Assessment of Fatty Acids and Phenolic Content of some *Citrullus colocynthis* Accessions seeds from Iran

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Abstract

Bitter melon (Colocynth) is a species of herbs, annual medicinal plant of family Cucurbitaceae which are mainly grown in warm regions of Iran. Fatty acids of the seeds of this plant are considered as important materials, medicinally. In this study, essential fatty acids of seed collected from nine different regions of Iran were evaluated. The composition of fatty acids such as oleic, linoleic, stearic and palmetic acid were measured by GC (Gas Chromatography). The total fatty acid content in the seed was varied from 35% to 40%. The highest content of linoleic fatty acid was found in seeds of Orzooiyeh accessions (72.4%) and the maximum amount of oleic acid was in Khorasgan (13.8%%). Kerman accession was important in case of phenolic content which was 8810.9 mg GAE/g based on results fatty acids contents were significantly differed among different accessions and there were valuable accessions considering unsaturated fatty acids and total phenolic compounds. These plant materials could be used for improving breeding programs and selection to plant as valuable accessions.

Keywords: Colocynth; Linoleic acid; Oleic acid; Palmetic acid

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Introduction

The Bitter melon (*Citrullus Colocynthis* Schrad.) is an annual medicinal plant belongs to Cucurbitaceae family which grows widely in arid desert regions of Iran. Cucurbits seed oil are rich source of protein and oil content about 35% to 50% (Achu et al., 2005), while Colocynths seed oil is rich in protein and fatty acid compounds (Abbah et al., 2014).

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The amount of oil in seeds of this plant is about 35 to 40 percent (Kulkarni., 2012). The main fatty acids which comprise approximately 90% of oil content are linoleic, stearic, oleic and palmitic acid, which 50% of that counted as linoleic acid content (Stevenson, 2007). Bioactive secondary metabolites included phenolic contents, alkaloid, flavonoids, saponin and ascorbic acid. High content of unsaturated oils and fatty acids have been proved in *Citrullus* genus, especially in *Citrullus lanatus* by the world Food and Agriculture Organization (FAO) (Ziyada and Elhussien, 2008). Mass cultivation and production of Bitter melon has not been reported anywhere, but the only report is from Nigeria, in

which 347,000 tons of seeds yielded in 2002 from 361,000 hectares of area (Giwa, 2010).

Dried fruit of this plant is mainly effective in treating diabetes, its seed oil is also important in modern medicine, for the treatment of prostate cancer and for treatment of atherosclerosis, for regulation of digestive system and for prevention of vitamin breakdown (Delazar, 2006). The Colocynth seeds contain high level oil and in ancient times, this oil was used for lamps and despite the extremely bitter taste of the seeds which is mainly due to Cucurbitacin compounds, its oil is considered edible (Ziyada, and Elhussien, 2008).

Since most useful genomes, such as biotic and a-biotic stress resistance genes and genes responsible for medicinal product quality and performance normally found in diversity centres of crop diversity, so, appropriate and accurate information about genetic resources could lead to better utilization of plant genetic diversity. Lack of knowledge for genetic resources and favorable genes is a reason for limited breeding programs of medicinal plants in Iran, while, this country is a rich source of plant genetic pool for most medicinal crops. Colocynth and other melons has long been regarded as medicinal and edible, but so far, little researches have been done in order to identify local populations, study their properties and cultivations as field crops (Levi et al., 2001). Different climatic conditions of Iran are a positive point for selection of new crops with important traits. Colocynth is a plant genus that is reported to be grown widely in different part of Iran, mainly in arid desert regions. The active substances might be differently produced from a plant genus being in different places with varied environmental conditions.

Therefore the aim of this research was to evaluate the phytochemical properties such as fatty acid content and total phenolic compounds which are engaged with medicinally properties of Colocynth accessions collected from different regions of Iran.

Method and Materials

Fatty acid analysis

Dried samples of 9 accessions (Table 1) were extracted with chloroform: methanol (2:1, v/v) according to the method of Folch (Folch et al., 1957). Solid and non-lipid material were removed, then the solvent was evaporated under nitrogen gas. Fatty acid methyl ester was prepared by methylation the total lipids, as described by Joseph and Ackman, 1992. Methyl esters were separated by gas chromatography (GC), (Varian 3400 capillary GC with a flame ionization detector, Varian, Walnut Creek, CA, USA and SP-2560, 100 m × 0.25 mm i.d., Supelco Inc., Bellefonte, PA, USA) where the detector temperature was 280 ℃, the injection port temperature was 260 ℃, and the column temperature was 180 ℃. Carrier gas (hydrogen) flow was 1 mL/min with a nitrogen flow of 30 mL/min. To identify each fatty acid, each retention time was compared with the standard (Supelco 37 fatty acid methyl esters).

Table1
List of 9 *Citrullus colocynthis* accessions used in this study with their collection site

No.	City	Province
1	Kerman	Kerman
2	Khorasgan	Esfahan
3	Yazd	Yazd
4	Shiraz	Fars
5	Mashhad	khorasan
6	Bandarabas	Hormozgan
7	Ahvaz	Khuzestan
8	Orzooiyeh	Kerman
9	Jiroft	Kerman

Total Phenolic Compounds

The total phenolic content was determined by the Folin–Ciocalteau assay (Thaiponga, 2006) using Gallic acid as a calibration standard. A 0.5 ml extract was added with 2.5 ml of Folin- Ciocalteu reagent followed by addition of 2 ml sodium carbonate (Na_2CO_3) (75g/l). The sample was then incubated for 5 min at 50 °C. The absorbance was measured at 760 nm using Spectrophotometer. The results were expressed as mg Gallic acid equivalents per gram of extract (mg GAE/g) that was derived from a calibration curve.

Results

The result of variance analysis showed that there was a significant difference among accessions in fatty acids linoleic, stearic acid and phenolic content at 0.05% level of erroe probality (Table 2).

The highest amount of saturated acids, palmetic acid was found in Yazd and Jiroft accessions (11.42% and 10.87%, respectively) and also stearic acid (7.3%) the other saturated fatty acid was high in Khorasgan accession (Table 3). The lowest amount of saturated acid linoleic was found in Shiraz accession (62.56%), oleic in Khorasgan (11.01%), palmetic in Ahvaz (9.47%) and stearic in Shiraz accession (6.31%). Analysis of unsaturated fatty acids revealed that linoleic acid content was in maximum range in Orzooiyeh (72.44%) accession and oleic acid in Shiraz accession (17.25 %) (Fig.I).

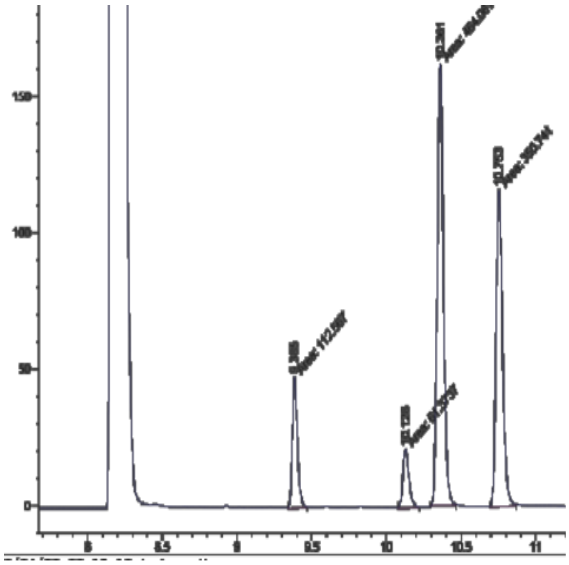

Fig. I. Gas chromatogram Metil Ester of fatty acid in Orzooiyeh accession

For the phenolic content as well, great variation was among the accessions and the highest value of phenol compounds was in Kerman accession with 8810.9 mg GAE/g and the lowest phenolic compounds (4040 mg GAE/g) were in Jiroft, Hormozgan and Ahvaz accessions (Table 3).

Discussion

In a study the linoleic acid content of colocynth seeds was reported as 66% that proves that linoleic acid is the highest compound of fatty acids in colocynth seeds (Kulkarni, 2012).

Physicochemical characteristics and fatty acid composition of crude oil and lipoxygenase activity of six varieties of pumpkin and melon seeds were investigated. The major fatty acid in total lipid was representing 68.7% for Citrullus lanatus (Chinese), 65% for C. colocynthis, 63.7% for C. lanatus (Iranian), 62% for C. lanatus (Egyptian), 53% for Cucurbita moschata, and 43% for Cucurbita pepo. Lipoxygenase activities varied among seeds. The residual enzyme activities after roasting were different among the six varieties and were in the range of 0−60% of the original activity (Al-Khalifa, 1996).

Colocynth seeds also could be a main and rich source of essential fatty acids and phenolic compounds and be considered as nutritional for medicinal aspects. The fatty acid composition depends on several factors such as variety, growing region, climate and fruit and seed maturity (Murkovic et al., 1999). Protein and fatty acid content of Bitter melon seeds was about 28% and 72%, respectively and this plant introduced as a source for fatty acids (Abbah, 2014).

These results reflect the variation which

Table 2
Analyse mean variance in 9 accessions of Colocynth.

Source	DF	Linoleic	Oleic	Stearic	Palmetic	Phenol
Block	2	9ns	9ns	9*	7.14*	8082640.14ns
accession	8	15.7**	11.11*	0.35**	1.71*	9503813.45*
Error	16	0.00	0.00	0.00	0.148	2466962.21

** Significant at 1% * significant at the 5% level ns non-significant

in many cases can also be influenced by environmental conditions or pollen that can affect the quality and quantity of seed and fruit. In a recent study 96 populations of *Citrullus* have been evaluated for their seed oil contents and oil content between different populations of Colocynth was reported about 35% and linoleic acid content was about 71%, which sets a high value for this plant (Jarret and Levy, 2012). Different climatic conditions, but were grown in the same environmental conditions. High variability in genotypes was evaluated for the considerable increase in the amount of oil, seed oil fatty acids, linoleic acid and tocopherols in cucurbits as an extensive selection program (Winkler et al., 2002).

The differences observed in this study, provides more beneficial features and desirable characteristics of native plants in the national selective and breeding programs.

The studied medicinal plant in part has potential bioactive compounds which could be used for therapeutic purpose and/or as precursors for the synthesis of useful drugs. The current study is the first report comparing the Iranian Colocynth genetic resources and demonstrated that a considerable accessions exists for phytochemical (fatty acid and phenolic content) traits in germplasm sources. There were more variation in phenolic content and oil composition of seeds among accessions at result selection of useful germplasm among these collections could be useful for the future breeding programs. As a result the accessions of Shiraz, Kerman and Orzooiyeh had the highest quality of phytochemical characters which are recommended for cultivation as valuable medicinal accessions of colocynth.

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Table 3
Mean comparisons of fatty acid and phenol content of 9 Colocynth accessions in this study.

Accessions	Palmetic	Stearic	Oleic	Linoleic	phenol
Kerman	10.27bc	6.9d	12.87e	69.98f	8810.94a
Khorasgan	10.05cd	7.31a	11.01i	71.63c	4063.05dc
Yazd	11.42a	7.1b	14.74b	66.47i	7024.72b
Shiraz	9.92cd	6.31i	17.25a	66.52h	4040cd
Mashhad	10.72b	6.73f	13.75c	68.8g	6460b
Hormozgan	9.94cd	6.36h	11.77g	71.93b	4040d
Ahvaz	9.47d	6.88f	12.23f	71.42d	4109.15cd
Orzooiyeh	9.54cd	6.46g	11.56h	72.44a	3636.66c
Jiroft	10.87a	7c	13.12d	71.23b	4120.67cd

Fatty acid % and phenolic content per unit is mg GAE/g.

Similar letters in each column are not statistically different at 5% level of probability using Duncan test

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