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Phytochemical Screening, Antioxidant Activity and Total Phenols of "*Sonchus Oleraceus Asteraceae*" and *"Ruta Graveolens"* from Libya

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Abstract: ***This study includes phytochemical screening, antioxidant activity, and total phenols whither qualitative and quantitive analysis of primary and secondary metabolites such as carbohydrates, tannin, flavonoids and phenol from "Sonchus Oleraceus Asteraceae" and "Ruta Graveolens" leaves. The results of qualitative phytochemical screening and the quantities revealing that the chemical constituents in the crude plant extracts (Methanolic and chloroform) show the presence of flavonoids, alkaloids, tannins, cardiac glycosides, and phenols were not present in the methanolic extracts, while saponins, Alkaloids, Cardiac glycosides, Carbohydrates, saponins, Phenols were not present in the chloroform extract of "Sonchus Oleraceus Asteraceae" , and presence of flavonoids, alkaloids, tannins, cardiac glycosides, Carbohydrates and phenols in the methanolic crude extract of "Ruta Graveolens" while chloroform crude extract revealing the presence of Tannin, flavonoid and phenols. The quantitative analysis of the plants leaves "Sonchus Oleraceus Asteraceae" for alkaloids, flavonoids and saponins were 17, 10 and 12 % respectively. Whereas the percentage yield for methanolic and aqueous extracts 15 and 4% correspondingly. And the quantitative analysis of the "Ruta graveolens" for alkaloids, flavonoids and saponins were 14, 7 and 3 % respectively. While the percentage yield for methanolic and aqueous extracts were 16 and %5 correspondingly. The total phenols of methanol and chloroform crude extract concentrations in "Sonchus Oleraceus Asteraceae" were more than the concentration of phenol in the "Ruta graveolens" (3.34mg/g) also note that the concentration of phenols in methanol extract was more than the concentration of phenols in chloroform extract.***

Keywords: *"Sonchus Oleraceus Asteraceae"*, "*Ruta graveolens"*, Qualitative and Quantitative, Phytochemical Screening, Total Phenol, Antioxidant Activity

1. Introduction

Plants include of prodigious significance owing to their beneficial importance and as a main source of medications. The Asteraceae family is the major and the furthermost multicultural of the blossoming plants and is perhaps the greatest widespread in the Mediterranean [1]. *"Sonchus Oleraceus Asteraceae" ("S. Oleraceus")* is recognized locally for its traditional remedial properties, with uses as for the treatment of diseases (Headaches and General Pain-Relief) in Libya. *"S. Oleraceus"*, was a plant that had effectively used in traditional Libyan medicines for centuries. Abundant remedial plants were used as a nutritional adjunct and in the treatment of many illnesses without appropriate acquaintance of their purpose [2]. In the earlier studies, the species of *"S. Oleraceus"* were well-known for its varied activities and had used in relieving disorders such as hepatotoxicity [3]. *"S. Oleraceus" is* used as a herb for diuretic and purgative [4]. The thorough of free radicals by antioxidants possibly will condense the fibrosis development in the tissues. *"Ruta Graveolens" ("R. Graveolens")* is one of the oldest recognized therapeutic plants which remains charity in traditional medication in earliest nations, it is natural to Mediterranean area of Northern Africa and southern Europe. It is a well-known remedial herbal used for treating several illnesses such as eczema, hypertension, cough, seizure, ulcers, inflammatory conditions, arthritis, the antidote for venoms and insect repellent [5]- [9]. In addition *"R. Graveolens"* was used in the treatment of many diseases and has an effective material such as alkaloids; phenols also contain oxidative stress, which makes the plant is of great significance. These resources derived from medicinal plants more effective than the same manufactured resources, further the side effects that are manufactured substantial moreover, medicinal plants found free and can be grown at low cost [10].

Intended for the antioxidant activities, as well as the iron-chelating activities and radical Scavenging effects were reported for genus *"Sonchus"* [11]. Flavonoids, alternatively, show Antioxidant activities, were known that antioxidant activity and their effects on human health and diet are significant. The mechanisms of achievement of flavonoids are through scavenging or cheating process [12]. Since this result may be antioxidant activities owing to the attendance of flavonoids contents. Phenolic compounds are a class of antioxidant agents, which act as free radical terminators. The activities of these plants include digestive properties, anthelminthic, antitussive antimicrobial, antidiabetic activities and also to treat infertility. Furthermore, these activities are attributed to some phytochemical classes with polysaccharides and other ingredients [13] - [17]. Flavonoids and phenols are the greatest possible of which could be incapable to necessitate scavenging activity of DPPH radical. The flavonoid which is the most various and comprehensive spread group of natural compounds acts as main antioxidants [18]. Furanocoumarins are the leading active constitute of *"R. Graveolens"* which is an effective antioxidant [19] – [20]. Correspondingly, in traditional remedies were mostly used for respiratory diseases, menstrual and problems gastrointestinal

disorders [21] – [22]. Likewise, various scientific reports about the *"R. Graveolens"* were considered to have spasmolytic, hypotensive, anti-inflammatory, acting as an anti-carcinogenic agent, sperm motility-inhibitory, and antioxidant [23] – [29]. The objective of this work is to elucidate the phytochemical screening, quantitative analysis and antioxidant activity of methanol and chloroform extracts of *"S. Oleraceus"* and *"R. Graveolen"* effect.

2. Experimental

2.1 Plant material:

The test plants *("S. Oleraceus"* and *"R. Graveolen")* were collected in February/ March 2015 from the wild nearby Al-khums (120 Km East to Tripoli), Libya. The species were Authenticated and identified by Department of Botany, Education College, El- Mergeb University Alkhums Libya.

2.2 Preparation of the extracts:

The Fresh plant leaves *("S. Oleraceus"* and *"R. Graveolen")* washed with tap water, air-dried in shadow at room temperature (24 ±2 °C), after which it was ground to a uniform powder. Mean particle size d = 0.388 mm was determined using sieve sets (Erweka, Germany). All extracts (Methanol and chloroform) were prepared by soaking 20 g dry powdered plant material in 500 mL of appropriate solvent at room temperature for 3 days. The extracts were filtered through a Whatman No. 1 (122 mm) filter paper, concentrated using a rotary evaporator and were dried at 60 °C to the constant mass. The dried yield was stored in the refrigerator at 4 °C until further use.

2.3 Phytochemical analysis:

The phytochemical analysis of the dry leaf was carried out to determine the presence of the following bioactive components using the standard qualitative and quantitive procedures [30] – [36].

2.3.1 Qualitative Analysis:

Detection of Tannins:

Ferric Chloride Solution: To 5 ml of crude extract 1 drop of 5% $FeCl_3$ was added. The presence of deep blue-black color indicated the presence of tannins.

Sodium Chloride Solution: sodium chloride solution (0.9 %) was added to 2 ml of the methanolic extract and heated in a water bath (50 °C), the solution was filtered and 1% $FeCl_3$ solution added to the filtrate. The appearance of blackish green color indicates the presence of tannins.

Detection of cardiac glycosides:

Methanol crude extract:
5 ml of aqueous crude extract was boiled with 0.5ml of diluted H_2S0_4 in a test tube for 5 min then filtered while hot, cool and added an equal volume of C_6H_6 and $CHCl_3$, shake well and separate the organic solvent and add NH_3. The ammoniacal layer turned pink or red if cardiac glycosides were present.

Chloroform crude extract:
5 ml of alcoholic extract was mixed with 1 ml pyridine and 1 ml of sodium nitroprusside. Pink to red color will appear. Extract (5 ml) was treated with about 1 ml of glacial acetic acid containing a trace amount of $FeCl_3$ and 1 ml of concentrated H_2S0_4 was also add to the side of the test tube. The persistent blue color appeared in the acetic acid layer if cardiac glycosides were present.

Detection of Alkaloids:

Dragendroff's test: To 5 ml of each of the sample solution taken in a test tube, few drops of Dragendroff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids.

Hager's test: To 5 ml of each of the sample, few drops of Hager's reagent (Picric acid) was added, the yellow precipitate was formed reacting positively for alkaloids.

Wagner's test: To 5 ml of the sample solution, Wagner's reagent (Iodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.

Detection of carbohydrates:
5 ml of crude extract, treated with 3-5 drops of molasses reagent (10% alcoholic solution of α- Naphthol) was added and stirred for 5 mints. At that moment 5 ml of conc. H_2SO_4 was added. Violet ring was formed at the junction of the two liquids, indicated the presence of carbohydrates.

Detection of Flavonoids:

Alkaline Reagent Test: To 5 ml of crude extract 2-3 drops of NaOH solution was added. Development of a deep yellow color, which turns to colorless on the addition of a few drops of dill. H_2SO_4 indicated the presence of flavonoids.

Shinoda Test: To 5 ml of the crude extracts a few fragments of magnesium ribbon and Conc. HCl was added. The appearance of magenta color after few minutes indicates the presence of flavonoids.

Lead acetate test: 5 ml of crude extract was mixed with 1-3 drops of 5% lead acetate solution, white precipitates appeared indicated the presence of flavonoids.

Detection of saponins:

Foam test: 10 ml of crude extract was shaken with a small amount of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Froth Test: Crude extract (1 ml) was mixed with 9 ml of distilled water and shook vigorously for 15 seconds. The extract was allowed to stand for 10 min. Formation of stable foam (1 mm) indicates the presence of saponins.

Detection of Phenols: 5 ml of the crude extracts were mixed with 1-3 drops of ferric chloride solution. Creation of bluish black color indicates the presence of phenols.

2.3.2 Quantitative Analysis:

The quantities of the phytochemicals present were determined using the methods of Harborne J.B., 1973 and Obadoni B.O., Ochuko B.O, 2001 and D. Krishnaiah 2009. [37] – [39] as shown below; The extracts were weighed after separating the solvents by evaporated under reduced pressure and dried using a rotary evaporator at 55 °C then a percentage yield for each extract was calculated as:

$$Yield\ \% = \frac{Final\ wieight\ of\ extract}{Total\ weight\ of\ ground\ plant}\ X\ 100$$

Determination of saponins:
The samples were ground and 10 g of each were put into a conical flask and 50 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 100 ml 20% ethanol. The combined extracts were the purification process was repeated. 30 ml of n-butanol was added. The combined n-butanol extracts were 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 to a constant weight; the saponins content was calculated [37].

Determination of flavonoid:
20 g of finely powdered plant sample was extracted with 200 ml of 80% aqueous methanol repeatedly at room temperature. The whole solution was filtered through Whatman filter paper 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water-bath, the weight of the material and percentage quantity was calculated [37], [39].

Determination of total alkaloids:
Ten grams of the sample was weighed into a 500 ml beaker and 400 ml of 10% acetic acid in ethanol was added and covered then 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 [37], [40].

Determination of Total Phenol:
Total phenolic content was determined with the Folin-Ciocalteu reagent according to a procedure described by [8]. For a moment, 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 herbal material.

DPPH scavenging activity:
The free radical scavenging activity of the fruit extract was assessed by the declaration of a methanolic solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [32]. 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:

$$\%\ Scavenging\ Effect = [(ADPPH - AS) / ADPPH] \times 100$$

Where AS is the absorbance of the solution when the sample extract was added while ADPPH represents the absorbance of the DPPH. The extract concentration providing 50% inhibition (EC50) was calculated from the graph of scavenging effect percentage against the extract concentration. The antioxidant activity of standard concentration of vitamin E was assayed for comparison [41].

3. Result and Discussion

Table 1: Phytochemical screening of methanol and chloroform extracts of *"S. Oleraceus"* leaves:

Chemical Component		Observations	
		Methanolic Extract	Chloroform Extract
Alkaloids	Hager's reagent	+	-
	Wagner's reagent	+	-
	Dragendroff's reagent	+	-
Cardiac glycosides		+	-
Carbohydrates		+	-
Tannins	Ferric Chloride	+	+
	Sodium Chloride	+	+
Flavonoids	Lead acetate test	+	+
	Shinoda Test	+	+
	Alkaline Reagent Test	+	+
saponins	Foam test	+	-
	Froth Test	+	-
Phenols		+	-

Key: + = present; - = absent

Table 2: Phytochemical screening of methanol and chloroform extracts of *"R. Graveolens"* leaves:

Chemical Component		Observations	
		Methanolic Extract	Chloroform Extract
Alkaloids	Hager's reagent	+	-
	Wagner's reagent	+	-
	Dragendroff's reagent	+	-
Cardiac glycosides		+	-
Carbohydrates		+	-
Tannins	Ferric Chloride	+	+
	Sodium	+	+

	Chloride		
Flavonoids	**Lead acetate test**	+	+
	Shinoda Test	+	+
	Alkaline Reagent Test	+	+
saponins	**Foam test**	-	-
	Froth Test	-	-
Phenols		+	+

Key: + = present; - = absent

As shown in Tables 1 the phytochemical screening of methanol extract of *"S. Oleraceus"* levels were present of all constituents. And for chloroform extract also showed present the chemical constituents (tannins and flavonoids), whereas alkaloids, cardiac glycosides, carbohydrates, saponins, and phenols were not present. Table 2 showed the phytochemical screening of methanol and chloroform of extracts of *"R. Graveolens"* were present the chemical constituents except saponins were not present in the methanolic extract. And for chloroform extract presence only tannins and flavonoids remained the other chemical constituents not present.

Table 3: Percentage Yield of methanol and chloroform extracts of *"S. Oleraceus"* leaves

Plants Name	**Percentage yield %**	
"S. Oleraceus"	Methanolic extract %	Chloroform extract %
	15	4
	Flavonoids %	Saponins %
	10	12
	Alkaloids %	
	17	

Table 4: Percentage Yield of methanol and chloroform extracts of *"Ruta graveolens"* leaves

Plants Name	**Percentage yield %**	
"R.Graveolens"	**Methanolic extract %**	**Chloroform extract %**
	14	**5**
	Flavonoids %	**Saponins %**
	7	**9**
	Alkaloids %	
	14	

Furthermore, as shown in Table 3 the quantitative analysis of the plant *"S .Oleraceus"* for alkaloids, flavonoids and saponins were 17, 10 and 12 % respectively. While the percentage yield for methanol and chloroform extracts 15 and 4% correspondingly, and as shown in Table 4 the quantitative analysis of the *"R. Graveolens"* for alkaloids, flavonoids and saponins were 14, 7, 9 and3 % respectively, however, the percentage yield for methanol and chloroform extracts 14 and %5 correspondingly.

Table 5: Total Phenol of methanol and chloroform extracts of plant:

Plants Name	**Total phenols mg/g**	
"S. Oleraceus"	Methanolic extract	Chloroform extract
	3.34	1.31
"R. Graveolens"	3.16	2.9

Table 5 showed the total phenol concentration in the *"S. Oleraceus"* more than the concentration of phenol in the *"R. Graveolens"* (3.34mg/g) and also the concentration of phenol in methanol extract was more than the concentration of phenol in chloroform extract because the polarity of the solvent, whereas the phenolic compounds are different and its existence of a solvent to another at different rates.

Table6: Antioxidant Activity (DDPPH) Methanolic Extract of Plants:

Name Plant	**50 µL**	**100 µL**	**200 µL**
"S. Oleraceus"	59.35	63.05	64.35
"R. Graveolens"	69.73	73.18	74.71

Table7: Antioxidant activity (DDPPH) chloroform extracts plant:

Name Plant	**50 µL**	**100 µL**	**200 µL**
"S. Oleraceus"	40.13	45.11	49.66
"R. Graveolens"	50.20	55.32	60.22

Note from the Tables 6 and 7 that the efficiency of the antioxidants in *"R. Graveolens"* more than the *"S. Oleraceus"* at a concentration of 200 µl which stood in the methanol extract 74.71 either in chloroform extract 60.22 This according with phenolic content of the plant The higher the concentration of total phenol more in the *"R. Graveolens"* was increasing by oxidative stress more and similarly note from tables 5, 6 and 7 that increasing of antioxidants emphasis that's compatible with several previous studies. The results of this study have been agreed with the results of a study where the greater phenolic content increased antioxidant activity [42] – [43]. The extracts of *"S. Oleraceus"* were effective may explain the mechanism of action of the antipyretic effects of this plant reported in rats [44]. Various bioactive composites were known in the leaves of *"S. Oleraceus"* together with flavonoids, alkaloids, terpenes, steroids, tannins and phenols [45] – [46]. However, the possibility of *"S. oleraceous"* usage had incomplete to an insufficient quantity, for instance, medication, where it is an active component in the field of phototherapy owing to its antioxidant activity [47]. Such as, *"R. Graveolens"* as the treatment and based on these results the study suggested to exhibit anti-hyperglycemic and anti-hyperlipidemia possessions through their insulinogenic effects, reducing cholesterol absorption and intestinal glucose, improving peripheral insulin action, moving peacekeepers of insulin conflict, enhancing peripheral glucose acceptance and decreasing hepatic glucose productivity along with the ameliorating consequence on the antioxidant position in this circumstance [48].

4. Conclusion

In conclusion, our results exposed of the studied plants that can be used in medicine in the treatment of diseases such as diabetes, because it containing active ingredients and antioxidants, the phytochemical analyses revealed the presence of several compounds that might increase the potential efficiency of *"S. Oleraceus"* and *"R. Graveolens"*. Likewise, these natural products could be used as materials have an effective against bacteria and can be used as an alternative to chemical valleys are therefore advised numerous studies of these plants.

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Author Profile

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