



Stability of Polyphenols in Myrtle Berries Syrup, a Traditional Iranian Medicine

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Received: 17 May 2015

Revised: 26 May 2015

Accepted: 30 May 2015

Abstract

Myrtus communis L. is a medicinal herb that widely used in Iranian traditional medicine. Myrtle leaves extracts have been studied and a variety of products made from myrtle leaves in Iran and other countries, but only recently, the berries have been the object of scientific interest. In this study, we prepared herbal syrup from myrtle berries aqueous extract and standardized based on total phenols (Folin-Ciocalteu method) and gallic acid (Rhodanine assay) content. Stability tests including phytochemical assay and microbial limit tests were conducted during 3 months of the storage period and pH and viscosity variation of the product were recorded. pH and viscosity of syrup have not been significant changes during 3 months of storage. Evaluation of polyphenol and free gallic acid in myrtle berries syrup was investigated showing that myrtle syrup is microbially and phytochemically stable in the initial 3 months of the storage period.

Keywords: *Myrtus communis*, Myrtle, Phenolic Compounds, Gallic Acid, Syrup, Traditional Medicine

Citation: Mobli M, Haririan I, Amin Gh, Kamalinejad M, Hajimahmoodi M. **Stability of Polyphenols in Myrtle Berries Syrup, a Traditional Iranian Medicine.** Trad Integr Med 2016; 1(1): 35-9.

1. INTRODUCTION

Myrtus communis L. (Myrtle) is an evergreen shrub or small tree belong to Myrtaceae family. This herb is endemic in the

Mediterranean area and the Middle East. Ripe berries are eaten raw and it is widely cultivated for its edible fruit [1]. Different parts of this herb such as its berries, branches, and leaves are used for many medicinal purposes [2], [3]. Aerial parts of myrtle

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exhibited antioxidant [4], anti-inflammatory, analgesic [5], [6] and antimicrobial and antioxidative activity [7]. Leaves of myrtle showed antiproliferative, antigenotoxic [8], [9], anti-diabetic [10], antiviral [11] effects and effective in treatment of aphthous lesions [12], also berries exhibited neuroprotective effect [13] and is effective in treatment of peptic ulcers [14] and hypermenorrhea [15].

Myrtle fruit is a source of phytochemical compounds which can be used in both the food industry and for medicinal purposes. Essential oil, phenolic compounds such as gallic acid, flavonoids, and anthocyanins are the major phytochemicals in myrtle berries [16], [17]. Compounds such as anthocyanins and polyphenols are responsible for their antioxidant effects and because of these properties; myrtle could be used in dietary supplements and medicinal preparations [18].

Myrtle syrup is an Iranian herbal medicine obtained from myrtle berries, and it is characterized by dark-purple color with aromatic odor and astringent taste. The present study was carried out on the final product in order to discover stability of polyphenols and gallic acid, during 3 months of storage in accelerated conditions.

2. METHODS

2.1 Plant Materials

M. communis L. dried berries were purchased from local market in Tehran, Iran and identified by a botanist, voucher specimen (voucher number is 6632-TEH) kept at the herbarium of Faculty of Pharmacy, Tehran University of Medicinal Sciences, for further reference.

2.2 Preparation of Myrtle Syrup

Myrtle syrup was prepared according to the traditional Iranian recipe. 400 g of myrtle berries were coarsely ground and macerated in an appropriate amount of water for 24 hours, then boiled for 1-hour and filtered (extraction yield = 28.9%). 650 g sucrose and 500 g sorbitol were added to the extract in order to prepare the syrup. Methylparaben and propyl paraben is used as a preservative.

2.3 Stability Test

Prepared samples of syrup were transferred in dark bottles and stored in incubator 40 °C for 3 months. Monthly, samples were taken, and 10 ml of each sample was poured into petri dishes and left to dry under the hood.

2.4 Determination of Total Phenolic Content

The total phenol content was measured by the spectrophotometric determination with Folin-Ciocalteu method [5]. 1 ml of syrup sample (25 mg/ml) were added to 5 ml of Folin-Ciocalteu's reactive (10-fold diluted with distilled water). After 10 minutes, 4 ml of sodium hydrogen carbonate solution (7.5% w/v) were added the mixture was shaken after 30 minutes incubation period at room temperature, the absorbance was read at 765 nm with an UV-VIS spectrophotometer, against a blank. The total polyphenols content results, expressed as μg of gallic acid equivalents per mg of the dried sample, were obtained using a calibration curve of a prepared gallic acid standard solution (75-200 $\mu\text{g}/\text{ml}$).

2.5 Determination of Free Gallic Acid

Free gallic acid content was measured by rhodanin assay [6]. Dried syrup sample (1 g) is taken in a glass beaker of approximately 25 ml capacity. Ten ml of aqueous acetone (70%) is added, and the beaker is suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 18 minutes at room temperature. The contents of the beaker is then transferred to centrifuge tubes and subjected to centrifugation for 10 minutes at approximately 3000 g at 4 °C (if refrigerated centrifuge is not available, cool the contents by keeping the centrifuge tube on ice and then centrifuge at 3000 g using an ordinary clinical centrifuge). Collect the supernatant. Pipette 200 μl supernatant in a culture test tube (four tubes per sample). Remove acetone from the sample using a vacuum valve to dry the sample. Add to 200 μl of 0.2 N sulphuric acid to the tubes containing dried supernatant. To three tubes add 300 μl of the rhodanine solution and to the fourth tube 300 μl methanol. This fourth tube acts as a proper blank. After 15 minutes, add 200 μl of 0.5 N

potassium hydroxide solutions to all the tubes. Wait for 2.5 minutes and then add 4.3 ml of distilled water. After 15 minutes, measure absorbance at 520 nm against a proper blank. The free gallic acid content results, expressed as μg of gallic acid per mg of the dried sample, were obtained using a calibration curve of a prepared gallic acid standard solution.

2.6 pH Value

Calibrated digital pH meter was used to determine the pH of samples during 3 months.

2.7 Viscosity

The rheometer was used to determine the viscosity of samples during 3 months.

2.8 Microbial Limit Tests

To assess the microbial stability of the product, total aerobic microbial count and the total combined yeast/molds count, *Escherichia coli* and *Salmonella* tests based on Institute of Standard and Industrial Research of Iran methods was conducted.

2.9 Data Analysis

Statistical analysis was carried out using repeated measurement ANOVA with SPSS (version 19, SPSS Inc., Chicago, IL., USA). Data are expressed as mean \pm standard deviation. Statistical significance was set at $P < 0.05$.

3. RESULTS

3.1 Phytochemical Analysis

The results of total phenols and gallic acid analysis of myrtle berries syrup during 3 months of storage are shown in table 1. No significant variations were observed during 3 months of storage.

3.2 pH Value

pH of samples were measured each month, and as shown in table 2, no significant variations were observed during 3 months of storage.

3.3 Viscosity

The viscosity of samples were measured each month, and the results are shown in table 3.

3.4 Microbial Limit Tests

To evaluate the efficacy of added preservative, microbial limit tests conducted during 3 months of storage. No significant variations were observed during 3 months of storage. The results are shown in table 4.

4. DISCUSSION

Phenolic compound, flavonoids and anthocyanins are the major phytochemicals in *M. communis* berries, gallic acid is a type of phenolic acid and is major compound in the extract prepared following the traditional Iranian recipe for the preparation of myrtle syrup [16], [18].

Table 1. Total phenol and gallic acid amount of samples during 3 months of storage

Duration	Average of total phenol \pm SD	Average of gallic acid \pm SD
0 month	6.56 \pm 0.10	0.29 \pm 0.03
1-month	6.38 \pm 0.10	0.31 \pm 0.02
2 months	6.49 \pm 0.10	0.33 \pm 0.01
3 months	6.43 \pm 0.08	0.33 \pm 0.03

SD: Standard deviation

Table 2. pH value of samples during 3 months

	0 month	1-month	2 months	3 months
Sample pH \pm SD	5.00 \pm 0.01	5.00 \pm 0.01	4.90 \pm 0.04	5.00 \pm 0.01

Table 3. Viscosity of samples during 3 months

	0 month	1-month	2 months	3 months
Sample viscosity (pa.s)	0.14	0.13	0.14	0.14

Table 4. Microbial limit tests results of samples during 3 months

Tests	Standard	Acceptance criterion based on Iranian pharmacopoeia	Acceptance criterion based on British pharmacopoeia	Results	Units
TAMC	5272	Maximum acceptable count: 10^2	10^4	<1	cfu/ml
TYMC	10899-1	10	10^2	<1	cfu/ml
<i>Escherichia coli</i>	2946	-	Absence	Absence	cfu/ml
<i>Salmonella</i>	1810	-	Absence	Absence	cfu/25 ml

TAMC: Total aerobic microbial count, TYMC: Total combined yeast/molds count

Myrtle syrup is widely used in Iranian traditional medicine, and different effects such as; stomach tonic, astringent, wound healing, and hemostatic are mentioned in these manuscripts [21].

In this report, the evaluation of polyphenol and free gallic acid in myrtle berries syrup was investigated showing that myrtle syrup is microbially and phytochemically stable in the

initial 3 months of the storage period. pH and viscosity of syrup have not been significant changes during 3 months of storage. Due to the stability of polyphenol and free gallic acid in this period, these phytochemicals can be used for standardization of myrtle syrup.

6. CONFLICT OF INTERESTS

Authors have no conflict of interests.

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