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Full Length Research Paper

High hydrostatic pressure extraction of phenolic compounds from *Maclura pomifera* fruits

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High hydrostatic pressure processing (HHPP) is a food processing method, in which food is subjected to the elevated pressure which is mostly between 100 to 800 MPa. HHPP is seen not only in food engineering, but also have other application areas, such as extraction of active ingredients from natural biomaterials. In this study, several extraction conditions such as two different solvents [methanol and solvent cocktail (dH_2O:ethanol:methanol:acetone:CH_2Cl_2 - 1:2.5:2.5:2:2)], two different pressures for high hydrostatic pressure extraction (HHPE) (250 and 500 MPa), three different extraction methods (shaking at room temperature, soxhlet extractor and HHPE) and different extraction times for each extraction method (10 min for HHPE, 2 h for shaking and 14 h for soxhlet extraction) were used in order to extract phenolic compounds from *Maclura pomifera* fruits. The highest amount of phenolic compounds (913.173 µg gallic acid equivalent (GAE/mL) was observed in HHPE at 500 MPa using solvent cocktail, where the lowest amount (316.877 µg GAE/mL) was in soxhlet extraction using methanol. In terms of extraction efficiency, the highest amount of extraction is seen in the shortest time period. It was observed that HHPE in solvent cocktail was the most effective method when compared to the other methods tested.

Key words: *Maclura pomifera*, Osage orange, hedge apple, phenolic compound, high hydrostatic pressure extraction.

INTRODUCTION

Maclura pomifera (Raf.) Schneid. is a thorny, dioecious tree, which is classified under Moraceae family (Mulberry family) (United States Department of Agriculture [USDA], 2011; Wagner et al., 1999). It has some common names such as Osage orange (USDA, 2011), hedge apple, bois d'arc (Carey, 2011). The genus, *Maclura*, is named in honor of American geologist William Maclure. The species name, *pomifera*, refers to bearing pomes or apples, for the fruit (Starr et al., 2003). *M. pomifera* is mostly planted because of their hardiness, tolerance to drought, extremely hard wood, resistance to termites, and ability to grow in most types of soils (Starr et al., 2003).

The female *M. pomifera* flowers in ripening become very fleshy, forming a large multiple fruit or syncarp composed of 1-seeded drupelets. The ripe fruit, 7.6 to 15 cm in diameter, yellowish-green, resembles an orange and exudes a bitter milky juice when bruised (Bailey, 1935; Burton, 1990).

The *M. pomifera* fruits consist of some alkaloids, glycosides, carbohydrates, flavonoids such as morin and rutin (Nasacheva et al., 1973). Different metabolites have been isolated from the fruits and other parts of *M. pomifera* tree (López, 1993; Monache et al., 1994; Rudenskaya, 1995; Lee et al., 1998). Osajin and pomiferin are also two types of flavonoids extracted from *M. pomifera* fruits which show antimicrobial activity (Ismail et al., 2001). Fruit extracts are also used in animal

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Abbreviations: HHPP, High hydrostatic pressure processing; **HHPE,** high hydrostatic pressure extraction; **GAE,** gallic acid equivalent.

Table 1. Solvents used for active compound extraction Cowan(1999).

Water	Ethanol	Methanol	Dichloromethane	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Flavonols
Starches	Polyphenols	Terpenoids		
Tannins	Polyacetylenes	Saponins		
Saponins	Flavonols	Tannins		
Terpenoids	Terpenoids	Xanthoxyllines		
Polypeptides	Sterols	Totarol		
Lectins	Alkaloids	Quassinoids		
		Lactones		
		Flavones		
		Phenones		
		Polyphenols		

immunological studies (Ismail et al., 2001).

Extraction of active substances from *M. pomifera* fruit is not very easy because of its adhesive nature. Especially after condensing process, which is the step of evaporating the extraction solvent, the extracts stick inside the extraction medium such as Eppendorf tubes and huge amounts of active substance lost, which cause low amount of extraction yield.

In order to get high amount of extraction yield of flavonoids, one of the phenolic compounds, scientists try different types of extraction methods. High hydrostatic pressure extraction (HHPE) is one of those extraction methods.

HHPP is a non-thermal process that can effectively be used to extract active ingredients from natural biomaterial (Zhang et al., 2005). HHPP is cold isostatic super high hydraulic pressure that ranges from 100 to 800 MPa or more (US Food and Drug Administration Center [USFDAC], 2011).

HHPE does not need any heating process; it could be operated at room temperature (Altuner et al., 2006). According to the compression, a slight rise in temperature is also expected (Zhang et al., 2005). But some type of HHPP equipments could get rid of this rise with their cooling systems.

During HHPP, the solubility increases, as the pressure increases according to the phase behavior theory (Zhang et al., 2005; Richard, 1992; Le Noble, 1988). It is also known that pressurized cells show increased permeability due to the mass transfer theory (Zhang et al., 2005; Yan, 2002). This means the higher the hydrostatic pressure is, the more solvent can enter into the cell. As they enter, the more compounds can permeate the cell membrane which could cause the higher yield of extraction. A rapid permeation is observed under HHPP due to the large differential pressure between the cell interior and the exterior of cell membranes (Zhang et al., 2005).

Plants contain many compounds. These compounds could have varying polarities. The extraction solvents used in HHPE also vary according to the polarity of the compound of interest. Thus, HHPE can be applied to the extraction of strongly polar, weakly polar and non-polar compounds using different solvents (Zhang et al., 2005).

In this study, several extraction conditions such as two different solvents methanol (MeOH) and solvent cocktail (dH_2O:ethanol:methanol:acetone:CH_2Cl_2 - 1:2.5:2.5:2:2), two different pressures for high hydrostatic pressure extraction (HHPE) (250 and 500 MPa), three different extraction methods (shaking at room temperature, soxhlet extractor and HHPE) and different extraction times for each extraction method (10 min for HHPE, 2 h for shaking and 14 h for soxhlet extraction) were used in order to extract phenolic compounds from *M. pomifera* fruits.

It is also tested whether HHPE could be an effective method in order to extract phenolic compounds from plant materials having adhesive nature such as *M. pomifera* fruits.

MATERIALS AND METHODS

Phenolic compound extraction

M. pomifera fruits were collected from *M. pomifera* trees previously planted in the garden of Ankara University, Faculty of Science and identified by Ankara University, Department of Biology.

M. pomifera fruits were cut into small pieces and dried before extraction procedures. Dried samples were then grounded by mortar and pestle. Grounded samples were kept at -20°C until extraction. Three different types of extraction methods were applied according to the following procedures. In all extraction procedures, the ratio was kept as 1 g *M. pomifera* samples per 5 mL solvent.

Cowan (1999) has reviewed the solvents used for active compound extraction and these results are given in Table 1.

In order to extract the highest amount of phenolic compounds, methanol and solvent cocktail (dH_2O: ethanol: methanol: acetone: CH_2Cl_2-1: 2.5: 2.5: 2: 2) was chosen as the extraction solvent. All solvents were purchased from Merck (Germany).

Extraction by shaking at room temperature

10 g of ground samples were mixed with 50 mL of MeOH and

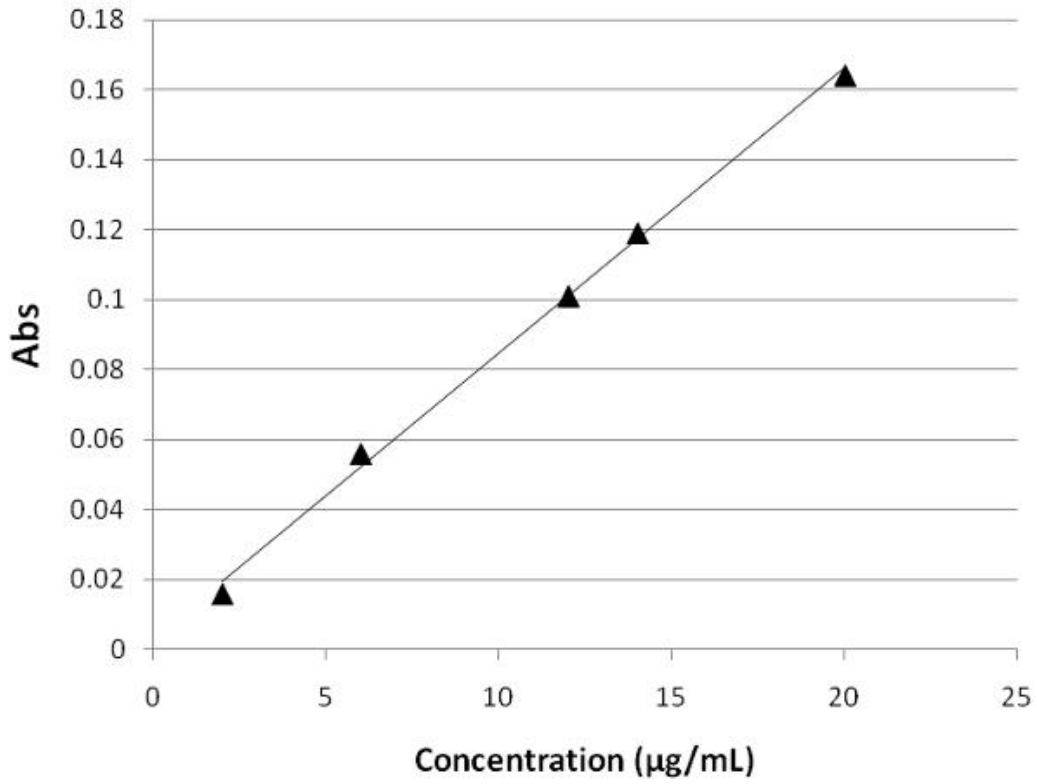

Figure 1. Calibration curve for gallic acid solutions.

solvent cocktail. Samples mixed with two different solvents were shaken for 2 h at room temperature to extract phenolic compounds from *M. pomifera* fruits.

Extraction by soxhlet extractor

20 g of ground samples were placed in a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor and 100 mL of solvents were put in the still pot. Phenolic compounds were extracted by soxhlet extractor for 14 h.

Extraction by high hydrostatic pressure

1 g of ground samples was mixed with 5 mL of MeOH and solvent cocktail. These samples were then processed by HPP equipment at 250 and 500 MPa for 10 min at room temperature.

High hydrostatic pressure was applied with an industrial high pressure system (SITEC CH-8124, Zürich, Switzerland). Samples were pressurized at 250 and 500 MPa at room temperature for 10 min. Water was used as the pressure transmitting medium. A built-in heating-cooling system (Huber Circulation Thermostat, Offenburg, Germany) was used to maintain and control the required temperature which is measured by a thermocouple. Pressurization rates were 400 MPa/min for 250 MPa and 300 MPa/min for 500 MPa. Decompression time was less than 20 s.

Measuring the extracted total phenolic compounds

The content of total phenolic compounds was measured by Folin-Ciocalteu's reagent which is also known as Gallic acid equivalence (GAE) method (Singleton et al., 1999). Gallic acid was used to establish standard curve for total phenolic content as defined by Koç et al. (2010). Gallic acid calibration solutions having final concentrations ranging from 2 to 20 µg/mL were used to plot calibration curve (Figure 1).

750 µl of Folin - Ciocalteu's phenol reagent was added on 100 µl of each extract and shaken. After 5 min, 750 µl of (6%) Na_2CO_3 solution was added to the mixture. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 765 nm with an UV-visible spectrophotometer. Total phenolic content was expressed as µg GAE/mL (Gayosa et al., 2004; Singleton and Rossi, 1965). All experiments and analysis were performed in triplicate.

Statistical analysis

The data were expressed as mean of 3 parallel studies. All values given here are mean values of these 3 parallel studies. Statistical analysis was performed using a non-parametric method Kruskal-Wallis one-way analysis of variance. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The effect of solvents on the extraction of phenolic compounds

Figures 2, 3, 4 and 5 show that solvent cocktail can be

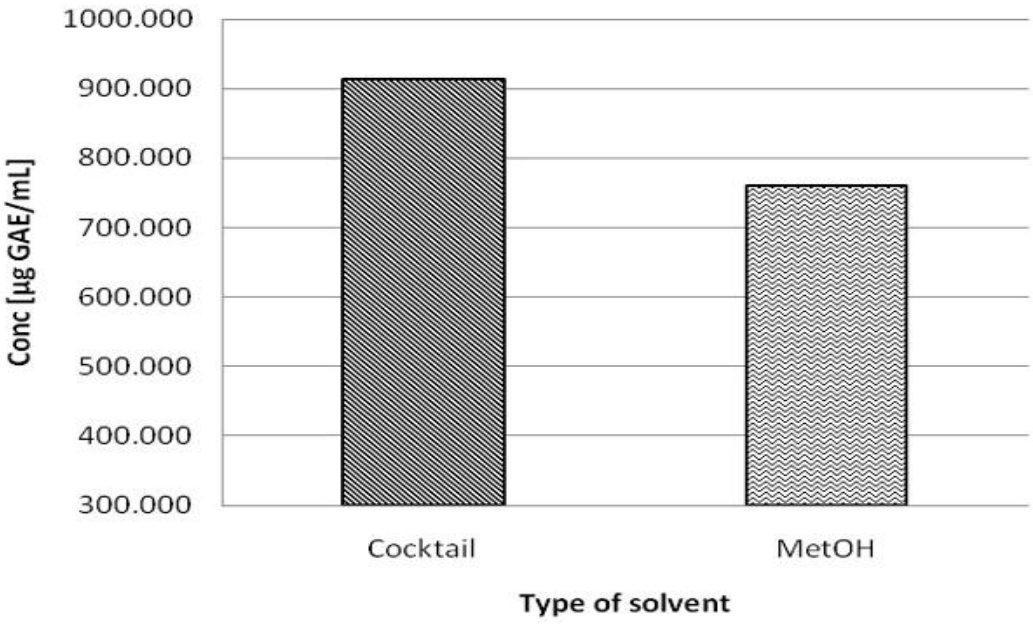

Figure 2. The effect of different solvents on the extraction of phenolic compounds in HHPP at 500 MPa.

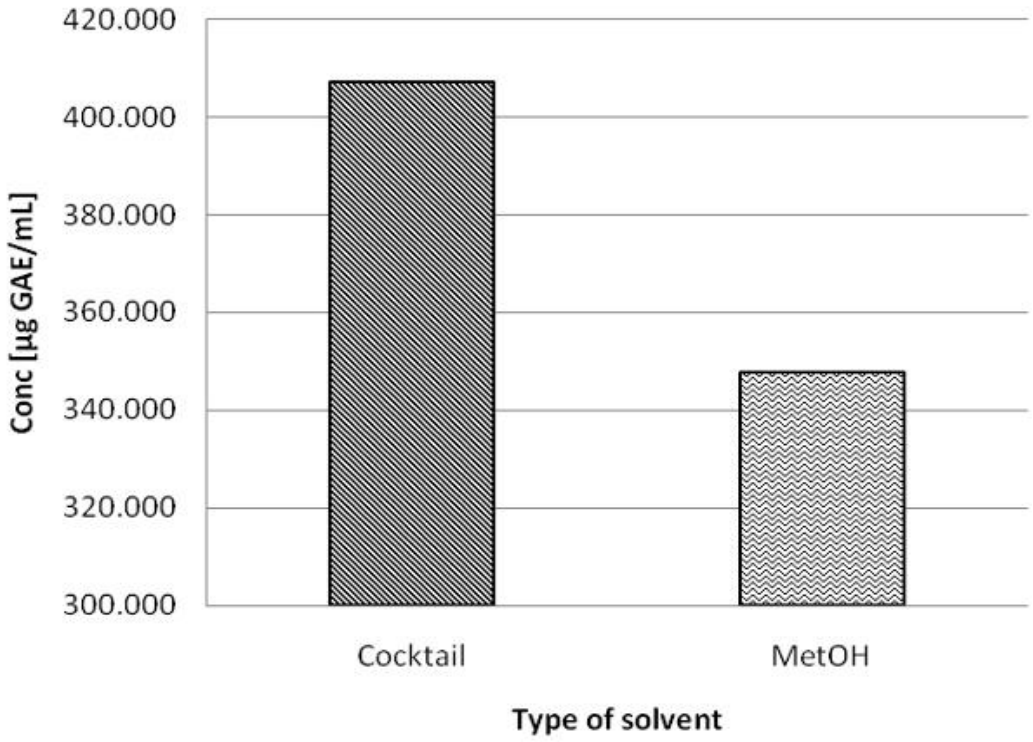

Figure 3. The effect of different solvents on the extraction of phenolic compounds in HHPP at 250 MPa.

used to obtain higher extraction of phenolic compounds instead of using MeOH in HHPP soxhlet extraction and shaking at room temperature.

The amount of phenolic compounds extracted is 1.20 times higher in solvent cocktail than in MeOH at 500 MPa, where it is 1.17 times higher in solvent cocktail than in MeOH at 250 MPa, 1.09 times higher in solvent cocktail than in MeOH by soxhlet extraction and 1.12 times

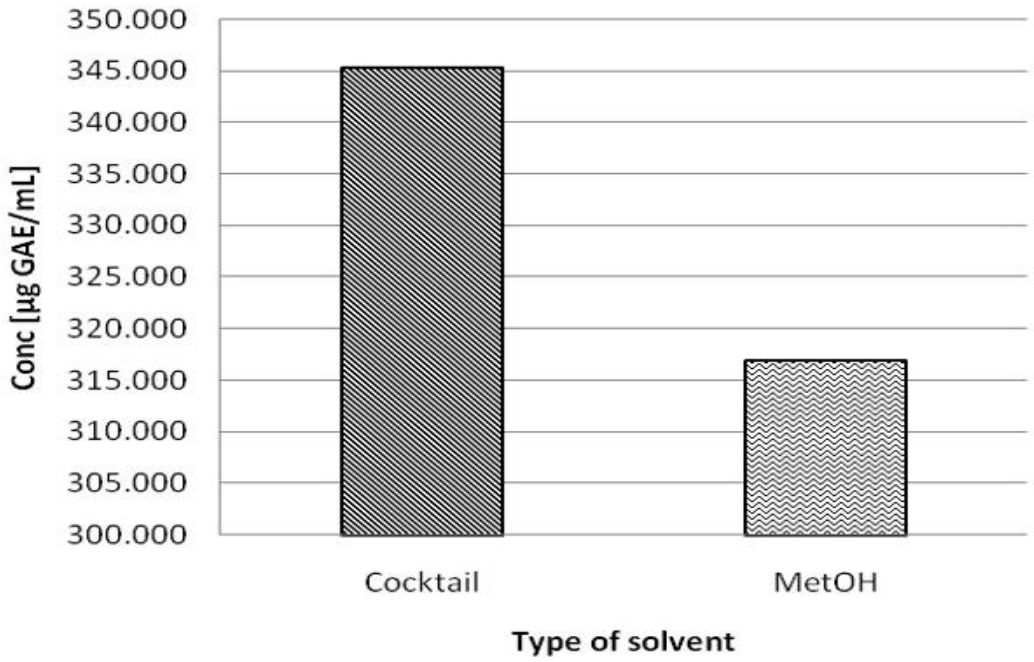

Figure 4. The effect of different solvents on the extraction of phenolic compounds in soxhlet method.

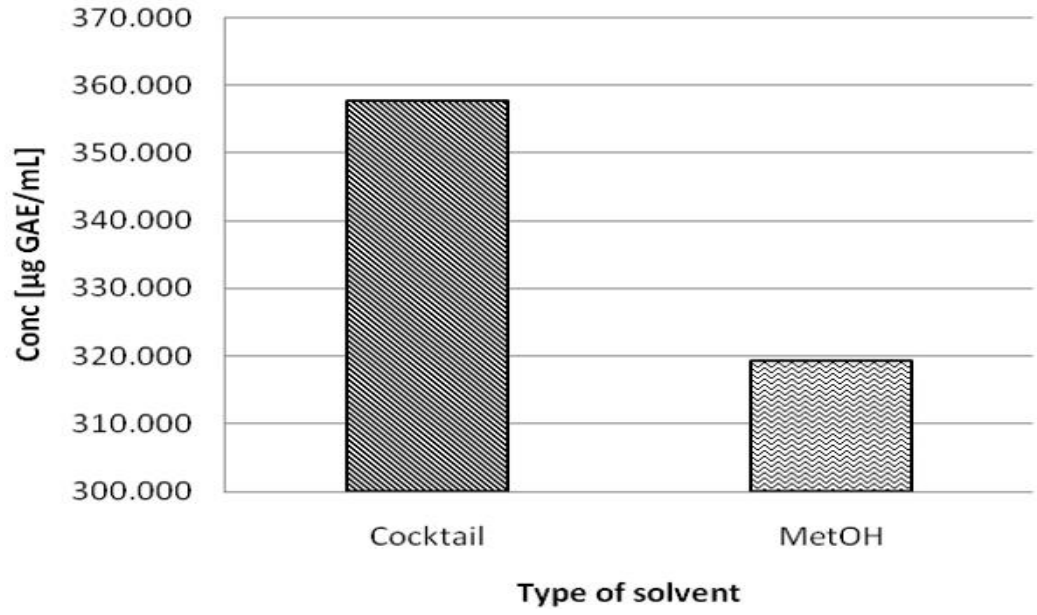

Figure 5. The effect of different solvents on the extraction of phenolic compounds in shaking method.

higher in solvent cocktail than in MeOH by shaking at room temperature.

The effect of HHPE on the extraction of phenolic compounds

Figure 6 shows how HHPE affected the amount of extraction of phenolic compounds from *M. pomifera* fruits. It is observed that as the pressure increases, the extraction of phenolic compounds increases. The amount of phenolic compounds extracted is 2.24 times higher at 500 MPa than at 250 MPa HHPP in extraction by solvent cocktail where it is 2.19 times higher at 500 MPa than at 250 MPa in extraction by MeOH.

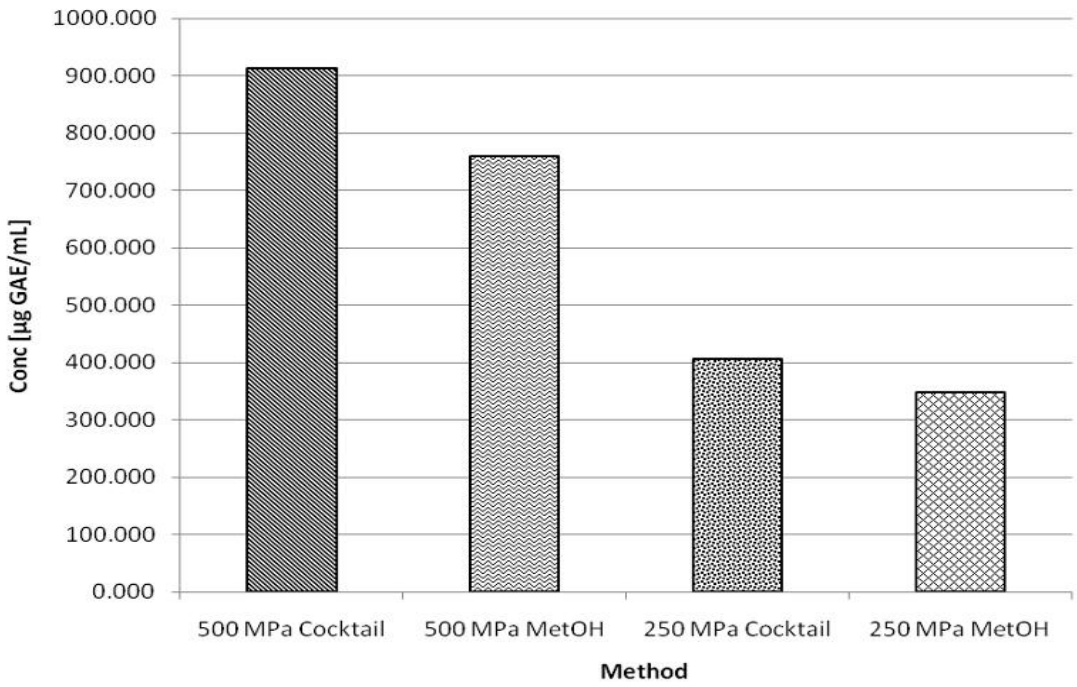

Figure 6. The effect of HHPE on the extraction of phenolic compounds.

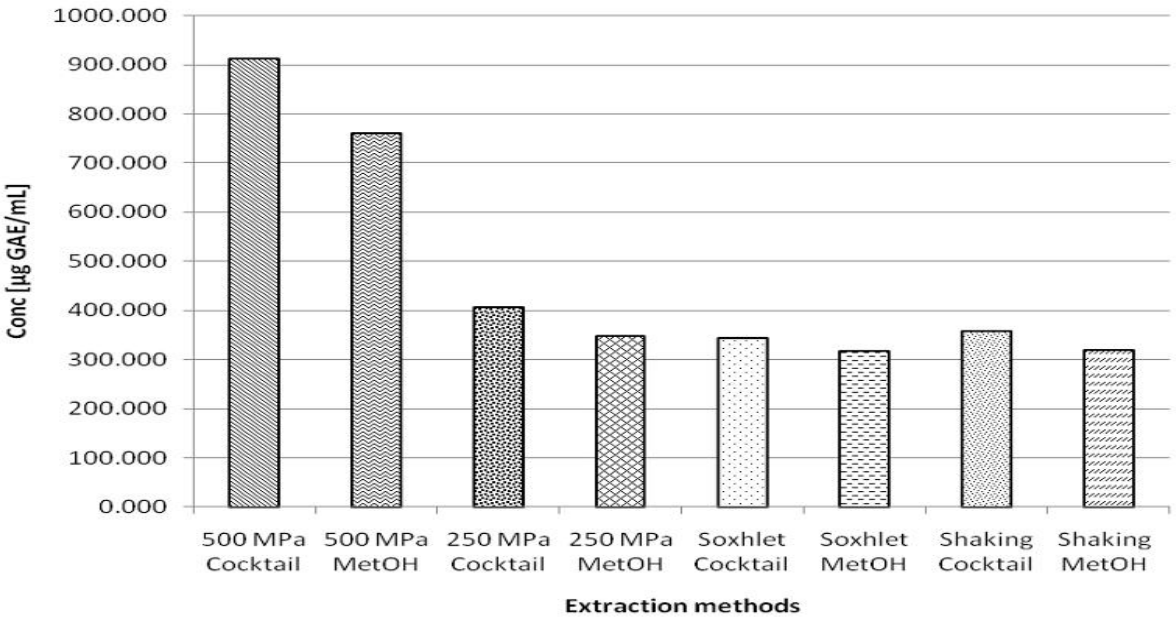

Figure 7. Comparison of HHPE and other extraction methods.

Comparison of HHPE and other extraction methods

Figure 7 shows the comparison of HHPE against other extraction methods in terms of the extraction of phenolic compounds. The highest amount of phenolic compounds extracted by eight different types of extraction methods is 913.173 µg GAE/mL extracted by 500 MPa HHPP by using solvent cocktail and the lowest amount of phenolic compounds extracted by eight different types of extraction methods is 316.877 µg GAE/mL extracted by soxhlet extraction by using MeOH.

According to Figure 7, we can set all methods in order from the highest amount to the lowest amount of phenolic compounds extracted as 500 MPa HHPP - solvent cocktail,

500 MPa HHPP - MeOH, 250 MPa HHPP - solvent cocktail, shaking at room temperature with solvent cocktail, 250 MPa HHPP - MeOH, soxhlet extraction with solvent cocktail, shaking at room temperature with MeOH and soxhlet extraction with MeOH. The amount of phenolic compounds extracted by all methods is given in Figures 2, 3, 4, 5, 6 and 7.

DISCUSSION

A well known saying about solubility is “like dissolves like” (Williamson, 1994). This means that a solute will dissolve best in solvent having similar chemical structure. The overall extraction capacity of a solvent primarily depends on its polarity.

In our study, two types of solvents were used for the extraction of phenolic compounds. According to the results, solvent cocktail yield higher extraction than MeOH in all extraction methods. As the solvent cocktail is composed of the mixture of solvents having different polarities, it could dissolve more solutes than MeOH.

Solubility increases, according to the pressure increase in HHPE process (Zhang et al., 2005; Richard, 1992; Le Noble, 1988). Figure 6 shows that 500 MPa extracted more phenolic compounds than 250 MPa. Under the process of high pressure, it is expected that the solubility is greater as the pressure increases (Le Noble, 1988; Richard, 1992), which is also observed in our study too.

Figure 7 shows that the highest amount of phenolic compounds is extracted in HHPE. As it is stated previously the solubility changes according to the pressure change. Solubility also increases according to the increase in the temperature up to 100°C for many solids (Hill and Petrucci, 1999). So, a higher amount of phenolic compound is expected when compared to shaking at room temperature. But Figure 7 clearly shows that shaking method extracts more phenolic compounds than soxhlet extraction.

Most of the phenolic compounds are very sensitive to several degradation factors such as temperature, presence of oxygen and light (Junior et al., 2010). As a result, soxhlet extraction method extracted lower amount of phenolic compounds when compared to the shaking method, as in some other traditional extraction techniques which expose phenolic compounds high temperature and oxygen (Vatai et al., 2009; Bleve et al., 2008).

As a result it can be inferred that HHPE could be a very effective extraction method for plant materials having adhesive nature such as *M. pomifera* fruits.

REFERENCES

Altuner EM, Alpas H, Erdem YK, Bozoglu F (2006). Effect of high hydrostatic pressure on physicochemical and biochemical properties of milk. Eur. Food Res. Technol. 222: 392-396.

Bailey LH (1935). The Standard Cyclopedia of Horticulture. Macmillan, NY.

Bleve M, Ciurliaa L, Erroi E, Lionetto G, Longo L, Rescio L, Schettino T, Vasapollo G (2008). An innovative method for the puri cation of anthocyanins from grape skin extracts by using liquid and sub-critical carbon dioxide. Sep. Purif. Technol. 64: 192-197.

Burton JD (1990). *Maclura pomifera* (Raf.) Schneid. Osage-orange. In: Burns RM, Honkala BH, technical coordinators. Silvics of North America: Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC. Vol. 2.

Carey JH (2011). *Maclura pomifera.* In: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Sciences Laboratory. Fire Effects Information System. http://www.fs.fed.us/database/feis, accessed 04.06.2011.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 564-582.

Gayosa C, Pomar F, Merino F, Bernal MA (2004). Oxidative metabolism and phenolic compounds in *Capsicum annuum* L. var. *annuum* infected by *Phytophthora capsici* Leon. Sci. Hortic-Amsterdam, 102(1): 1-13.

Hill JW, Petrucci RH (1999). General Chemistry 2nd edition. Prentice Hall.

Ismail AI, Radwan HM, Hussein AA (2001). Biodetrimental effects of the major flavonoidic constituents from *Maclura pomifera* on the European corn borer, *Ostrinia nubilalis* (Hub.). J. Egypt Ger. Soc. Zool. Entomol. 36: 59-66.

Junior MRM, Leite AV, Dragano NRV (2010). Supercritical Fluid Extraction and Stabilization of Phenolic Compounds From Natural Sources - Review (Supercritical Extraction and Stabilization of Phenolic Compounds). The Open Chem. Engr. J. 4: 51-60.

Koç E, İşlek C, Üstün AS (2010). Effect of Cold on Protein, Proline, Phenolic Compounds and Chlorophyll Content of Two Pepper (*Capsicum annuum* L.) varieties. GU. J. Sci. 23(1): 1-6.

Le Noble WJ (1988). Organic High Pressure Chemistry. Elsevier, Amsterdam.

Lee SJ, Word A, Maier C, Dixon R, Mabry T (1998). Prenylated flavonoids from *Maclura pomifera*. Phytochemistry, 49(8): 2577.

López LMI, Natalucci CL, Caffini NO, Curotto E (l993). Isolation and partial purification of serine proteinases present in the latex of *Maclura pomifera* ("osage orange") fruits. Acta Alimentaria (Budapest), 22(2): 131-142.

Monache CR, Scurina R, Vitall A, Botta B, Monacelli B, Pasua G, Palocci C, Cernia N (1994). Two isoflavone and a flavone from the fruits of *Maclura pomifera*. Phytochemistry, 37(3): 893.

Nasacheva EP, Anisimova KI, Snteinbok SD (1973). Rind (Pericarp) Substances of Pomegranate Fruits and Prospects for Its Use in Tanning Industry. Rast. Resour. 9(2): 267-270.

Richard JS (1992). High Pressure Phase Behaviour of Multicomponent Fluid Mixtures. Elsevier, Amsterdam.

Rudenskaya GN, Bogdanova EA, Revina LP, Golovkin BN, Stepanov VM (1995). Macluralisin - a serine proteinase from fruits of Maclura pomifera (Raf.) Schneid. Planta, 196(1): 174-179.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158.

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Method. Enzymol. 299: 152-178.

Starr F, Starr K, Loope L (2003). *Maclura pomifera*, Osage orange, Moraceae. http://www.hear.org/starr/hiplants/reports/pdf/maclura_pomifera.pdf, accessed 04.06.2011.

United States Department of Agriculture, Natural Resources Conservation Services, National Plant Data Center (2011). National Plants Database. http://plants.usda.gov, accessed 04.06.2011.

US Food and Drug Administration Center for Food Safety and Applied Nutrition (2011). Kinetics of microbial inactivation for alternative food processing technologies-high pressure processing. http://www.cfsan.fda.gov/, accessed 04.06.2011

Vatai T, Skerget M, Knez Z (2009). Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide. J. Food Eng. 90: 246-254.

Wagner WL, Herbst DR, Sohmer SH (1999). Manual of the Flowering

Plants of Hawai'i. Bishop Museum Special Publication, University of Hawai'i and Bishop Museum Press, Honolulu.
Williamson KJ (1994). Macroscale and microscale organic experiments (2nd ed.). Lexington, Mass.: D.C, Health.
Yan H (2002). Separation Engineering. China Petrochemical Press, Beijing.
Zhang S, Xi J, Wang C (2005). High hydrostatic pressure extraction of flavonoids from propolis. J. Chem. Technol. Biot. 80: 50-54.