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Effect of temperature and solvent on the total phenolic compounds extraction from leaves of *Ficus carica*

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ABSTRACT

The total phenolic compounds of Ficus carica extracted either by ethanol or by simple aqueous extraction were evaluated using the Folin-Ciocalteu assay and compared. The main parameters that affected the yield of phenolics included the condition of the Ficus carica, temperature of the extraction and solvent concentration. Generally, fresh frozen samples had the highest total phenolic contents. High extraction (about 80%) was obtained using ethanol as solvent and the percentage extraction could further be increased using a higher temperature of 80 ◦C.

Key words : Total phenolic compounds; Ficus carica; Solvent extraction; Folin-Ciocalteu assay.

INTRODUCTION

Ficus carica grows in tropical and subtropical regions of Iran. Different biologically active compounds were isolated form this plant. The barks, leaves, fruits are considered to be very effective in various treatments, such as diabetes, skin diseases, ulcers, dysentry, diarrohoea, stomachache, piles. Ficus constituted one of the largest general of medicinal plant with about 750 species of woody plants, trees, and shrubs primarily occurring in subtropical and tropical regions through out the world. Ficus carica is commonly referred as "Fig". The fig is a very nourishing food and used in industrial products. It is rich in vitamins, mineral elements, water, and fats. Ficus carica has been reported to include antioxidant, antiviral, antibacterial, hypoglycemic, cancer suppressive, hypotriglyceridaemic, and anthelmintic effects [1-3].The fig is a deciduous tree, to 50 ft tall, but more typically to a height of 10 - 30 ft. The large, wavy-margined leaves are usually 5 lobed but may have only 4 or 3 lobes. The leaves are conspicuously palmately veined.The leaves contain moisture, 67.6%; protein, 4.3%; fat, 1.7%; crude fiber, 4.7%; ash, 5.3%; N-free extract, 16.4%; pentosans, 3.6%. Ficus carica have numerous bioactive compounds

such as Mucilages, flavinoids, vitamins, enzymes, nicotinic acid, and tyrosin. Ficusin, bergaptene, stigmasterol, psoralen, taraxasterol, beta-sitosterol, rutin ,sapogenin, Calotropenyl acetate, lepeolacetate and oleanolic acid sistosterol are present in the leaf. The plant also contains arabinose, β-amyrins, β- carotines, glycosides, β-setosterols and xanthotoxol [4-6]

Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity[7]. The antioxidative phytochemicals especially phenolic compounds found in vegetables, fruits and medicinal plants have received increasing attention for their potential role in prevention of human diseases[8]. Several members of the genus Ficus are being used traditionally in a wide variety of ethnomedical remedies all over the world[9,10]. Phytochemical investigations of some Ficus species revealed that phenolic compounds constitute the major components of them[11-13]. Also, some studies reported the presence of antioxidant activity of some Ficus species which attributed the antioxidant activity to the phenolic content of them[14].

Many valuable natural materials have traditionally been extracted with organic solvents. However, some of the organic solvents are believed to be toxic, and the extraction conditions are often harsh. A simple method using ethanol instead of methanol was applied for the extraction of phenolic compounds from Ficus. In this study, the effects of the following parameters: the conditions of the leaves samples, effect of repeated extraction, different types of organic solvents, the concentration of the solvent and temperature of extraction were examined[15].

EXPERIMENTAL SECTION

Plant specimens of figs (leaves) were collected in Quchan city, Iran. Air-dried leaves were homogenized. To achieve a standard size of particles, the ground material was sieved through a 1mm metal sieve. Large particles remaining on the sieve were further ground. The process was repeated until all the material passed through the sieve. They were stored at −18 ◦C before any further treatments.

The Folin–Ciocalteu's reagent (FCR) is one of the oldest methods designed to determine the total contents of phenolics in foods or medicinal plants.[15] Phenolic compounds react with FCR only under basic conditions (adjusted by aqueous sodium carbonate 5–10%). Dissociation of a phenolic proton in basic medium leads to a phenolate anion, which is capable of reducing FCR in which the molybdate in testing system is reduced forming a blue coloured molybdenum oxide with maximum absorption near 760 nm. The intensity of blue colouration produced is proportional to the total quantity of phenolic compounds present in the testing samples.

The FCR for determination total phenolic compounds was freshly prepared according to the described method by [16]. The FCR is typically made by boiling (for 10 h) the mixture of sodium tungstate ($Na_2WO_4.2H_2O$, 10 g), sodium molybdate ($Na_2MoO_4.2H_2O$, 2.5 g), concentrated hydrochloric acid (10 ml), 85% phosphoric acid (5 ml), and water (70 ml). After boiling, lithium sulphate ($Li_2SO_4.4H_2O$, 1.5 g), 5 ml water and one drop of bromine were added followed by reflux for 15 min. Cool to room temperature and bring to 100 ml with water. About 1 hexavalent phosphomolybdic/phosphotungestic acid complex is formed.

The solvents, sulphuric acid and all chemicals used in the study were purchases from Merck Chemicals company.

Two systems: ethanol extraction and aqueous extraction were studied and compared. Frozen leaves powder (2 g) was placed in a 50 ml centrifuge tube, and 16 ml of solvent or aqueous phase was added. The preparation was left to stand at different temperature (various from 20 to 80 ◦C) for 3 h. The mixtures were then centrifuged using a Mistral 1000 centrifuge (MSE Labsupply Pierce, Loughborough, Leicestershire, UK) at 500×*g* for 10 min at room temperature. After centrifugation, the supernatants were filtered through Whatman No. 42 filter paper (Whatman Inc., Clifton, NJ, USA). Following filtration, a 10ml aliquot of the filtrate was concentrated by evaporation of the solvent, using a rotary evaporator (Rotary Vacuum Evaporator Laborota-4011, G6,Heidolph Co., Germany) under partial vacuum at 40 ◦C until less than 1ml of filtrate remained. The extract was then re-dissolved in 10 ml of Distilled water and stored at 4 ◦C prior to purification step. All the extracts were prepared in triplicate.

Sugars and organic acids can contribute to the absorbance measurement in the Folin-Ciocalteu assay.[16,17]. Purification of the crude extracts is necessary. Sugars and organic acids were removed from the crude extract using the method of [18] with some modifications.

Total pehnolic contents in leaves extract were evaluated using the Folin-Ciocalteu assay, which was adapted from [19] with some modifications as described by [20]. Briefly, 250μl of leaves extract (in triplicate), a gallic acid calibration standard, or Distilled water (as blank) was placed in a separate 25 ml volumetric flask, followed by the addition of 15 ml Distilled water and 1.25 ml Folin-Ciocalteu reagent. The contents were swirled to mix and allowed to stand for 5–8 min at room temperature. Next, 3.75 ml of a solution of sodium carbonate (7.5% w/v) was added. Then, Distilled water was added to the flask to volume. Solutions were mixed and allowed to stand for 2 h at room temperature before measurement of the absorbance at 765 nm using UV–vis Spectrophotometer (JASCO, V-550 UV/VIS Spectrophotometer, Japan). If any sample had an absorbance reading above the reading of 500 mg l^{-1} standard, it was diluted adequately and re-measured. Results are expressed as mean total phenol content (mg of gallic acid equivalents per 100 g of leaves of figs) ±S.D. for triplicates.

RESULTS AND DISCUSSION

Phenolic compounds were extracted with ethyl alcohol and pure water and the results are expressed in gallic acid equivalents (GAE). Various parameters, which can affect the recovery of phenolic contents from leaves of figs using solvent extraction, were studied. These parameters include the conditions of the leaves of figs samples effect of repeated extraction, different types of organic solvents used the concentration of the solvent and temperature of extraction.

Fig. 1 shows the comparison of the phenolic compounds recovery from leaves of figs by using oven-dried material, or using fresh frozen material. Fig. 1 indicates that there is 345.81 mg GAE of phenolic compounds obtained from 100 g oven dried leaves of figs; the recovery is higher than 151.36 mg GAE of phenolics from 100 g frozen material. After 24–26 h of oven-drying, the weight of the left material was approximately 25.18±1.60% of the original fresh frozen ficus. The weight reduction is mainly because of the evaporation of the water content. A 100 g fresh ficus converted to dry weight basis is 25.18±1.60 g, from which 151.36 mg GAE total phenolic content extracted. The drying process (temperature or long drying time) might destroy some of the phenols and the water present in plant cells may have assisted the extraction of phenols; on the other hand, in the dried material, all the components (e.g., membranes and organelles) in the cells adhere together in the absence of water, and possibly making the extraction with solvent more difficult, as a result, the overall recovery was lower. Thus, if leaves of figs are dried and then used for extraction, the recovery is much lower overall than using the fresh frozen material

for processing. Therefore, fresh frozen leaves of figs powder was used for extraction and all the results are presented on a fresh weight basis.

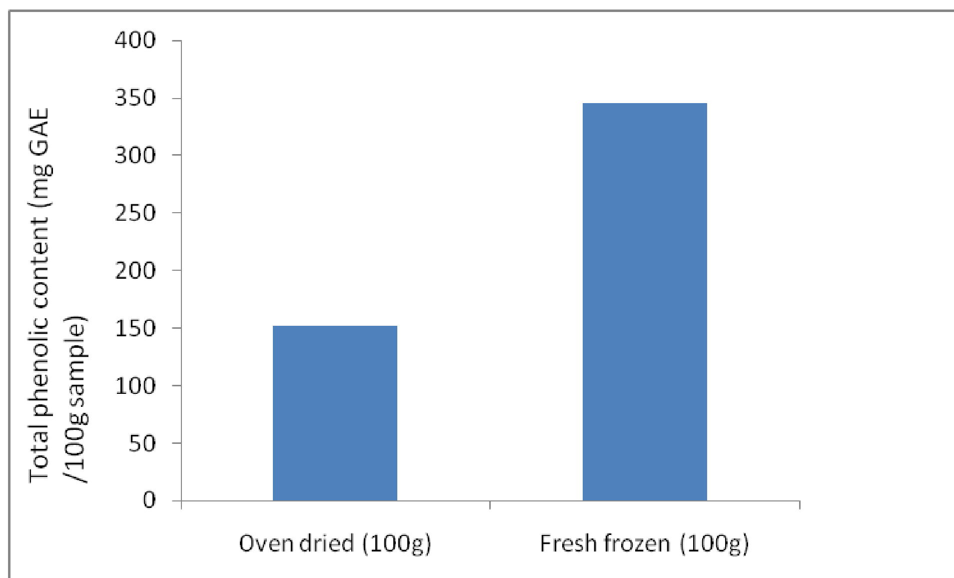

Fig. 1.Comparison of the total phenolic recovery from leaves of figs by using oven-dried material, with using fresh frozen material.

Fig. 2 compares the recoveries obtained from single-extraction of 3 h and double-extraction (2× for 1.5 h). The results show that a single-extraction results in higher recovery than a double-extraction, when using different concentrations of ethanol.

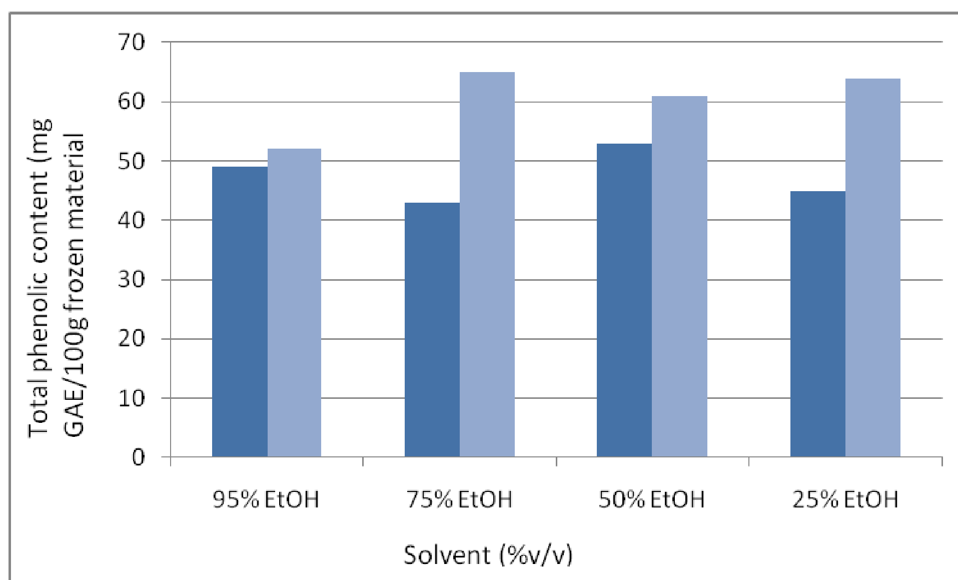

Fig. 2 Effect of repeated extraction on the recovery of total phenolics ( ) double-extraction;( ) single-extraction. Results are presented as mean ± S.D. for triplicate analyses.

Fig. 3 shows that the recoveries of total phenolics from leaves of figs, obtained by using 95% (v/v) ethanol and 95% (v/v) methanol were similar. However, methanol is a toxic and harsh organic solvent whereas ethanol is more acceptable for use in food industry. Thus, ethanol was used as the solvent of choice in all subsequent studies.

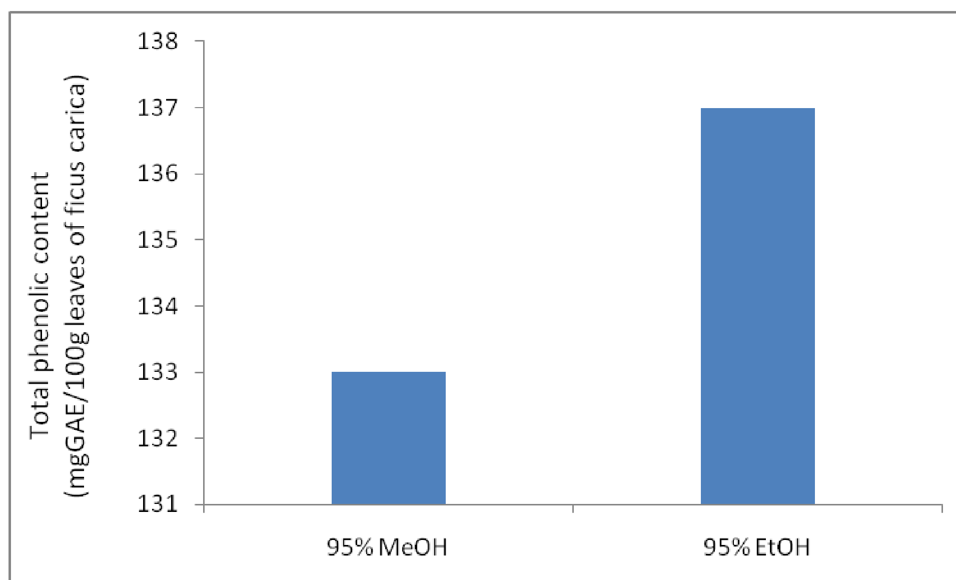

Fig. 3 Effect of types of organic solvents used for extraction. Results are presented as means±S.D. for triplicate analyses.

The effect of the ethanol concentration used in the extraction is presented in Fig. 4 and in Table 1. The recovery of total phenolics increased with increase in the ethanol concentration, until the concentration reached 85%; after which, the recovery reduced with the increase of ethanol concentration. The greatest recovery was achieved when using an ethanol concentration between 80% and 70% (v/v), even when the extraction time was increased to 6 h. Table 1 indicates that in the aqueous only extraction, phenolics were also extracted, although the amount was lower. Since there was little difference in extraction between 75% and 80% concentration, the lower concentration was chosen as the data indicated that the presence of water assisted the extraction. It should be noted is that the volume of water for each solvent concentration in real terms is a little higher due to the water present in the fresh leaves of figs tissue.

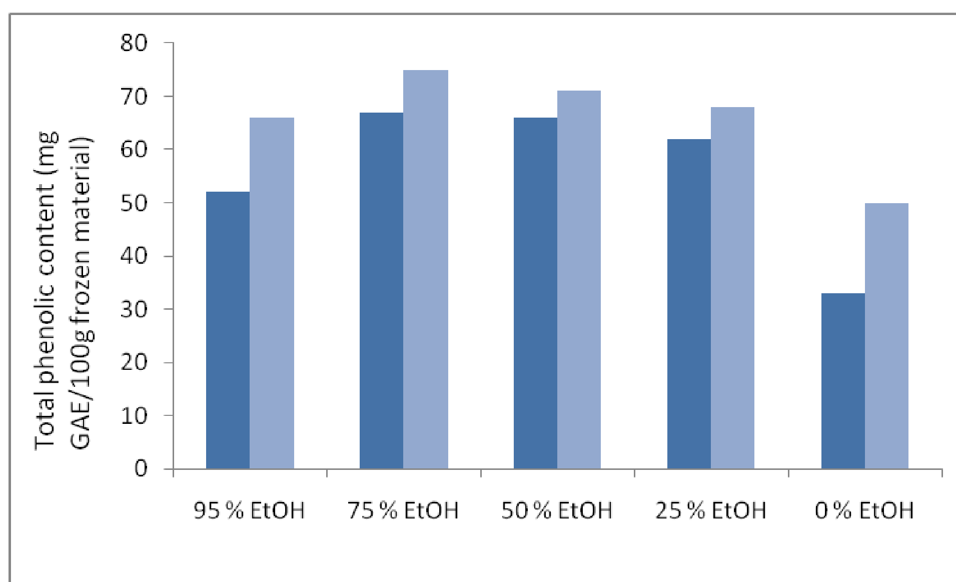

Fig. 4. Effect of ethanol concentration used as extraction media on the recovery of total phenolics from samples: ( ) sample extracted for 3 h; ( ) sample extracted for 6 h. Results are presented as means±S.D. for triplicate analyses

Table 1.Effect of ethanol concentration on extraction of phenolic from Ficus carica

Concentration of ethanol % (v/v)	Extracted for 3 h		Extracted for 6 h	
	TP content (mg GAE/100 g leaves of Ficus carica	Increase in recovery from aqueous[a] (%)	TP content (mg GAE/100 g leaves of Ficus carica	Increase in recovery from aqueous[a] (%)
0	33.30±1.11	-	50.12±2.10	-
25	62.92±2.45	51.37	68.15±1.32	28.41
50	66.23±1.14	60.54	71.65±1.54	45.63
75	67.89±1.50	75.21	75.68±2.01	59.84
95	52.41±1.64	24.61	66.55±1.58	32.45

Results are presented as means± S.D. for triplicate analyses. [a]Aqueous only extraction.

Generally, the higher the incubation temperature the greater is the total phenol recovery as observed in Fig. 5 An exception is incubation at 37 ◦C, where the recovery was lower than 19 ◦C. Higher incubation temperature may improve the recovery, because incubation in hot water can extract some pectic polysaccharides from cell wall [21], and weaken the cell wall integrity. Possibly, as a result, the solvent containing water can easily get in contact with the phenolic materials, and the recovery is improved.

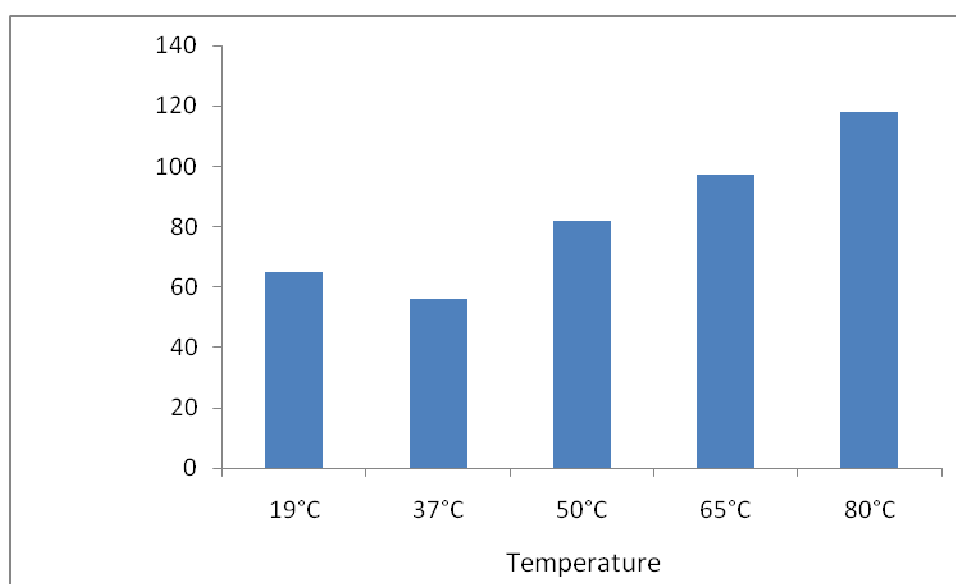

Fig. 5. Effect of extraction temperature on 75% (v/v) ethanol extraction system. Results are presented as means±S.D. for triplicate analyses.

CONCLUSION

The total phenolic contents of leaves of ficus citrus were affected by the method of leaves preparation, the type of the solvent and its concentration and the operating temperature. Ethanol was found to be the best solvent for the extraction. The variation of the phenolic contents depended on the extraction conditions. The recovery increased with the increase in ethanol concentration up to 80% ethanol (v/v), after which the recovery decreased. In general, the recovery increased with the increase in temperature of extraction. Solvent extraction gives reasonable recovery but it poses some disadvantages like the solvent need to be evaporated adding extra cost and possible loss of quality. Therefore, other methods should be considered to extract phenolic contents from plant materials.

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