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European Journal of Experimental Biology, 2013, 3(1):38-41

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Phenolics in Henna: Extraction and stability

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ABSTRACT

This study was designed to examine the percentage yield and total phenolic contents of various extracts (prepared by using solvents of varying polarity and different extraction methods) of Henna leaves. Total phenolic content in the extracts was determined spectrometrically applying the Folin-Ciocalteu assay and calculated as tannic acid equivalents. Maximum amount of extract (24.83%) was obtained with water, followed by methanol (23.06%) and ethanol (13.25%), while the maximum amount of phenolics (308.8 µg tannic acid equivalent per gramme of leaves) was obtained with methanol, followed by water (150.3 µgTAE gdw^{-1}) and ethanol (141.5 µgTAE gdw^{-1}) using the ultrasound method. Using ultrasound increased the total phenolic compounds of the Henna leaves extract. The total phenolic content was not affected by storage in dark conditions at -18 and 25 °C and in light conditions at 25 °C over a period of 10 days, while a significant reduction was observed for extracts at 25 °C either in dark or light conditions after 20 days and for all the extracts after 30 days storage.

Keywords: methanol extract, Henna leaves, sonication, phenolics

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INTRODUCTION

Phenolic compounds, widely distributed within plants [1], are commonly isolated, using aqueous or organic solvents. Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Many plants have been used for different purposes, such as food, drugs and perfumery [2]. Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [3]. In general, phenolic compounds possess ideal structural chemistry for free radical-scavenging and metal-chelating properties, and have been shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis [4]. There is intense interest in plant polyphenols as witnessed by the numerous papers devoted to various aspects of these compounds [5-7]. The use of plants, herbs as antioxidants in prossesed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants [8]. In vitro studies suggest that foods like vegetables, grains, seeds [9] and legumes [10] with antioxidants have protective effects against many diseases.

Due to these facts, it would be interesting to optimize an extraction process to obtain maximum yield of these substances. Several extraction techniques have been reported for extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, water, ethyl acetate and petroleum ether [11, 12]. Furthermore, supercritical CO_2 [13, 14] and solvent extraction by sonication have been applied for this purpose [15]. Henna (*Lawsonia inermis*) is a plant which grows wild in abandoned areas. This plant is a worldwide known cosmetic agent used to stain hair, skin and nails [16]. Alcoholic extracts of Henna leaves showed mild antibacterial activity against *Micrococcus pyrogenes* var *Aureus* and *Eschericia coli* [17]. This plant has been reported to have the antioxidative and anticarcinogenic effects [18]. However there is no study reported on the extraction of phenolics from leaves of Henna using ultrasound and optimizing the extraction process. Therefore, the present work was undertaken to study the extraction of phenolics from Henna leaves using solvent and ultrasound-assisted methods. Attempts were also made to study the stability of these extracts during storage.

MATERIALS AND METHODS

Plant material and chemicals

Henna leaves were obtained from the Kerman province of Iran. Leaves were dried and ground to give 40-mesh size powder. All chemicals and solvents were of analytical grade and obtained from Merck Chemical Company.

Ultrasonic extraction

The ultrasound assisted extraction procedure was used for the extraction of Henna leaves with different solvents (water, methanol, ethanol, acetone, chloroform and hexane). Thus 10 ml of solvent were added to 500 mg of powdered leaves, the mixture was sonicated in an ultrasonic bath for 15 min. The extract was filtered through whatman No. 41filter paper for removal of leaves particles and then centrifuged at 3000 × g for 10 min at 5 °C and stored in a refrigerator [19].

Solvent extraction

In this method 10 g of ground leaves were extracted by mixing using a magnetic stirrer, with 200 ml of methanol and also with 200 ml of water at room temperature overnight .The extract was filtered through whatman No.41 filter paper and the residue was re-extracted under the same conditions. The combined filtered was evaporated in a rotary evaporator below 40 °C. Then centrifuged at 3000 × g for 10 min at 5 °C and stored in a refrigerator [19].

Stability of Henna leaves methanol extract

Three 2-g samples of Henna leaves were extracted with methanol using the ultrasound-assisted method. The extracts were filtered and then centrifuged at 3000 × g for 10 min at 5 °C [19]. The methanol extract from Henna leaves was divided into three (10 ml) aliquots. The first aliquot was stored in dark condition under freezing (-18 °C), the second in dark condition at room temperature (25 °C), and the third was stored in light conditions at room temperature (25 °C) for 30 days. The total phenolic content was determined periodically over 30 days for each aliquot.

Determination of total phenolic content

The concentration of phenolics in the extracts was determined by the method of Singh *et al*. 2002 [11], and results were expressed as tannic acid equivalents per gramme dry weight of sample (TAE/gdw). Five milligrams of each dried Henna leaves extract was dissolved in a 10 ml mixture of methanol and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of 10-fold-diluted Folin–Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate solution; after standing for 30 min at room temperature, the absorbance was measured at 765 nm using a UNICAM 8620 UV–Vis spectrophotometer. The estimation of phenolic compounds in the extracts was carried out in triplicate, and the results were averaged.

Statistical analysis

Experimental data was analyzed using analysis of variance (ANOVA) and significant differences among means from triplicate analyses at ($P < 0.05$) were determined by Duncan's multiple range test (DMRT) using the SPSS System (SPSS).

RESULTS AND DISCUSSION

Extraction

Table 1 shows the percentage yield of Henna leaves extract obtained after ultrasoundig ground Henna leaves with different solvents; i.e. water, methanol, ethanol, acetone, chloroform and hexane and refluxing ground Henna leaves with methanol and water. The maximum amounts of Henna leaves extracts (24.83%) and (25.33%), were obtained with water followed by methanol (23.06%) and (23.10%), using Ultrasonic extraction and Solvent extraction respectively. Higher percentage yield were obtained with an increase in polarity of the solvents. There was no significant difference ($p < 0.05$) in the percentage yields between the extracts of two mentioned methods with both water and methanol (solvent and ultrasound-assisted solvent methods). Jacques *et al*. 2005 [20] and Mohagheghi Samarin *et al*, 2012 [21] in their investigations on *Ilex paraguariensis* leaves and potato peels extracts observed that there was no significant difference in the percentage yields between the solvent and ultrasound-assisted extraction methods.

Table 1. Percent yield of Henna leaves extract obtained with different solvents.

Extraction methods-solvent	Henna leaves extract yield (%)
Water	25.33 ± 0.67 a
Methanol	23.10 ± 0.70b
Ultrasonic-water	24.83 ± 0.33a
Ultrasonic-methanol	23.06 ± 0.34b
Ultrasonic-ethanol	13.25 ± 0.25c
Ultrasonic-acetone	4.65 ± 0.15d
Ultrasonic-chloroform	3.94 ± 0.06e
Ultrasonic-hexane	00.00 ± 0.00f

Values with different letters (a, b, c, d, e, f) were significantly different (P < 0.05, Duncan's multiple range test). Values expressed are means ± SD of triplicate measurements.

Phenolic acids in the extracts

The phenolic contents of water, methanol, ethanol, acetone, chloroform and hexane extracts were found to be 150.3, 308.8, 141.5, 50.4, 30.3 and 0.0 µg TAE/g, respectively. The amounts of phenolic compounds in the methanol extracts (in either solvent or ultrasound-assisted extraction method) were highest and total phenolic concentrations in the six solvents were in the order: methanol > water = ethanol > acetone = chloroform > hexane. There was a significant difference (p <0.05) in the phenolic contents between the extracts of the two mentioned methods (Table 2). Sonication improved the total phenolic compounds of the both water and methanol extracts of Henna leaves and shortened the extraction times. The results of investigation of phenolic acids in the extracts agreed with those of Liu *et al.* 2000 [22], Goli *et al.* 2005 [23] and Mohagheghi Samarin *et al.* 2012 [21] who reported that use of ultrasound extracted more phenolic compounds than refluxing method. Therefore stability of the extract of highest phenolic compounds (methanol) was tested in different conditions.

Table 2. Total phenolic content extracted from Henna leaves by different extraction methods and solvents.

Extraction methods-solvent	Phenolic content (µgTAE/gdw)
Water	150.0 ± 1.35c
Methanol	256.3 ± 1.70a
Ultrasonic-water	150.3 ± 1.25d
Ultrasonic-methanol	308.8 ± 2.45b
Ultrasonic-ethanol	141.5 ± 1.44d
Ultrasonic-acetone	50.4 ± 0.55e
Ultrasonic-chloroform	30.3 ± 0.50e
Ultrasonic-hexane	0 ± 0.00f

Values with different letters (a, b, c, d, e, f) were significantly different (P < 0.05, Duncan's multiple range test). Values expressed are means ± SD of triplicate measurements. TAE, Tannic acid equivalent.

Stability of the methanol extract

Total phenolic content of methanol extract of Henna leaves was determined on day 0 (256.3). Methanol extracts from Henna leaves stored in the dark at -18 and 25 °C and extracts stored in light conditions at 25 °C over a 10 day period did not show any change in the total phenolics (Table 3). Samples stored at -18 °C did not show major changes in total phenolic content after 20 days storage, samples stored at 25 °C either in dark or light conditions did. There was a significant (p <0.05) reduction in the amount of total phenolics from day 20 to day 30 of storage in all

extracts (Table 3). The results showed that among the different mentioned conditions, dark condition under freezing (-18 °C) within 20 days is the best condition for storage of methanol extract of Henna leaves and after this time there would be degradations in phenolic acids. Similar results were reported by Rodriguez de Sotillo *et al.* 1994 [24] and Mansour and Khalil 2000 [25] who indicated that the phenolic acids in potato peel extract were degraded into other compounds during storage at room temperature.

Table3. Changes in phenolic content extracted from Henna leaves by methanol during storage.

Storage condition	Phenolic content (µg GAE gdw^{-1})			
	Storage period, day			
	0	10	20	30
Freezing(-18 °C)	256.3 ± 1.70a	242.1 ± 1.75a	207.4 ± 1.35a	145.7 ± 1.44c
Dark(25 °C)	256.3 ± 1.70a	245.1 ± 1.60a	210.1 ± 1.44b	176.4 ± 1.60d
Light(25 °C)	256.3 ± 1.70a	253.2 ± 1.54a	246.4 ± 1.45b	223.4 ± 1.25e

Means within a column with different superscript are significantly different (P < 0.05). TAE, Tannic acid equivalent

CONCLUSION

Methanol extract was found to have high phenol contents (308.8 µg/gdw of sample), so the best method for extraction of phenol compounds was ultrasonic extraction with methanol. The results showed that among the different mentioned conditions, dark condition under freezing (-18 °C) within 20 days is the best condition for storage of methanol extract of Henna leaves and after this point there would be degradations in phenolic acids.

Acknowledgements
We would like to express our appreciation to the Sabzevar Azad University for partially funding this research project.

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