



Original Article

Oral Administration of an Ethanolic Extract from *Cynara cardunculus* var. *scolymus*: Its Effects on Serum Enzymes in Animals and Assessment of Its α -glucosidase and α -amylase Inhibitory Activity

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Abstract

The positive attributes of the edible *Cynara cardunculus* L. subsp. *scolymus*, commonly referred to as globe artichoke, have long been known due to the amount of bioactive chemicals found in it including inulin and polyphenols. Artichokes, which were washed under tap water, were picked manually and consumed as edibles and commercially viable. They were then frozen at -20 °C and then freeze dried after the removal of stems and outer bracts. Removal of the outer bracts had to be done thrice. Cynaran and caffeic acid were the metabolized compounds. Octyl α -D-glucopyranoside, Salvadoraside, 1, 4-o-Caffeoyl quinic acid, 5-Feruloylquinic acid, Apigenin 7-O-rut, and several others. The comparative values with respect to 2 α -amylase were (39.00 \pm 2.09, 71.81 \pm 5.83, 85.00 \pm 6.07, and 32.93 \pm 2.01) in regard to the type of extract (ethanol fraction, ethyl acetate fraction, water fraction and acarbose as standard). The α -glucosidase inhibitory power was determined to be (61.09 \pm 4.00, 41.00 \pm 2.98, 30.05 \pm 1.99, and 20.00 \pm 0.67), respectively. Researchers studied the effect of the SGPT, SGOT, and ALP in the blood of rats by oral administration of *Cynara cardunculus* var. *scolymus* extract in a controlled environment. The outcomes of these experiments that involved ethyl acetate extract of *Cynara cardunculus* var. *scolymus*, Di-(2-ethylhexyl) phthalate, and Control (0.5 ml/kg corn oil) were (90.00 \pm 4.86, 103.54 \pm 5.67, and 37.00 \pm 2.98), respectively.

Keywords: *Cynara cardunculus* var. *scolymus*, Serum Enzymes, Animals, α -glucosidase, α -amylase.

Introduction

Perennial *Cynara cardunculus* L. subsp. *scolymus* is an edible native Mediterranean plant. The antioxidant effect of Polyphenolics is not the only example of the numerous benefits of these

compounds to human health whose effects on human well-being have been well documented. The major chemicals believed to cause such benefits are the polyphenols and especially the dicaffeoyl quinic acids, which are very prevalent



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in the extracts. The metabolites being produced by cynara spp. are varied with glycosides being the most prevalent being produced but flavonoids, apigenin, luteolin and their glycosides were also found¹. The phytochemical content is not fixed since metabolic variability determined by physiological and environmental factors is a natural phenomenon; this also includes; genotype and cultivar, weather, soil traits, and growth stage. The profile and concentrations of artichoke secondary metabolites can be highly influenced by the various factors. Traditional foods employ the so-called "Carciofo di Paestum a Romanesco - type PGI (Protected Geographical Indication) food, which has been widely studied in terms of its nutritional and health-promoting qualities and is the most renowned and widely cultivated type of them. Such studies showed great disparity in polyphenol quali-quantitative profiles and antioxidant activity; they used nuclear magnetic resonance spectroscopy and/or mass spectrometric analysis to give metabolomic fingerprints of the extracts which were used to compare landraces and selected clones². Operating bioactivity tests and chemical characterizations of different cultivars would be helpful to know more about the unique characteristics of agri-food products. This information can be applied to select variety/clone and valorisation in the mother country. Carciofo di Procida has never undergone a study of chemical profile or biological characteristic^{3,4}. We point out the particular metabolite content in each component, the heart and inner bracts, commonly used in homemade and commercial food preparation, and the outer bracts and stem, which comprise half and a half of commercial artichoke fresh, respectively, and are reportedly wasted in some homemade and commercial food preparation and canning.

C. scolymus has medicinal properties and nutritional value which have promoted its cultivation all over the world. It has been known all over the world and since time immemorial, as a drug. It positively affects biliary tract, digestive activity and scurvy and anaemic treatment and atherosclerosis. It is also a popular and interesting herbal remedy^{5,6,7} in addition to being a healthy food that has a nice bitter taste. Artichoke leaf extracts (ALE) are also used to emulsify alcoholic and soft drinks and also in the preparation of herbal medicine either alone or mixed with other herbs. The active ingredients of this plant are said to be the poly-phenolic substances that are present in the leaf but not the artichoke head of the plant.

Already in the fourth-century B.C. people were exploring the artichoke plant as a culinary and as a remedy. The botanical description was given by a student of Aristotle called Theophrastus at the time. Eating artichokes as a digestive, and a stimulative^{8,9}, Romans then appeared to forget about them until the 16 th century, when they were found again beneficial in the treatment of jaundice and liver problems. The leaves of the artichoke plant were also used in European traditional medicine as a diuretic and choleric plant respectively to increase liver and gall bladder secretion of more liver and bile respectively.

Materials and Methods

Artichoke and Sample Collection Process

The artichokes were then washed manually with the use of tap water after they had fallen and were of commercial and edible size. It had isolated stems and external bracts, which were freeze-dried in a consecutive manner after freezing them in -20 C°.

Cynara cardunculus var. *scolymus* IR Spectroscopy.

The FTIR spectra of the native and defatted GLVs were obtained with the help of the FTIR instrument (Model/Make: IFS 25, Bruker, Germany) and a PC-based program. In this manner the two types of FTIR spectra of the GLVs were retrieved. To prepare the FTIR analysis, a small amount of the powder of the leaf was pelletized with KBr and a thin layer formed by pressing the mixture. To finish the process of collecting laboratory data on infrared light transmittance, various specified wave numbers will be used e.g. 4000 cm⁻¹ to 500 cm⁻¹. The three samples that formed part of this study were individually tested with the untreated KBr pellets being used as a control. The structure of functional groups that occurred in the sample was determined through the comparison of the spectral data in the laboratory and the standard reference.

Alpha-Amylase Inhibition Assay In vitro

The extract and fractions that were used were subjected to standard practical tests in a controlled laboratory environment where their α -amylase inhibitory activities were tested. It should be mentioned that already certain minor changes had been implemented to the procedures in the lab. Twenty liters of extract (dosing 0.5 mg/mL and varying doses) were put into a fifty liters of water

with the addition of laboratory phosphate solution (100 mM, pH 6.8). Moreover, ten actual liters of alpha-amylase were placed in the mixture with the concentration of two units per milliliter. Meanwhile, the mixture was added to a 96-well plate and pre-incubated at 37°C during twenty minutes. The temperature had been kept at 37°C before the pre-incubation process could take place. Next, 20 liters of a substrate with Comprised of one percent soluble starch at one hundred mM phosphate buffer with a pH of 6.8 were put into the mixture. Once the components have been added back to the lab, the mixture is incubated at 37 degrees Celsius, after 30 minutes. This was followed by the addition of 100 liters of DNS colour reagent and the liquid was practically heated after 10 minutes of heating under a constant estimated pressure. The 540 nm absorbance value had been revealed through the lab using Multiplate Reader before. This experiment was conducted in controlled environment to make sure that it was possible to measure the absorbance of the resultant combination accurately. Since it was usable as a standard, acarbose was used at a concentration of 0.1 to 0.5mg/ml. To have a control, there was the development of a drug that had not been tested experimentally in parallel to the experimental method. Moreover, to be more exact, every experiment was repeated three times.

An Enzyme Inhibition Assay of 3-Phosphoglycosidase.

The α -glucosidase inhibition method is used to determine the activity of a single type of enzyme, α -glucosidase. In order to neutralize the acidic pH of the solution and deactivate the inhibitor of the enzyme, the test tube was added with 100 ml of 0.05 M NaHCO_3 . Then, 5.0 ml of 1.0 M G 3 was added to the test. The analysis of the level of α -glucosidase inhibitory activity of extract and fractions were determined. This analysis was done using the conventional approach with some modifications here and there. A 96-well plate was pre-incubated a reaction mixture at 37 degrees Celsius, fifteen minutes. The phosphate buffer (H_2O) of 6.8 pH and 100 millimolar concentration; alpha-glucosidase (1 U/mL) of ten liters; different fractions and extracts (0.5 mg/mL) were used. These were all contained in the reaction mixture. The temperature kept at a constant of 37 degrees Celsius was used in the incubation procedure to ensure preincubation. In order to quantify the absorption of just released

nitrophenol, a multiplate reader at the wavelength of 405 nm was used in this experiment. Meanwhile, acarbose was used as a control and was present in the test sample in a 0.5mg/mL concentration.

Experiments in Living animals

The experiments were done using male albino rats that weighed 200-250 grammes; these were all healthy. They were fed their normal laboratory feed in polypropylene cages. The temperature of the room was also kept at a constant of 23.2degrees Celsius. The rats were also given at least eight hours to become familiar with the lab environment prior to the commencement of the tests.

Pharmacological Dosage and Animal Testing

The albino rats were used in the experiments and they weighed between 200 and 250 grams or 5 grammes. They originated in the same location as the animals did- the breeding facility. They were exposed to normal weather conditions that consisted of 12 hours of darkness and 12 hours of light besides having enough ventilation in their cages. They were also fed on normal rat food. A sample of twelve rats was used and four animals were used in each group. The animals in group I were administered maize oil as a control although later the same oil was used as a vehicle control. In each of the separate experiments which involved the *Apium graveolens* methanol fraction, the positive control was 100 mg/kg Di-(2- ethylhexyl) phthalate. *Cynara cardunculus* var. *scolymus* Vat was found in Groups 2 and 3 with concentration of 0.50 and 0.75 mL/kg respectively and Group 1 contained 100 mg/kg of Di-(2-ethylhexyl) phthalate.

Assessment through Statistical Methods

The graphics were designed and evaluated by the use of GraphPad Prism (version 10.3.1, GraphPad Software, San Diego, CA, USA). One-way ANOVA and multiple comparison tests were used to compare the groups in a sequence manner. To determine the differences among the parts of artichokes, we conducted partial least squares discriminant analysis (PLS-DA) and log-transformed and Pareto-scaled data on metabolites. The MetaboAnalyst Version 6.0 was used to generate a biplot that was effective in showing the separation of the samples and also to identify the main metabolites that caused the separation.

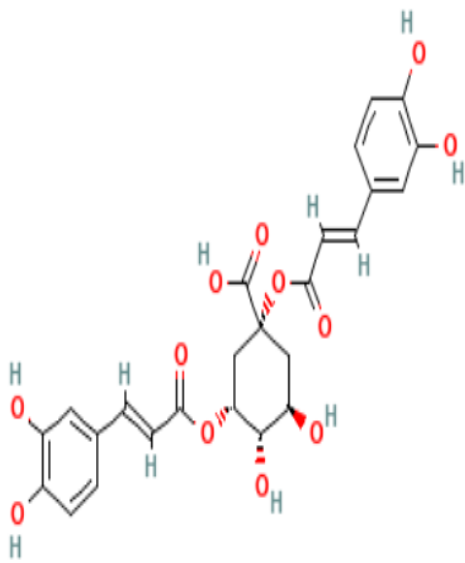
Results and Discussion:

The GC-MS technique is commonly used to determine and analyze lignin, volatile organic compounds (VOCs), and fatty acids in chemical study of *Cynara cardunculus*. To describe the oil that is extracted out of *C. cardunculus* seed and cell cultures, we make use of gas chromatography-mass spectrometry. The fatty acids present are oleic, palmitic, linoleic and stearic acids that are predominantly found. This analysis can help establish whether the oil can be exploited in producing bio-diesel or to be used by man. It can be employed to determine volatile compounds and oils of fresh and dried flower and leaf samples^{10,11,12,13}. Substances such as hexadecanoic acid, methyl hexadecanoate and 3-hexen-1-ol can be utilized in improving the

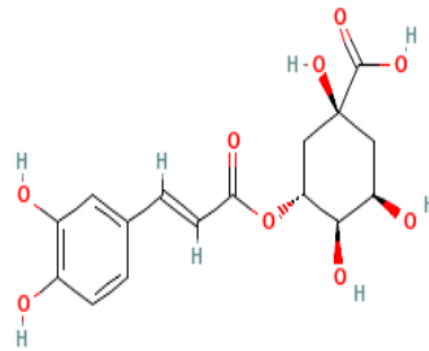
fragrances used in food or utilized in cosmetic fragrances as well. More polar phenolic compounds such chlorogenic acid and cynarin, which play a large role in the high medicinal and nutraceutical properties of the plant, are normally analyzed using strategies like (UHPLC-HRMS) and (HPLC-DAD) whereas GC-MS is also important in these particular compounds^{14,15}. The metabolites were caffeine and cynaran based on the chemicals contained in them. Some of the compounds include octyl alpha-D-glucopyranoside, Salvadoraside, 1, 4-o-Caffeoylquinic acid, Apigenin 7-O-rutinoside, Cis- 5-Caffeoylquinic acid, 5-Feruloylquinic acid and chlorogenic acid.

Table 1. Metabolite profile of *Cynara cardunculus* using GC-MS.

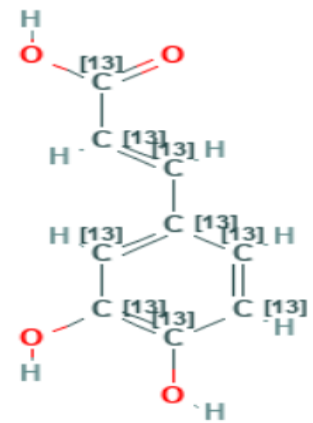
Compound	Molecular Formula	Molecular Weight	Compound	Molecular Formula	Molecular Weight
Cynarin	MF: C ₂₅ H ₂₄ O ₁₂	MW: 516.4 g/mol	chlorogenic acid	MF: C ₁₆ H ₁₈ O ₉	MW: 354.31 g/mol
caffeic acid 1	MF: C ₉ H ₈ O ₄	MW: 189.09 g/mol	Cis-5-Caffeoylquinic acid	MF: C ₁₆ H ₁₈ O ₉	MW: 354.31 g/mol
4-o-Caffeoyl quinic acid	MF: C ₁₆ H ₁₈ O ₉	MW: 354.31 g/mol	5-Feruloylquinic acid	MF: C ₁₇ H ₂₀ O ₉	MW: 368.3 g/mol
Luteolin 7-O-rutinoside	MF: C ₂₇ H ₃₀ O ₁₅	MW: 594.5 g/mol	Apigenin 7-O-rutinoside	MF: C ₂₇ H ₃₀ O ₁₄	MW: 578.5 g/mol
5-O-(3,4-Dihydroxycinnamoyl)-L-quinic acid	MF: C ₁₆ H ₁₈ O ₉	MW: 354.31 g/mol	Methyl alpha-D-glucopyranoside	MF: C ₇ H ₁₄ O ₆	MW: 194.18 g/mol
Octyl alpha-D-glucopyranoside	MF: C ₁₄ H ₂₈ O ₆	MW: 292.37 g/mol	1,5-O-trans-dicaffeoylquinic acid	MF: C ₂₅ H ₂₄ O ₁₂	MW: 516.4 g/mol
Salvadoraside	MF: C ₃₄ H ₄₈ O ₁₈	MW: 744.7 g/mol	Agavasaaponin C	MF: C ₄₅ H ₇₂ O ₁₉	MW: 917 g/mol



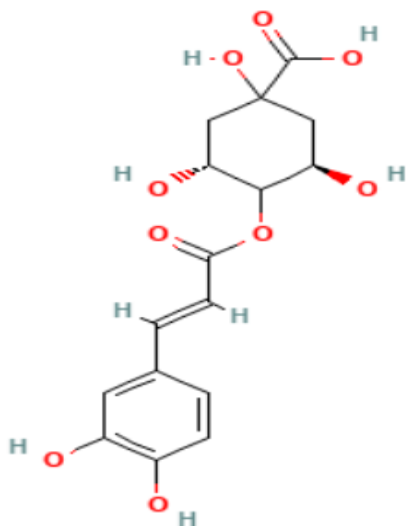
Cynarin



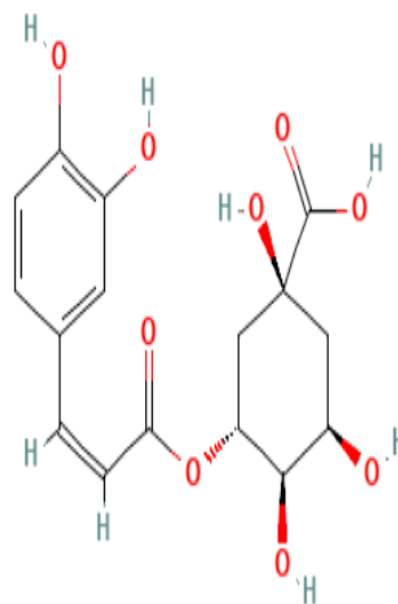
chlorogenic acid



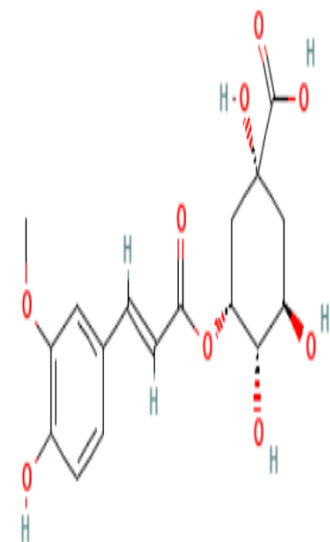
Caffeic acid-13C9



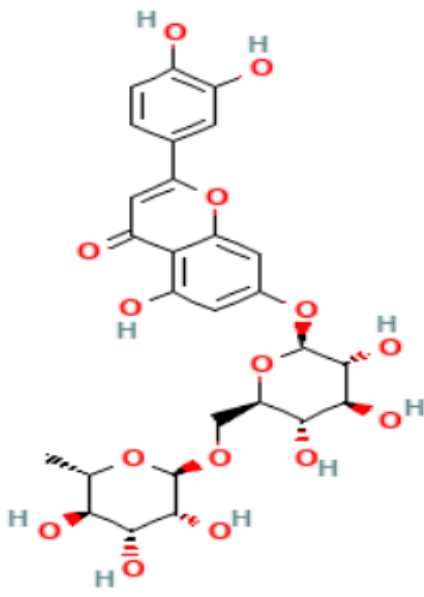
4-o-Caffeoyl quinic acid



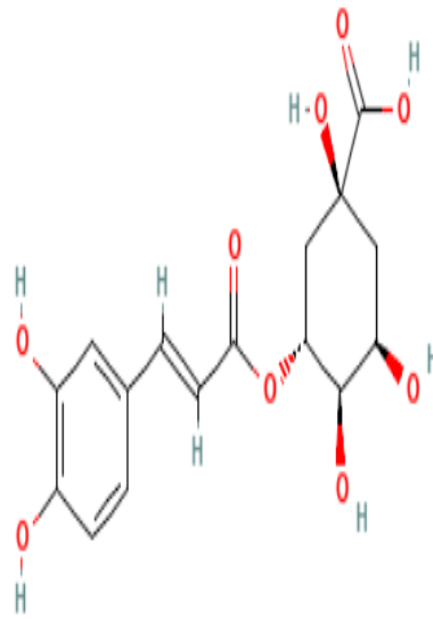
Cis-5-Caffeoylquinic acid



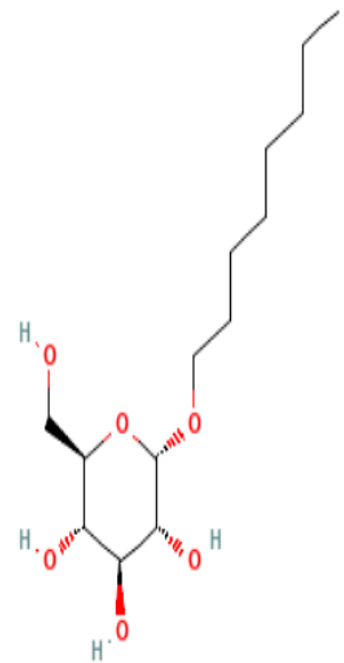
5-Feruloylquinic acid



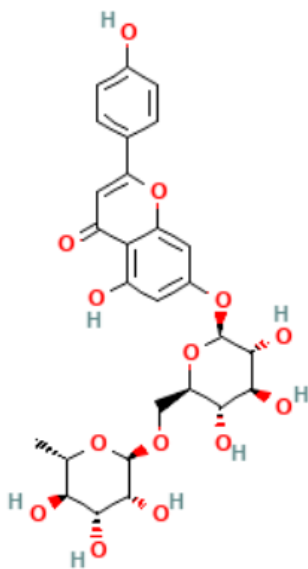
Luteolin 7-O-rutinoside



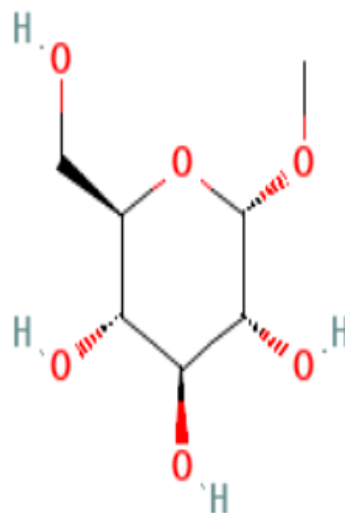
**5-O-(3,4-Dihydroxycinnamoyl)-
L-quinic acid**



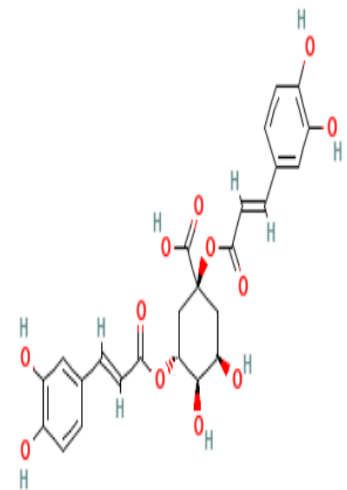
**Octyl alpha-D-
glucopyranoside**



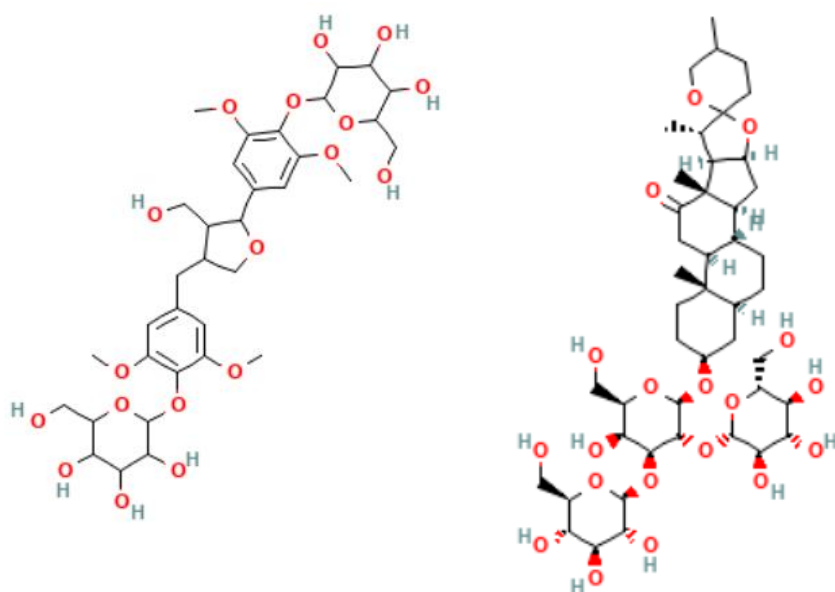
Apigenin 7-O-rutinoside



**Methyl alpha-D-
glucopyranoside**



**v1,5-O-trans-
dicaffeoylquinic acid**



Salvadoraside

Agavasaponin C

The inhibitory power of the extract against α -amylase was also determined in a different way depending on the type of extract (Ethanol fraction, Ethyl acetate fraction, Water fraction, and Acarbose as a standard): (39.00±2.09, 71.81±5.83, 85.00±6.07, and 32.93±2.01), respectively. Meanwhile, the inhibitory effect against the activity of the 3-glucosidase was measured to be (61.09±4.00, 41.00±2.98, 30.05±1.99, and 20.00±0.67) respectively (Figure 1 and 2). The

effect of *Cynara cardunculus* var. *scolymus* extract on blood enzyme SGPT, SGOT and ALP was conducted with the help of rats developed in a controlled laboratory environment. The values of *Cynara cardunculus* var. *scolymus* ethyl acetate extract were (90.00±4.86, 103.5±45.67 and 37.00±2.98), those of Di-(2- ethylhexyl) phthalate they were (141.06±7.45, 169.00±8.07 and 46.09±2.05) and that of the Control (vehicle) (0.5ml/kg Corn oil).

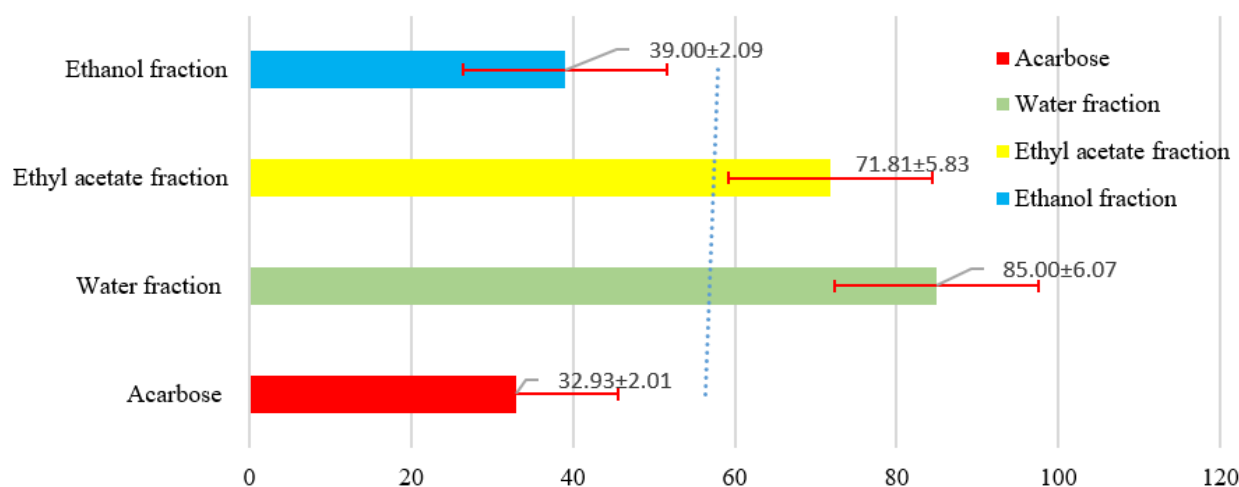


Figure 1. Antidiabetic activity of *Cynara cardunculus* var. *scolymus* extract (Ethanol, Ethyl acetate, Water fraction and Acarbose as standard) against α -amylase

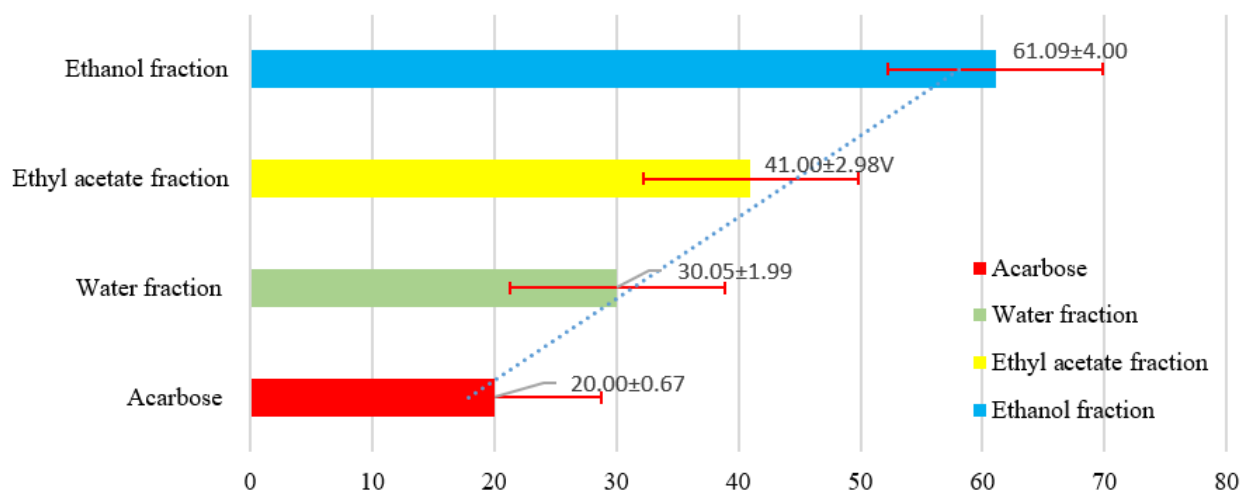


Figure 2. Antidiabetic activity of *Cynara cardunculus* var. *scolymus* extract (Ethanol, Ethyl acetate, Water fraction and Acarbose as standard) against α -Glucosidase

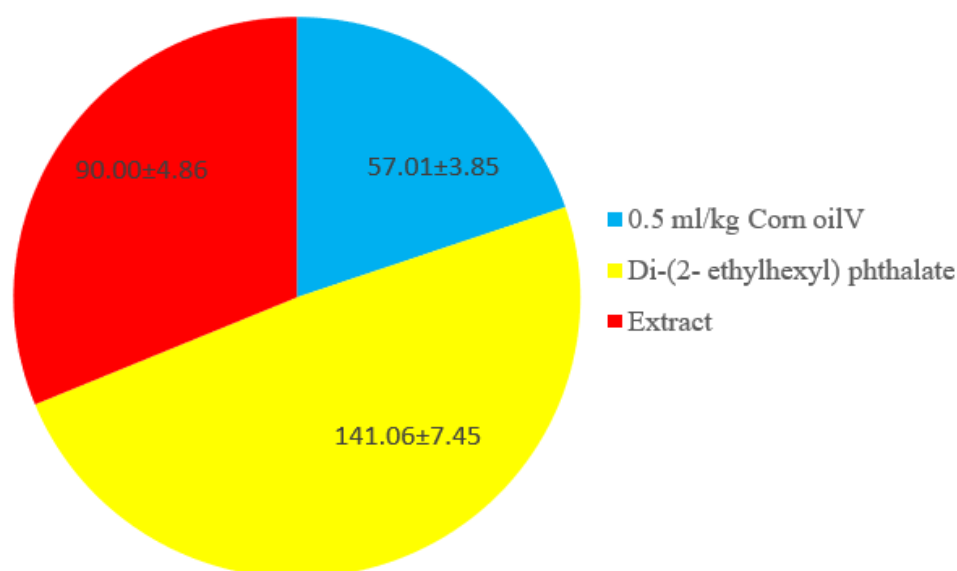


Figure 3. Oral administration of *Cynara cardunculus* var. *scolymus* extract on the blood enzymes SGPT

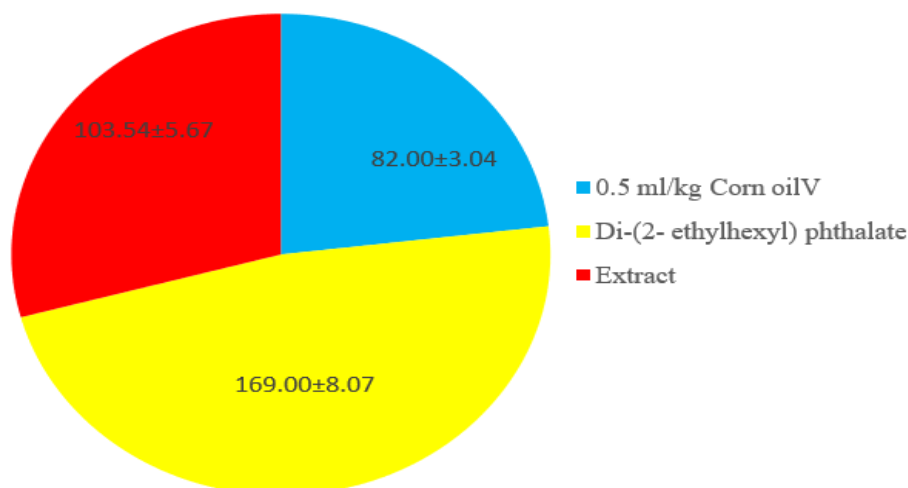


Figure 4. Oral administration of *Cynara cardunculus* var. *scolymus* extract on the blood enzymes SGOT

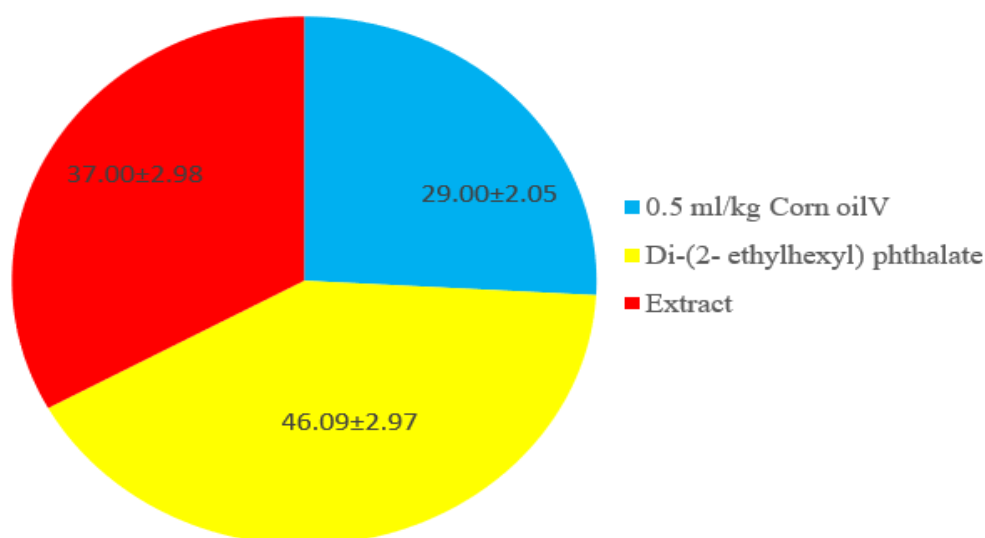


Figure 5. Oral administration of *Cynara cardunculus* var. *scolymus* extract on the blood enzymes ALP

Chemical composition of the leaves of artichoke has been well researched and has been found to contain high concentrations of polyphenolic chemicals. The two caffeic acid molecules, 1, 3-di-o-caffeoyl quinic acid (cynaran) and 5-o-caffeoyl quinic acid (cyrogenic acid) fuse with 1, 3-o-quinic acid to yield the significant antioxidant chemicals in *cynara cardunculus* var. *scolymus*. It is a biological molecule derived out of artichoke extract and has small quantities of fat. It contains also minerals (potassium, sodium and phosphorus), vitamin c, fiber, polyphenol flavone, inulin and hydroxycinnamates which are derivatives of caffeoylquinic acid^{16,17}. An artichoke has the following chemical composition; 21.6% crude protein, 17.1% crude fiber, 24.5% crude oil, and 3.8% ash. Artichoke leaves and heads contain several nutrients including minerals, fiber, inulin and polyphenolic chemicals. ALE has been shown to have antioxidant, antibacterial, anti-HIV, bile-expelling, hepatoprotective, urinate and choleric effects as well as having the capacity to suppress the synthesis of cholesterol and LDL oxidation. Even though the medicinal history of artichoke extract has a long history, it has not been fully studied regarding its antibacterial properties. Contrarily, other scientists have demonstrated that these extracts possess strong antibacterial effects and may be used in the food industry. In the procedure of extraction, very much depends on how the polyphenol is extracted.

The predominant phenolic compounds are caffeic acid derivatives which include caffeoylquinic acid derivatives. Moreover, artichoke leaves and stems do have the chemical 1, 3-O-dicaffeoylquinic acid (cynaran), but the concentration of this chemical is insignificant in methanolic extracts (approximately 1.5 percent). This compound is majorly present in the pulp of the leaves. High cholesterol was found to increase the risk of atherosclerosis and coronary heart disease. It is true that ALE lowered the level of cholesterol. Efforts toward the exploration of this idea began in 1970s when the researchers started experimenting with cynaran and cholesterol. According to the latest studies, the active component luteolin plays a key role in the inhibitory effect of ALE on the production of cholesterol.

Apparently this was occurring at a lower rate than that of HMG-CoA reductase, which is the most critical enzyme in cholesterol production. Other interactions are likely to cause the HMG-CoA reductase effects observed by the overall extracts, including the activation of inhibitory processes or inhibition of activating processes. Apparently, ALE blocked the process of lipid synthesis at several levels. Thus, in comparison to some of the synthetic lipid-lowering drugs, ALE did not possess the possibilities to produce unwanted sterol molecules. Animal research also indicated that ALE reduced lipids and extensive clinical

evidence supported these results in the literature, but it was not only an animal study that determined this conclusion. The results indicated that the antiatherogenic effect was due to caffeoylquinic acids and luteolin glucosides. Luteolin, which is one of the components of the ALE, was believed to inhibit the novo cholesterol production. The individuals were administered artichoke extract or a placebo once and then over several hours, the output of the bile was measured using special methods. Also, the artichoke extract group had significantly higher degree of bile secretion. The ensilage of the artichoke by products generated following the industrialization process is a cheap and environmentally friendly method of avoiding waste releases and costs incurred when managing waste. These are good in smell, can be utilized in the production of silage and can be easily ensiled. This silage can be consumed by ruminants rather than feeds that are more coarse such as hay. There was an increase in proportion of omega-3 fatty acids in the fat reserves of lambs that were fed on meal that contained bracts of artichokes. Fat and total free fatty acids in cheese prepared using dairy ewes fed on artichoke silage were lower than those of control animals, but did not affect milk production¹⁸. Specifically, the proportion of organic matter had in the forage was extremely high (86% digestible) and ensilage is the most appropriate method of preserving it over long period of time. A different study established that whole cardoon seeds placed at the ratio of approximately 10 in the diets of the withers had the benefit of the digestive action. The presence of high fibre content of the seed and low protein content of the low grade protein led the above researchers to warn against feeding on ruminant more than 10 percent of seed in the ruminant diets. This was due to the fact that the seed could reduce the productivity of the already rather productive animals. These days it is known that the polyphenolic compounds present in artichoke extract are strong antioxidants. One such caffeoylquinic acid is caffeoylquinic acid; over the last few years, there have been various interesting studies investigating this particular

substance, which is why there is the possibility of new uses. Also, there is a need to study more on the liver glucose metabolism. It is characteristic of non-insulin-dependent diabetes that strangely high levels of glucose release out of the liver are normally elevated in the values; this GI-6-P inhibitor could be beneficial in lowering the values. The carcinogenic activity of toxic substances involved chlorogenic acid and various animal experiments proved that it is useful in the reduction of the carcinogenic reaction. These findings lead to the fact that this natural chemical can be one day considered in cancer chemoprevention. The neutralizing effect of chlorogenic acid on peroxy nitrite is one among the numerous interesting characteristics of chlorogenic acid. An example is chlorogenic acid which inhibits the oxidative damage of DNA by eliminating peroxy nitrite which is a cytotoxic agent generated by the body in response with superoxide and nitric oxide. According to recent research, peroxy nitrite is one of the largest and most harmful oxidants that are created by humans. Since the threat of free radicals or reactive oxygen species (ROS) always exists in the background and threaten our tissues, it is better to have some mechanism to counteract and prevent the damages or, at least, reduce them. Imperative elements of vital defensive mechanisms include antioxidants such as glutathione, vitamin C, and vitamin E, and enzymes such as superoxide dismutase. After the formation of the harmful radicals in surplus of the ability of the antioxidant defenses to respond, the oxidative stress kicks in. Chlorogenic acid might perhaps provide much of the zest to these defenses¹⁹. In the foreseeable future, scientists most probably will continue excavating peroxy nitrate and chlorogenic acid. The potential applications of chlorogenic acid, which is in artichoke leaves, as a treatment of cancer and diabetes is under investigation. Chlorogenic acid is as well antioxidant of peroxy nitrite which is very harmful oxidative and has been linked to cardiovascular disease, neurological disorders and inflammatory illnesses.

Conclusions

Based on all these findings, the numerous applications of this study in the implementation of basic physiological processes makes it a harmless and completely natural means to sustain and enhance the overall wellbeing. In order to define whether artichoke leaves have a positive or negative impact on human health, the in vivo application of a set of metabolic tests (e.g., diabetes, cardiovascular) can be performed with the help of this study. Among the phenolic acid compounds, 5-caffeoylquinic acid, the chlorogenic acid is one of the most available caffeoylquinic acids. Cynaran 5-caffeoylquinic acid 1, 5-dicaffeoylquina acid has numerous significant and medicinal applications besides being an antidiabetic and anti-inflammatory agent. In addition, 1, 5-dicaffeoylquinic acid is a newly-discovered crucial ingredient in the treatment of diabetes. These researches demonstrate the presence of 5-caffeoylquinic acid (5-CQA) and 1,5-dicaffeoylquinic acid (1,5-diCQA) in the highest concentration in infusion and decoction because of the interesting behavior of the given compounds. It should be mentioned that flavonoid glycosides might be extracted in a better manner with the help of the ethanolic. It is safe to use as a nutritional supplement to regular treatments since it is not only an anti-inflammatory but also a nutritional supplement.

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