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Phytochemical Screening, Total Phenols and Total Flavonoids Content of Cynara cornigera roots

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ABSTRACT

The aim of the current study is to detected the phytochemical compounds in the methanolic extract of Cynara cornigera roots. In addition quantify the total phenol content (TPC), total flavonoid contents (TFC) using spectrophotometric methods. The total phenolic compounds determined by using Folin Ciocalteu. While, the total flavonoid contents of the extract determined using quercetin reference standard method. Phytochemical analyses of the extract was carried out using standard methods to assess the major classes of phytochemicals alkaloids, flavonoids, phenolic, tannins, saponins, steroids, terpenoids, carbohydrate and glycosides. Phytochemical screening revealed that all the tests were positive except alkaloids, saponins, and steroids. The total phenol and flavonoids content of the methanolic extract of Cynara cornigera roots show high concentration. The estimated phenolics and flavonoids content are 17.59 mg/g and 18.62 mg/g respectively. These findings suggest that the methanol extract of Cynara cornigera roots has potential invitro antioxidant activities. According to the obtained results, the roots of Cynara cornigera considered as a rich source of natural antioxidants since its methanolic extract contains many effective chemical compounds that demonstrate the highest free radical scavenging capacity.

1. INTRODUCTION

Plants such as vegetables, fruit, spices medicinal herbs, etc., have been used to relief many diseases since ancient time. Although synthetic drugs are readily available and highly effective in relieving various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. These Plants consist of various kinds of chemical constituents known as phytoconstituents or secondary metabolites, which is include flavonoids, alkaloids, terpenoids, steroids. In addition phenolic compounds, such as nitrogen compounds, carotenoids and ascorbic acid. Previously study have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antiviral, and anti-diabetic effect(1).

The phytochemical compounds have scavenger activities against reactive oxygen species (ROS). Many scientists have suggested that the antioxidants could help to protect the cells from a damage caused by oxidative stress and to strengthen the defense system against degenerative diseases. These antioxidants substances, particularly phenolic compounds can save the body from the damage caused by the free radicals, has been linked to many diseases. In fact, a recent study has shown that the decrease of premature death and mortality from cancer or and other chronic diseases are associated with antioxidant-rich diet including fruits and vegetables(2).

Cynara cornigera L (Asteraceae) commonly called as Gaamool in Libya. This plant is widely grown in Mediterranean countries. It is rich in natural antioxidants mainly polyunsaturated fatty acids , vitamins C, A, E Also it has been that they are rich in polyphenols and flavonoids compounds(3).

Cynara cornigera roots contain antioxidant and antidiabetic compounds such as vitamins C, K, α-Tocopherol and β-Carotene. Also rich in polyphenols mainly Caffeoylquinic acids and Luteolin. A study has proven That the cynara cornigera roots have a safe antidiabetic agent and might help in preventing diabetic complications . It can serve as a good adjuvant in the present armamentarium of antidiabetic drugs(4).

A previous study showed that various plant organs (roots, leaves and flowers) of Cynara cornigera had an

important and interesting power against several human pathogenic bacteria and fungi, possibly, due to their specific phenolic composition. The major phenolic compounds are Chlorogenic acid, p- hydroxyl benzoic acid, p-coumaric acid and ferulic acid (major phenolic acids) in addition to, catechin and quercetin as the major flavonoids(5).

High phenolic contents are thus an important factor in determining the antimicrobial activity of this plant. It was reported that an antimicrobial action of phenolic compounds were related to the penetration of the substance into the cell, cause membrane permeability changes. Increased membrane permeability is a major factor in the mechanism of antimicrobial action, where compounds may disrupt membranes and cause a loss of a cellular integrity and eventual cell death(6).

Furthermore, *Cynara cornigera* is an herbal drug that has a strong hypolipdaemic effect. Previous study evaluates the lipid lowering effect of aqueous roots of *cynara cornigera* extract. This extract show reduction of total cholesterol, triglycerides and LDL-cholesterol levels, and increasing HDL-cholesterol levels(3).

The present investigation comprises phytochemical screening as well as estimation of total phenol and flavonoid contents from methanol extracts of roots of *cynara cornigera*.

2. MATERIALS AND METHODS:

2.1. Collection of plant materials

Fresh roots of *cynara cornigera* were collected from al lawifia area in Benghazi-Libya during March of 2019. The plant was identify at the botany department of the science college, University of Benghazi.

2.2. Chemical Reagents

Ascorbic acid, Folin-Ciocalteu reagent, methanol, aluminum chloride , potassium acetate, quercetin, pyrogallol, Dragendoff's reagent**,** sodium hydroxide, ferric chloride, chloroform, sulphuric acid, acetic acid, Molish reagent, and other chemicals were obtained from the biochemistry laboratory of chemistry department-Benghazi University.

2.3. Preparation and Extraction

The roots of *cynara cornigera* cleaned and dried at room temperature. After complete drying, the roots grounded Into powdered form. In the next step, powdered plant (20g) transferred into a dark-colored flask and mixed with 1L of methanol. The mixture kept in the shaker for 48 hours and then was filtrated. After that the filtrate mixed with methanol second time and kept in the shaker for another 48 hours. The mixture was filtrated. These steps repeated for third time. The methanol that collected after each time of filtration evaporated by rotatory at 40C◦.

2.4. Phytochemical qualitative analysis

The methanolic extract of the plant were screened for the presence of phytochemical classes by using the standard following methods (Table 1) [7].

Table 1 Protocol for phytochemical screening.

Component exit test	Protocol for test	Result for confirmation
Alkaloids	1.0ml extract + 3.0 drops of Dragendoff's reagent	Orange-red precipitate
Flavonoids	1.0ml extract + few drops of dil. NaOH	Intense yellow colour
Total phenol	1.0ml extract + 2.0ml water + few drops of 10% FeCl3	Blue-green colour
Tannins	100mg solvent free extract + 1ml 5% $FeCl_3$	Bluish-black precipitate
Saponins	1.0ml extract + 20ml water + agitation for 15min	1cm layer of foam
Steroids	1.0ml extract + 10ml $CHCl_3$ + 10ml conc. H_2SO_4	Upper layer – red and lower layer – yellow-green
Terpenoids	5.0ml extract + 2.0ml $CHCl_3$ + 3.0ml conc. H_2SO_4	Reddish brown precipitate
Carbohydrate	2.0ml extract + 2 drops of Molish reagent (95% α-napthol in ethanol) + 2.0ml conc. H_2SO_4	Purple colour at junction
Glycosides	5.0ml extract + 2.0ml $CHCl_3$ + 2.0ml CH_3COOH	Violet, blue to green colour

2.5. Antioxidant activity

2.5.1. Determination of total phenol content in the plant extract (TPC)

Total phenolic content (TPC) in the plant methanolic extract was determined using spectrophotometric method. 1 mg/ml aqueous solutions for methanolic extract were prepared for the analysis. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10 % FolinCiocalteu's reagent (FCR) which dissolved in water and 2.5 ml of 7.5% of NaHCO3 aqueous solution. The sample mixture was thereafter incubated in a thermostat at 45 ºC for 45 min. The absorbance then determined by the spectrophotometer at wave length = 765 nm.

The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solutions of pyrogallol and the calibration line was construed. Based on the measured absorbance, the concentration of pyrogallol equivalent expressed in terms of pyrogallol mg of /g of extract). Total phenolic content of the extracts was expressed as mg pyrogallol

equivalents (pyrogallol E) per gram of sample in dry weight (mg/g)(8).

2.5.2. Determination of total flavonoid content in the plant extract (TFC)

Total flavonoid content was determined from the calibration curve of quercetin and expressed as milligram of quercetin Equivalent per gram of extract (mg QE/g extract). Variou concentrations of standard quercetin (500, 400, 300, 200, and 100 µg/ml) were prepared. (0.5 ml) Of each concentration was mixed with 3 ml methanol, 0.2 ml of 10% Al Cl3, 0.2 ml of potassium acetate (1M) and 5 ml of distilled water. The mixture was incubated at room temperature for 30 minutes. After that, the absorbance was measured at 415 nm. Solution of distilled water with methanol, 10% AlCl3 and potassium acetate was used as a blank. Total flavonoid in extracts was expressed in terms of quercetin equivalents (mg of quercetin /g of roots of cynara cornigera extract). Total flavonoid content of the extracts was expressed as mg quercetin equivalents (QE) per gram of dry extract (mg/g)(9).

3. RESULT

3.1. Qualitative Phytochemical Analysis

The results of the phytochemical screening test (positive and negative) obtained from the methanolic extract of the roots of *cynara cornigera* are presented in Table (2)

Phytochemicals compounds	Methanolic extract
Alkaloids	-
Flavonoids	+
phenols	+
Tannins	+
Saponins	-
Steroids	-
Terpenoids	+
Carbohydrate	+
Glycosides	+

3.2. Total phenolic content

Absorbance of standard compound (pyrogallol) at λmax =765nm.

Table 3: Absorbance of standard compound (Pyrogallol) at λmax = 760 nm.

Table 2 Phytochemical screening tests for the methanolic extract roots of cynara cornigera

Pyrogallol concentration(µg/ml)	Absorbance (mean value) at λmax = 760 nm
100	0.410
200	0.799
300	1.323
400	1.828
500	2.105

Standard curve of pyrogallol indicated the equation of y = 0.0044x + 0.0327 and

R2 = 0.9911 clarified in Fig.1

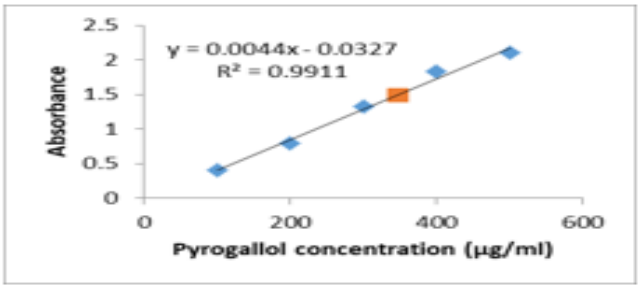

Figure 1 Standard calibration curve for quantification of total phenolic content.

3.3. Total flavonoid content

Table 5 show the total flavonoid content of the methanolic extract of cynara cornigera roots and table 4 show the absorbance of the standard compound (quercetin) at different concentrations**.**

Table 4 Absorbance of standard compound (quercetin) at λmax = 415nm.

Quercetin concentration(µg/ml)	Absorbance (mean value) at λmax =415
100	0.478
200	0.701
300	0.923
400	1.429
500	1.706

Standard curve of Quercetin indicated the equation of y = 0.0032x + 0.0922 and

R2 = 0.9757 clarified in Fig.2

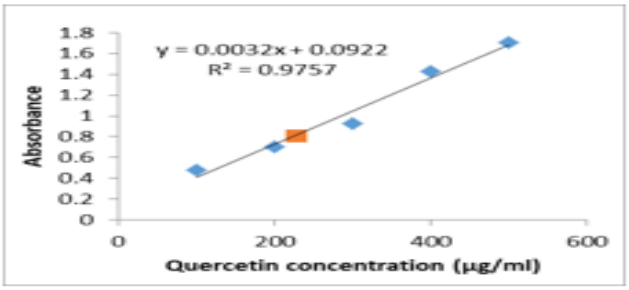

Figure 2 Standard calibration curve for quantification of total flavonoid content.

Table 5 presented the contents of total phenols that were measured by Folin Ciocalteu reagent (FCR) in terms of

pyrogallol equivalent and total flavonoid content expressed as (mg/g) quercetin equivalent**.**

Roots of cynara cornigera	Methanol extract
Total phenolic (mg pyrogallol/g)	17.59 mg/g
Total flavonoid content (mg QE/g)	18.62mg/g

4. DISCUSSION

4.1. Antioxidant activity

In this study, methanolic extract was chosen based on polarity. Extraction in highly polar solvents resulted in a higher extract yield but a lower phenolic and flavonoid content compared to non-polar ones. Alcohol solvents are more capable of increasing the permeability of cell walls and facilitating the extraction of a greater number of molecules with both medium and low polarity[10,11]. After the successful extraction of *cynara cornigera* roots the phytochemical study revealed that methanol extract contains flavonoids, phenols, tannins, terpens, carbohydrate and glycosides, while, alkaloids, saponins and steroids were not detected (Table 2). These phytochemical compounds are known to have bioactive effects including antioxidant effect. Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidant activity, as they have an aromatic ring that allows the stabilization and relocation of the unpaired electrons of their structure. This is facilitate the donation of hydrogen atoms and electrons from their hydroxyl groups[12]. However, the benefits of medicinal and food plants may arise from a synergy of certain antioxidants such as flavonoids.

The content of phenolic compounds in *cynara cornigera* roots extract was determined from regression equation of the calibration curve of pyrogallol (Figure 1) and expressed as milligrams equivalent of pyrogallol per gram of extract (mg PYR/g). Similarly, total flavonoid content was determined from regression equation of the calibration curve of quercetin (Figure 2). Flavonoids content was expressed as milligrams equivalent of quercetin per gram of extract (mg QE/g). Total phenolics and flavonoids calculated in this study are presented in Table 5.

5.conclusion

In conclusion, The phytochemical screening of this investigation revealed the presence of several secondary metabolites with known biological antioxidant activities, Phenolic and flavonoids compounds are known as powerful chain breaking antioxidants, which may contribute directly to reduce the oxidative action caused by free radicals.

6. REFERENCES

1. Tinky Sharma, Binjita Pandey, Bishnu Kumar Shrestha, Gayatri Maiya Koju, Rojeena Thusa and Nabin Karki (2020); Phytochemical Screening of Medicinal Plants And Study of The Effect of Phytoconstituents In Seed Germination. Tribhuvan University Journal, 35: 1-11.
2. Arumugam Kathirvel, Venugopal Sujatha (2012); Phytochemical studies, antioxidant activities and identification of active compounds using GC–MS of Dryopteris cochleata leaves. Arabian Journal of Chemistry, 9: 435- 442.
3. Mohamed Ahmida (2011): Antidiabetic, Antihyperlipedemic and Antioxidant Effects of Aqueous Extract of the Roots of Cynara cornigera in Alloxan-induced Experimental Diabetes Mellitus. International Journal of Pharmacology, 7: 782-789.
4. Pandino, G., S. Lombardo, G. Mauromicale and G. Williamson (2011); Phenolic acids and flavonoids in leaf and floral stem of cultivated and wild Cynara cardunculus L. genotypes. Food Chem., 126: 417-422.
5. Bidaawat S., Ruchinag and Nag T. N. (2011); Antimicrobial principles from tissue cultures of Balanites aegyptiaca. Romanian Biotechnological Letters, 16 (2): 6120-6124.
6.Aicha Debib, Mohamed Nadjib Boukhatem (2017): Phenolic Content, Antioxidant and Antimicrobial Activities of "Chemlali" Olive Leaf (Olea europaea L.) Extracts; International Journal of Pharmacology, Phytochemistry and Ethnomedicine; 6: 38-46.
7. C F Kairupan, F R Mantiri, R R H Rumende (2019); Phytochemical Screening and Antioxidant Activity of Ethanol Extract of Leilem (Clerodendrum minahassae Teijsm. & Binn(as an Antihyperlipidemic and Antiatherosclerotic Agent, IOP Conf. Series: Earth and Environmental Science (217) 012016.
8. Nidal Jaradat1, Fatima Hussen and Anas Al Ali (2015): Preliminary Phytochemical Screening, Quantitative Estimation of Total Flavonoids, Total Phenols and Antioxidant Activity of Ephedra alata Decne. J. Mater. Environ. Sci. 6 (6) (2015) 1771-1778.
9. Laxman Bhandari and Meena Rajbhandari (2014): PETALS, Estimation of Total Phenolic, Total Flavonoid And Antioxidant Activity of The Different Parts Of Rhododendron Arboreum Smith Laxman Bhandari,Central. Department of Chemistry, Tribhuvan University, Kathmandu, Nepal.
10. Qing-Wen Zhang , Li-Gen Lin and Wen-Cai Ye (2018); Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med., 13: 20.
11. Haq Nawaz , Muhammad Aslam Shad, Najiha Rehman, Hina Andaleeb and Najeeb Ullah (2020); Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. Brazilian Journal of Pharmaceutical Sciences, 56:e17129.
12. Patricia Cosme, Ana B. Rodríguez, Javier Espino and María Garrido (2020); Plant Phenolics: Bioavailability as a Key Determinant of Their Potential Health-Promoting Applications. Antioxidants, 9, 1263.