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Qualitative phytochemical analysis of Cynara scolymus leaves extract

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ABSTRACT: The leaves of globe artichoke, Cynara scolymus, Family Asteraceae/ compositea have long used in traditional medicine and now included in British and European Pharmacopeia, the British Harbal Pharmacopeia and complete German Commission E monographs.The active groups contained inCynara scolymusleaves underlieits therapeutic potential. Therefore, this study aimed to clarify these groups using standard phytochemical screening tests. Results revealed presence of saponin, tannins, gallo or catecho tannins (hydrolysable or condensed), gallic acid, respectively and flavonoid (table 1). Whiles, phlobatannin, alkaloid, glycoside, carbohydrate-, cardiac glycoside(s), anthraquinones glycosides, resins, steroid, Protein compounds, terpenoids, oils/ fats and gums/mucilage were not detected content was suspect. These findings may prove Cynara scolymus is a rich source of active groups, suggesting that it may be a nutraceutical-based medicine in the pharmaceutical and cosmetic industries.

KEYWORDS: Artichoke, Cynara scolymus, Poly phenolic acids, Chlorogenic acid, Cynarin.

I. INTRODUCTION

The importance of the plant Cynara scolymus which is called artichoke or globe artichoke steamed from its used as edible material for nutrition and from its content of phenolic acid constituent in particular cynarin and chlorogenic acid (19),(26).

The leaves of globe artichoke, Cynara scolymus L.Family Asteraceae / Compositae, have been longused in traditional medicine and now included in British and European Pharmacopeia (BP / EP), the British Herbal pharmacopeia (BHP) and the Complete German Commission E Monographs (18).

The plant Cynara scolymus L. originally comes from Mediterranean region and north Africa and also cultivated around the world (26).

The flowers are used worldwide with nutrition purposes and the leaves with medical purposes, broadly used in Phytotherapy preparations with special indication in hepatic affections (18). The plant is widely distributed in Egypt. The plant flourishes in winter and harvested in February and March.

The leaves of C. scolymus are characterized by the composition and high content of bitter phenolic acid compounds whose choleretic, hypocholestremic and heptatoprotective activity attributed. At least to the antioxidant potential of artichoke extracts and of their phenolic compounds. Constituents which are around 2% such as: caffeic acid, chlorgenic acid and cynarin, flavenoids (0.1 – 1 %) and essential oil (18).

Pharmacological studies demonstrated that the extracts of C. scolymus and active principle cynarin (1,3 di-caffeoyl quinic acid (C25H24O11) possesscholeretic and hypocholesterolemic activity (6). The extract of the plant also protected hepatocytes treated with Thioacetamide from hepatic cellular necrosis. This activity is related to the powerful antioxidant effect of phenolic acids (12). Recently,published pharmacological investigations and clinical reports showed the efficacy and safety of artichoke extracts in treatment of hepatobiliary dysfunctions and abdominal pain (17).

In a previous study, we have found that the extracts of C. scolymus have anti-oxidative effect against Thioacetamide induced liver injury. In other separate studies conducted by me and colleagues have been proven C. scolymus extract to have hepatoprotective, hematinic, antioxidant effectsin Thioacetamide model of liver injury.

Therefore, this study is conducted to clarify the active principle group(s) related to such actions. To achieve this aim, the standard preliminary tests have been performed on the following chemical groups:alkaloids, glycosides, anthraquinones, cardiac glycosides, saponins, tannins, phlobatannins, flavonoids, resins, gums, terpenoids, coumarins, oils and proteins.

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II. MATERIALS AND METHODS

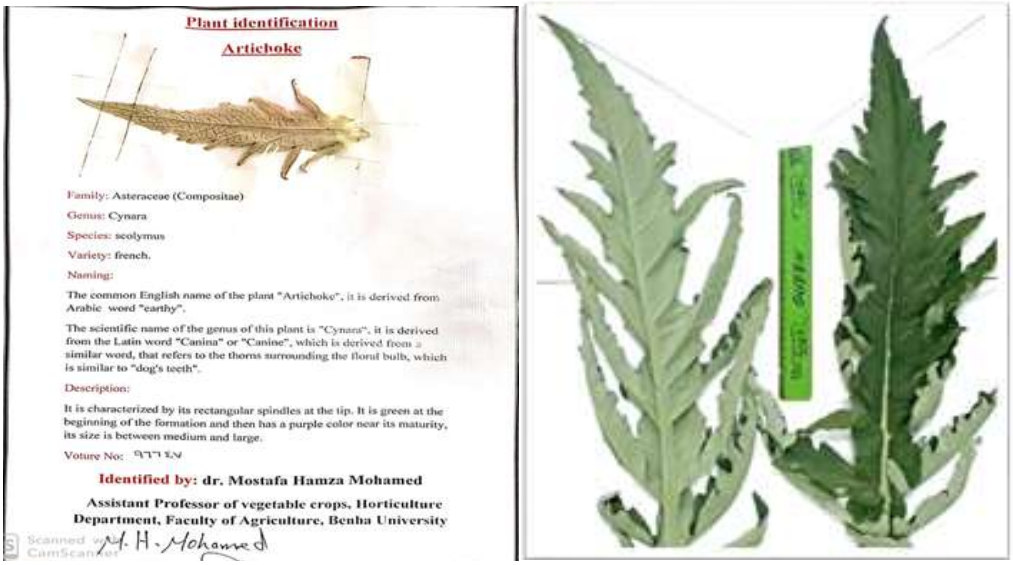

Fig. 1& 2:Plant identification&Leaves of Cynara scolymus used for extraction.

Plant part used

The plant is a traditional vegetable crop of the Mediterranean basin. The cultivated variety: Cynara cardunculus L. var. scolymus L (globe artichoke), grown for its fleshy capitula. It is belonging to the family of Asteraceae(9)

The leaves of Cynara scolymus were purchased from medicinal plants farm, Faculty of Agriculture, Banha university, Egypt. The leaves were collected in September 2022.

The plant was identified by Dr. Mostafa Hamza Mohamed, Assistant professor of vegetable crops, Horticulture department, Faculty of agriculture, Benha University, Voucher number 96647 (Figures 1 & 2).

Equipment

Beakers, test tubes, pipettes, water bath, hot flame, filter papers, Digital scale, cotton mesh paper, glass rod, dropper, test tube holder,spatula, mortar and pastel.

Chemicals and reagents

Reagents used for detection of different phytochemical groups were prepared as follows:

- Mayer's regent: Mercuric chloride (1.36 g) and potassium iodide (5.0 g) were dissolved separately in 60 and 20 mL of water, respectively; then both of the prepared solutions were then mixed and completed up to 100 mL with distilled water.
- Wagner's reagent: Potassium iodide (2 g) and then Iodine (1.27 g) were dissolved in 5 mL of distilled water and the solution was completed to 100 mL with distilled water.
- Hager's reagent: Saturated solution of picric acid, heated, filtered, and cooled.
- Dragendorff's reagent: The stock solution was prepared by mixing bismuth sub-nitrate (1.7 g) with distilled water (80 mL) and acetic acid (glacial, 20 mL). Potassium iodide solution (50% w/v, 100 mL) was then added, and the mixture was shaken until dissolved and then kept in a dark bottle. The working solution was prepared by mixing the stock solution (100 mL) with glacial acetic acid (200 ml) and made up to volume of 1 liter with distilled water in a dark bottle.
- Tannic acid reagent: Ten g of tannic acid powder in 100 mL of distilled water.
- Molisch's reagent: Ten% solution of α-naphthol in alcohol.
- Fehling's reagent: Is a mixture (50:50 v/v) of two solutions that should be mixed only upon use; solution-I was prepared by dissolving 6.3 g of copper sulfate-($5H_2O$) in distilled water containing a few drops of dilute sulfuric acid; solution-II was prepared by dissolving 35 g of potassium tartrate and 15.4 g of NaOH in 100 mL of distilled water.
- Benedict's reagent: Prepared by dissolving Sodium citrate (86.5 g) and anhydrous sodium carbonate (50 g) in 400 ml distilled water; and then boiling and cooling till clearance. After cooling, copper sulphate-($5H_2O$) (8.65 g) was

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dissolved in 75 ml distilled water was then added to the clear solution.

- Vanillin-hydrochloric acid reagent: Prepared by mixing vanillin (1 g) in alcohol (10 mL) and then adding 10 mL of concentrated hydrochloric acid.
- Wilson's reagent: Boric and citric acids in anhydrous acetone.
- Millon's reagent: Equal parts of mercury (or mercuric nitrate) and fuming HNO_3, diluted with water up to twice of the original volume.
- Biuret reagent: It consists (per 100 mL final volume) of 0.9 g Sodium potassium tartrate, 0.3 g Copper sulfate.$5H_2O$, and 0.5 g Potassium iodide, all dissolved in order in 40 ml 0.2 M NaOH. Then the mixture was brought to final volume (100 mL) by 0.2 M NaOH.

Experimentation

Phytochemical screening of Cynara scolymus for the presence of different active principle groups, including alkaloids, glycosides, anthraquinones, cardiac glycosides, saponins, tannins, phlobatannins, flavonoids, resins, gums, terpenoids, coumarins, oils and proteins was carried out. All tests were performed as triplicates and given marks from (-) to (+++) according to the strength of the color or precipitate that appeared.

✓ Detection of alkaloids

About one g of the Cynara scolymus leaves were extracted with 10 mL of diluted 1% HCl, with aid of heat; and then the mixture was filtered (11). In clean and dry test tubes, two mL of the filtrate were treated, separately, with a few drops of Mayer's or Wagner's or Hager's or Dragendorff's or tannic acid 10% reagents. Creamy, brown, yellow, deep yellow, buffy precipitation with those detecting reagents, respectively, was judged as indicator for alkaloidal substance content.

✓ Detection of glycosides/carbohydrates

Five grams of the Cynara scolymus leaves were mixed with 30 mL of distilled water and heated. The watery extract was then decanted and the supernatant was tested for its content of a carbohydrate and/or a glycosidal substance following the classical procedure reported by (5) and (3), with minor modifications, in the following tests:

Molisch's test: About 0.2 mL of α-naphthol alcoholic solution 10% was added to two mL of the tested water filtrate in a clean and dry test tube; then by addition of 2 mL of sulphuric acid onto the inside wall of the tube, a bluish violet zone formation denotes presence of glycosides and/or carbohydrates.

Fehling's test: Equal amounts of the concentrated extract and Fehling's reagent were mixed and heated for a few minutes. Precipitation with changing of color ranging between yellow to brown, indicates the presence of certain glycone as a part (or not) from glycosides and/or carbohydrates.

Benedict's test: Equal aliquots of the concentrated extract and Benedict's reagent were mixed in a clean and dry test tube and heated for a few minutes. Precipitation with change of color to any degree from yellow to red brown, denotes the presence of reducing sugar(s) as a part (or not) from glycosides and/or carbohydrates.

To differentiate if the constituent is a glycoside or carbohydrate; Fehling's and Benedict's tests were repeated twice, the first with aqueous extract of Cynara scolymus leaves. While the second with the acidulated (H_2SO_4) extract (that was then neutralized by 5% NaOH solution); stronger color in the second trial indicates a glycoside in general. Special tests to detect special glycoside categories were performed as follows:

Baljet's test: A few drops of sodium picrate solution were added to 1 mL of the concentrated extract. Orange discoloration denotes presence of cardiac glycoside(s).

Killer-Killiani test: Two mL of acetic acid (glacial) with a drop of ferric chloride solution were added to five mL (100 mg/mL in methanol) of the Cynara scolymus leaves extract in clean and dry test tube. One mL of conc. sulfuric acid was added to form a zone above the prepared mixture. Formation of a bluish-brown ring at the interface indicates deoxy- sugar that is characteristic for cardiac glycosides (7).

Schonteten's Reaction (Borax test): To 2 mL of the aqueous cynara scolymus leaves extract (1 g/10 mL), 0.1 g of Borax was added and heated until dissolved. A few drops of the liquid were poured into a test tube almost full of water; a green fluorescence indicates anthraquinone glycoside.

✓ Detection of saponins

Foam (Froth) test: The ability of saponin to produce froth upon shaking and to produce emulsion with oil was used as a test for its detection (11). About two g of the crushed Cynara scolymus leaves were heated in 20 mL of distilled water in a water bath for five minutes and then filtered. In a clean dry 25 cm cylinder, ten mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously, and froth formation was observed. To complete the test, then three drops of olive oil were mixed with

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the formed froth (if any), shaken vigorously and observed for emulsion formation. At least ten cubic mm height of froth that stands for at least 10 minutes indicates saponin; and emulsion formation confirms it.

✓ Detection of tannins/phenols

About 2 g of Cynara scolymus leaves were extracted in 20 mL ethanol (50 %) by heating in water bath for 10 minutes at 70 °C and tested for presence tannins and/or other phenolic compounds in the root and rhizomes extract using the following tests (21).

Gelatin test: Equal amounts of the extract and 1% gelatin solution in sodium chloride (0.85%) were mixed in a clean dry test tube. Formation of white/cloudy/buffy precipitate indicates the presence of tannins (in general) in the root and rhizomes extract.

Lead acetate test: Two mL of 10% lead acetate filtered, clear solution, were added to 2 mL of the extract. A bulky white precipitate indicates the presence of tannin and/or phenolic compounds.

Ferric Chloride test: A few drops of $FeCl_3$ solution (1%) were added to an aliquot of 2 mL of the prepared extract, formation of bluish-black or greenish color denotes the presence of gallo- or catecho- tannins (hydrolysable or condensed), respectively.

Hydrochloric acid test: Half g cynara scolymus leaves were boiled with 5 mL of 1% HCl for 10 minutes; the formation of a red precipitate indicated the presence of phlobatannins (7).

Vanillin test: Two mL of vanillin-HCl reagent were added to an aliquot of five mL of the alcoholic root and rhizomes powder extract (1 g seed/10 mL alcohol). Formation of a red or pink deposit denotes the presence of gallic acid (hydrolysable tannin).

✓ Detection of flavonoids

Shinoda's (Cyanidin) test: Two mL of 10% ethanolic extract (1 g/10 mL; w/v) were mixed with 0.5 ml of HCl (10%) and a few mg of magnesium metal turnings. Development of a reddish color denotes the presence of flavonoids (7).

Wilson's test: Some flavonoids (5-oxyflavones and 5-oxyflavonoles) with Wilson's reagent develop brightly yellow color with yellowish-green fluorescence, if present in Cynara scolymus leaves extract.

Lead Acetate test: A few drops of clear lead acetate solution (10%) were added to two mL of Cynara scolymus leaves ethanolic extract in a clean and dry test tube. Appearance of a yellow-colored precipitate denotes presence of flavonoids.

Alkaline reagent test: Two mL of Cynara scolymus leaves aqueous extract were treated with 10% solution of ammonium hydroxide; observation of yellow fluorescence denotes the presence of flavonoids.

✓ Detection of resins

Fifty mL of 95% ethanol were added to about 5 g of the dry grind of Cynara scolymus leaves. The mixture was heated in shaking water bath for about 20 minutes, then decanted or filtered. Formation of a precipitate upon the addition of about 5 mL of distilled water denotes a resinous content (11).

✓ Detection of Gums/Mucilages:

One g of Cynara scolymus leaves were dissolved in 10 mL of distilled water in a large clean dry test tube. Twenty-five mL of absolute alcohol were then added gradually with constant stirring. Appearance of white/cloudy precipitate denotes the presence of gums/mucilages (28).

✓ Detection of terpenoids/steroids

Presence of terpenoids and derived steroids in dry leaves of Cynara scolymus was carried out by the following tests:

Salkowski's test: One hundred mg of Cynara scolymus leaves were extracted in 2 ml of chloroform, and then 3 ml of concentrated H_2SO_4 were carefully added onto the wall of the test tube. After standing for minutes, appearance of reddish coloration at the lower layer confirms the presence of steroids; while turning it into yellow indicates terpenoids (11).

Libermann-Burchard test: The chlorformi extract was treated with a few drops of acetic anhydride and then heated. After cooling, equal amount of concentrated H_2SO_4 was added carefully onto the inside wall of the test tube. Appearance of a brown ring at the interface and turning of the upper layer into green indicate presence of steroids, while formation of a dark red color indicates terpenoids.

✓ Detection of Fixed oils

Spot (Stain) test: Petroleum ether or benzene Cynara scolymus leaves extracts were tested for presence of fixed oils/fats. A small amount of an extract was pressed in between the folds of a filter paper. Appearance of oil stains denotes content of fixed oil/fat (15).

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✓ Detection of Proteins/Amino acids

Cynara scolymus leaves (1 g) was mixed with 10 ml of distilled water in a clean dry test tube and filtered through Whatmann No.1 filter paper. Then, the filtrate was subjected to tests for proteins and/or free amino acids, including:

Millon's test: A few drops of Millon's reagent were added to two mL of the prepared root and rhizomes filtrate. A buffy white precipitate that turns red upon heating denotes the presence of proteins (22).

Biuret test: An aliquot of 2 mL filtrate was treated with a few drops of Biuret reagent (see above). Turning of the light blue color into violet/mauve color denotes the presence of peptide bonds/proteins (10).

III. RESULTS

As shown in table (1), phytochemical screening revealed presence of saponin, tannins, gallo or catecho tannins (hydrolysable or condensedrespectively,), gallic acid, and flavonoids. On the other hand, phlobatannin, alkaloids, glycosides, carbohydrates, cardiac glycoside(s), anthraquinone glycosides, resins, steroids, protein compounds, terpenoids, oils/ fats and gums/mucilage were not evident (Table 2).

Active group	Test	Result
Saponin	Froth	+
Tannin	Gelatin	+++
	Lead acetate	+++
	$FeCl_3$ test	++
Phlobatannin	Hydrochloric acid test	-
Gallic acid(phenolic acid)	Vanillin test	+++
Flavonoids	Shinoda's (Cyanidin) test	+++
	Wilson's	+++
	Lead acetate	++
	Alkaline reagent	+++

Table (1). Results of Saponin, Tannin/phenols/special Tannins and Flavonoids detection ofCynara scolymus leaves extract.

Active group	Test	Result
Alkaloids	Mayer's	-
	Wagner's	-
	Hager's	-
	Dragendorff's	-
	Tannic acid 10%	-
Glycosides/ Carbohydrates	Molisch's	-
	Fehling's	-
	Benedict's	-
Cardiac glycosides	Baljet's	-
	Kileer-Killiani	-
Anthraquinone glycosides	Schonteten's	-

Table 2:Results of Alkaloids, Glycosides/Carbohydrates and special Glycosides detection of Cynara scolymus leaves extract.

IV. DISCUSION & CONCLUSION

The richest bioresource for medications used in traditional and modern medical systems, and chemical components for synthesized drugs is found in medicinal plants. Because they are widely accessible, inexpensive, safe, and backed by public trust, traditional herbal medicines are encouraged,

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and promoted in national health care programmes by the World Health Organization (WHO) (20).

There are currently 121 active chemicals that are believed to be produced from plants, accounting for approximately 25% of all medications given around the world. 11% of the 252 medications on the WHO's list of essential medicines is solely derived from plants (23). For their primary healthcare, about 80% of people in Asia and Africa rely on traditional medicines. In India, about 80% of the rural population uses medicinal herbs or traditional medical practices. The Indian herbal business uses 960 plant species in total, 178 of which are employed in significant volumes exceeding 100 metric tonnes annually (24). According to reports, plant-based medications have been used successfully to treat skin conditions, AIDS, cancer, diabetes, jaundice, hypertension, tuberculosis, and many other infectious diseases (14).

Plant medications differ from synthetic drugs in that they have unique qualities. They frequently lack knowledge of the active principle and contain many active compounds. Consider the more than 2000 chemicals found in the Chinese medicinal plant Huang-qin (Scutellaria baicalensis) (25).

There are many attempts made in order to find a natural substance like silymarin or better for treating liver diseases. So, the aim of this study was to clarify the modulatory effect of TAA on hepcidin, blood picture as well as the possible improving potential of Cynara scolymus leaves extract (CSE) on TAA-induced liver injury. CSE was selected as it is well growing in Egypt, and it has been reported to have good antioxidant properties that have been also investigated here.

Artichoke has been used for centuries in folk medicine for its choleretic activity and more recently the C. scolymus extract has been proposed for its antioxidant properties against liver complaints. Commercially available extracts do not always show the same therapeutic activity, depending on their relative content in active principles (27).

The flavonoid content of the plant has antioxidant activity, and it was up- regulated endothelial type Nitric- oxy synthesis gene expressions in human endothelial cells. N. oxide (NO) produced by endothelial nitric oxide synthesis (eNOS) represent and antithrombotic and anti- atherosclerotic principle in vasculature which led to provide protection against cardiovascular diseases (18).

In the present study, phytochemical analysis of C. scolymus was relieved positively appearance of flavonoids, as shown in table (1). These yields are compatible with those of (16) who stated that ethanolic extract of C. scolymus has flavonoids as active groups in farm of Radmilovac of the Faculty of Agriculture, University of Belgrade. And compatible with those of (2) who stated that the phytochemical constituents of C. scolymus L. rhizomes methanolic extract proved to be rich in flavonoids.

Analysis of C. scolymus extract revealed that presence of phenolic acid (gallic acid) as shown in table (1). This result agrees with (13)who showed the phenolic composition of the extracts obtained from the different extraction methods applied. The phenolic profile was quite different, both qualitatively and quantitatively, depending on the extraction method used. The richest extract in phenolic compounds was obtained using methanol: water (60% v/v) with ultrasound application. Methanol was much more efficient than water for extracting hydroxycinnamic acids. According to (1), who also recorded a similar observation analysis of C. scolymus showed the presence of several categories of compounds including phenolic acids (gallic acid, quinic acid, chlorogenic acid, rosmarinic acid.

Phytochemical analysis of C. scolymus relieved positively appearance of tannin, as shown in table (1). This result is compatible with those of (4) who reported that the determination of tannin, the EtOH extract of C. scolymusleaves extract had the highest amount of these compounds in comparison with other extracts, which were an agreement with the result of (2) who showed that EtOH extract exhibited the maximum amount of tannin from the leaves of Cynara scolymus.

Phytochemical analysis of C. scolymus relieved positively appearance of saponin, as shown in table (1). This result is compatible with those of (8) who detected saponin in artichoke extracts (roots, bracts, flowers, and leaves) using HPLC-DAD-ESI-MS. These phytochemicals can be utilized as an external medicinal supplement and have a variety of bioactive qualities. The various phytochemicals found in the leaf of C. scolymus are now being identified and isolated, and their antioxidant and anti-cancer properties are being investigated. There is some compatibility recorded between our finding and other one of (4).However, may be different with(2), this differencemay be attributed to different research environments.

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CONCLUSION

According to phytochemical screening, C. scolymusleaves extractswere detected to contain abundant in flavonoids, tannins, gallo or catecho tannins (hydrolysable or condensed), phenolic acid (gallic acid), as well as saponin. These findings make the extract of Cynara scolymus a potential pharmaceutical agent of nutritional origin.

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