieve pore. (Erweka, Germany) (Handa et al. 2008)

Extraction:

The active chemical components were separated from the leaves of the selected medicinal plants by using soaked as brif :- 20 g from a fine powder of each plant's leaves wer esoaked separately, in stoppered Erlenmeyer flask (1000 ml capacity) then a 500 ml of an appropriate solvent (Methanol, Chloroform, separately) were added and left to stand at room temperature for 72 hours with frequent agitation, separately. after that, the mixture was filtered off through a Whatman No. 1 (122 mm) filter paper, the obtained crude extracts were concentrated by using a rotary evaporator (45°C). The dried yield was stored in the refrigerator at 4 °C until further use.

Phytochemical analysis

The phytochemical analysis of the dried yields was carried out to determine the presence of the following bioactive components using the standard qualitative and quantitative procedures (Kolkata CK.2005),(Ansari .SH 2006),(Edeoga HO, 2005) .

Qualitative Analysis

1 - Detection of Tannins : a) Ferric Chloride Solution. b) Sodium chloride solution. 2 -Detection of cardiac glycosides. 3 - Detection of Alkaloids a) Dragendorff's test. b) Hager's test. c) Wagner's test. 4- Detection of carbohydrates. 5 -Detection of Flavonoids: a) Alkaline Reagent Test . b) Shinoda Test. c) 6- Lead acetate test : a) Foam Test . b) Froth Test .

7-Antibacterial Activity

Through Disk Diffusion Method, extracts are examined against the activity of Gram-negative pathogenic bacteria Escherichia coli. .

Disk Diffusion Assays were applied for the antibacterial examination. Where, a pathogenic Gram-bacterium Escherichia coli were used against the selected crude extracts (Bauer et al, 1966). Bacterial strain and extracts were inoculated on a blood agar plate.

Bacterial growth inhibition test of plant extracts by the Disk Diffusion Method

The dried crude extracts were dissolved in methanol, separately, after those were filter sterilized by using 0.45 µm membrane filters (Whatman Co. UK). A 5 µl of each extracts 66 mg/mL (the disk concentration was 0.33 mg/disk) were impregnated into 6 mm, sterile filter paper disks. With each extract were control disks were also prepared with methanol, water, and chloroform. Subsequent, culturing bacteria on blood agar, the disks were fixed on the same plates and incubated for 24 hours at 37°C. The diameters of the inhibition zones were estimated in millimetres and compared with those of the control disks. The controls were the solvents used for each extract and they showed no inhibitions

8-Determination of Total Phenol

The total phenolic content was determined with the Folin-Ciocalteu reagent according to a procedure described by (Singleton, V. L, Rossi,J.A.,1965) A short time, 0.50 ml of the diluted sample was reacted with 2.5 ml of 0.2 Mole/L Folin-Ciocalteu reagent for 4 min, and then 2 ml of saturated sodium carbonate solution (About 75 g/L) was added to the reaction mixture. The absorbance readings were

taken at 760 nm after incubation at room temperature for 2 h. Gallic acid was used as a reference standard, and the results were expressed as milligram Gallic acid equivalent (mg GAE) /g dry weight of the plant material.

9-DPPH scavenging activity

The free radical scavenging activity of the crude extract was evaluated by using a methanolic solution of 2, 2- diphenyl-1-picrylhydrazyl (DPPH), (Lee et al.,2002). The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. The DPPH scavenging effect was calculated by using the equation: % Scavenging Effect = [(ADPPH – AS) / ADPPH] × 100 Where AS is the absorbance of the solution when the sample extract was added while ADPPH represents the absorbance of the DPPH

Results and Discussion

Detection of Tannins

a) Ferric Chloride Solution:- The presence of a deep blue-black colour b) Sodium chloride solution:- The appearance of blackish-green colour indicates the presence of tannins.

Detection of cardiac glycosides

Methanol crude extract:- The ammoniacal layer turned pink or red if cardiac glycosides were present

Chloroform crude extract:- The persistent blue colour appeared in the acetic acid layer if cardiac glycosides were present~

Detection of Alkaloids

a) Dragendorff's test :- A reddish-brown precipitate was observed indicating the presence of alkaloids.

b) Hager's test:- The yellow precipitate was formed reacting positively for alkaloids.

Wagner's test:- Reddish-brown precipitate indicating the presence of

Detection of carbohydrates

Violet ring was formed at the junction of the two liquids, indicated the presence of carbohydrates.

Detection of Flavonoids.

Alkaline Reagent Test:- Development of a deep yellow colour, the presence of flavonoids

b) Shinoda Test:- The appearance of magenta colour after a few minutes indicates the presence of flavonoids.

c) Lead acetate test:- White precipitates appeared indicating the presence of flavonoids

Detection of saponins

Foam test:- If foam produced persists for ten minutes it indicates the presence of saponins.

Froth Test:- Formation of stable foam (1 mm) indicates the presence of saponins.

.Table 1: The Phytochemical Analysis of Plants for this study

Plant's Names Constituents & Reagent	<i>L. shawi</i>		<i>C. citratus</i>	
	MeOH	CHCl ₃	MeOH	CHCl ₃

Flavonoids	<i>Lead acetate</i>	+++	+++	+++	+++
	<i>Alkaline</i>	+++	+++	++	++
	<i>Shinoda</i>	++	++	++	++
Alkaloids	Wagner's	++	++	+++	++
	Mayer's	++	++	++	+++
	Dragendorff's	+	+	+++	++
Cardiac Glycosides		+++	+++	+++	+++
Saponins	Foam	++	+++	++	+++
	Froth	+++	+++	++	+++
Tannins	Ferric Chloride	+++	+++	+++	+++
	chloride solution	+	-	+	-
Carbohydrates'		+	+	+	+

Key: - = none, + = present, ++ = average existence, +++ = abundant

As shown in Table 1, the phytochemical revelation of the active chemical components contained which are flavonoids, alkaloids, cardiac glycosides, saponins, Tannins and carbohydrates, in the leaves of the two plants showed that these components ranged in presence from few, moderate and to abundant. Generally, saponins, alkaloids, carbohydrates, cardiac glycosides, flavonoids, and Tannins are the major active constituents of, *L. shawii*, *C. Citratus*, plants and which are mostly rich by it.

Antibacterial Activity

Table 2: Antibacterial activity of leaves extracts of selected studied plants:

Plant's Names	Plant's Extracts	Bacterial Name & Diameter of the zone of inhibition (mm)
		<i>Escherichia coli</i>
<i>L. shawi</i>	MeOH	9
	CHCl ₃	7
<i>C. citratus</i>	MeOH	12
	CHCl ₃	-

mm = millimeter.

The biological effectiveness of the plant depends on various circumstances, similar to soil conditions, geographical source, the moisture and the period time of harvest.

Standard Antibiotics Amikacin 30 Mg (17 mm)

As showed in Table 2 the results from the current investigation revealed that at most limited one of these selected plants exhibited strong antibacterial activity activities against the gram-negative pathogenic bacteria *Escherichia coli*, where, the methanol fraction extract revealed exhibited a broad

spectrum of activity, whereas, the chloroform extracts revealed a weak inhibition effect result against the same sort of bacteria.

Among the uses of the *L. shawii* plant to treat many diseases, its use to treat high blood sugar level, that meaning this plant has the hypoglycemic potential or a decrease in glucose level in blood. On the other hand, from the phytochemical analysis results of *L. shawii* and which showed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, carbohydrates and coumarins, such these groups belong to natural products own deferent biological activities, also such compounds were earlier mentioned to possess anti-diabetic effects (Malviya N,etal.2010), (Liu EH,etal.2010)

In addition to what was previously mentioned, medicinal plant and herbs are considering as a sources of medications were used since more than a horn in the treatment of different microbial contagion, particularly, when are used perfectly, and hence by avoiding its toxicity effect. *C. Citratus* very described as a treatment as anti-microbial and for anti-diabetic also (Melo SF.2001) .

Antioxidant capacity

Total phenol concentration (TPC) in the methanolic:

Table 3: the concentration of total phenols in the methanolic extract

Plant's Names	Total phenols (mg/g)
	MeOH
<i>R. chalepensis</i>	3.164
<i>L. shawi</i>	3.385
<i>C. citratus</i>	16.737

Gallic acid was employed as calibration standard, and results were calculated as Gallic acid equivalents (GAE) per gram of dry weight basis (mg/g) And as shown in Table 3 that the total phenols in methanolic extract's results of measuring samples were, 3.385 and 16.737 mg/g, *L. shawi*, *C. citratus*. That is, the higher the concentration of the phenols, the less concentration of DDPH.

Specifically the higher the concentrations of the phenols are the less concentration of DDPH. (Deriding, et al. 2007). Therefore, and undoubtedly, these leaves of the selected plants in this study are contained antioxidants ingredients.

Table 4 : DPPH radical-scavenging activity

Sample concentration	1,69/10ml	
	<i>L. shawi</i>	<i>C. citratus</i>
50 ml	59.39	69.73
100 ml	63.60	70.88
200 ml	64.75	74.71

The natural antioxidants found in medicinal plants have a very large role in preventing various diseases in the human body. The free radical scavenging activity and the antioxidant were estimated by many standard processes using a spectrophotometer. The DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) is a free radical that receives an electron or hydrogen radical to become a permanent diamagnetic particle (Soares JR,etal.1997). The reduction ability of DPPH radical was defined by the decrease in absorbance caused by plant antioxidants.

Statistical analysis

Correlations test

To find out the strength of the relationship between the variables and the direction of this relationship

Table 5: Correlations Anioxidant concentration of L.Shawi

Correlations			
Descriptive	Descriptive Statistics	Anioxidant concentration	L.shawi
Anioxidant concentration	Pearson Correlation	1	0.873
	N	3	3
L.shawi	Pearson Correlation	0.873	1
	N	3	3

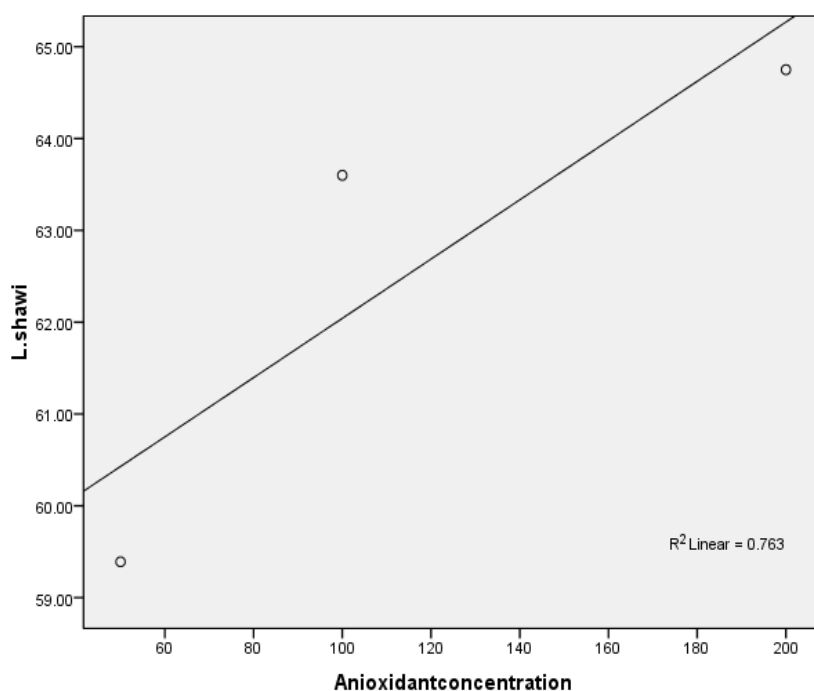


Fig 1: correlation analysis of (L.shawi)

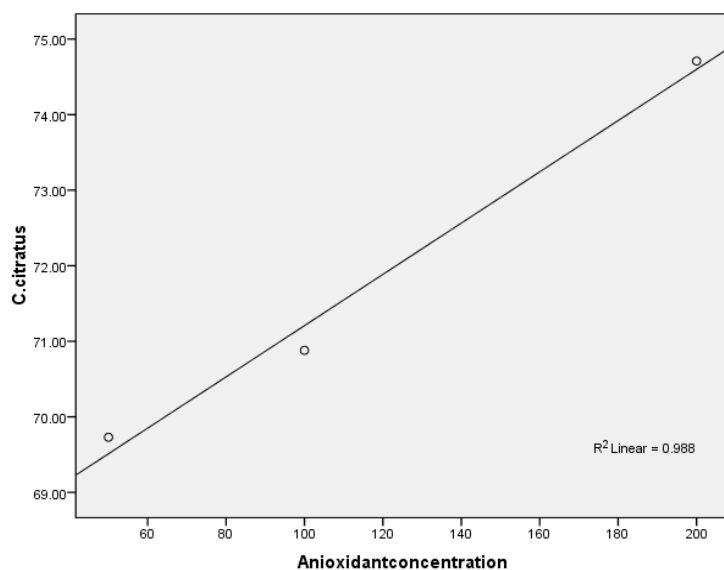
According to the results in table 4.6 the value of correlation is 0.873 and the value of R2 is 0.763 that means There is a strong significant positive relationship, between Anioxidant concentration and L.shawi

Correlations of C.citrus

Table 6 Correlations Anioxidant concentration of C.citrus

Correlations			
		Anioxidant concentration	C.citrus
Anioxidant concentration	Pearson Correlation	1	0.994
	N	3	3
C.citrus	Pearson Correlation	0.994	1
	N	3	3

The results showed in table 4.8 that the value of correlation is 0.994 and the value of R2 is 0.988 that means There is a strong significant positive relationship, between Anioxidant concentration and C.citrus



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(Fig2 correlation analysis of C.citrus

The results showed in table 4.8 that the value of correlation is 0.994 and the value of R2 is 0.988 that means There is a strong significant positive relationship, between Anioxidant concentration and C.citrus

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Search title	Page number
Allelopathic effect of Rhazya stricta plant residue on carbohydrate contents of Raphanus sativus (Radish) of 30-day-old Salma Mohammad Abad	6
Study of Phytochemistry, Biological, activity,total phenol And Antioxidant Activities Of L shawi . f and C Citratus Plants in Libya 1 Fatma Kahel . 2 Ismael Abd-Elaziz	13