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Determination of Suitable Solvents for Extraction of Different Fruit Parts of Bitter Melon (*Momordica charantia* L.)

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Abstract

This study was conducted to determine suitable solvents in regard to extract rate for extraction of different parts of ripe and unripe fruits in bitter melon (*Momordica charantia* L.) grown in Pazar/Rize conditions in the Black Sea Region. The fruit parts of the plant were separately assessed for extraction with solvents, methanol, ethanol and hexane. The extract values (g/g) obtained by solvent extraction in 2014 were determined as percentage. Significant differences were found on the performance of solvents used in extraction depending on the ripeness condition of fruits. Among the fruit sections, the highest ($p<0.01$) extract rate was obtained from seed coat of ripe fruits through methanolic (73.7 %) and ethanolic (68.5 %) extraction. On the other hand, for the seed coat of unripe fruits, the highest extract rate (16.9 %) was determined in only methanolic extraction. The highest extract rate was obtained from ethanol with 35.1 % ($P<0.01$) for seeds of ripe fruits. On the other hand, the highest extract rate ($p<0.05$) for seeds of unripe fruits was obtained with methanol and hexane, 27.7 % and 21.3 % respectively. Methanol was the best one ($p<0.01$) among the solvents used in extraction of fleshy parts of both ripe and unripe fruits, and extract rates occurred as 41.3 % and 34.8 % respectively. The results obtained from the study showed that, among the solvents for high extract yield, ethanol could be used for extraction of ripe seed and methanol for other fruit sections.

Keywords: Fruit sections, extract rate, seed coat

INTRODUCTION

Momordica charantia L. from *Cucurbitaceae* family generally known as “bitter gourd” and “bitter melon” is an annual and climber plant commonly cultivated in most tropical and subtropical regions of the world [13]. It is known as “Pelinsenk”, “Acayip Elması”, “Mucize Elması”, “Papara” and specially “kudret narı” in Turkey [1]. The plant bitter melon which is grown in many provinces of Turkey and widely used in traditional medicine is a valuable cultural plant easily adaptable to the ecological conditions of Rize province [31].

The fruits of the plant are used as medicinal vegetable in different countries [16]. In addition, bitter melon has a potantial to increase the value of various foods because it contains rich fenolic compounds [19]. Bitter melon has valuable pharmacological activities such as antidiabetic [34, 15, 3, 1], antioxidant [25], anticancer [37], antimicrobial and antiulcer activities [25, 27]. In addition, it is used to treat some skin diseases and hemorroids [17].

The fruits and leaves of bitter melon possess also laxative, antibilious, emetic and stomachic effects [6].

Unripe fruits, seeds, leaves and all aerial parts of *Momordica charantia* L. have been used to heal diabetes in many parts of the world. The fruit juice or seed powder taken orally causes a reduction in fasting blood glucose and improves glucose tolerance in animals and humans [28]. Acording to Sezgin [32], for a patient with chronic lymphocytic leukemia, ulcerative colitis and enteropathic arthritis, bitter melon extract (after several herbal drugs) was advised to be taken as a complementary therapy. At the end of the third month, a significant decline was observed in the patient’s complaint.

It was reported that extracts obtained from fruit pulp, seeds, leaves and whole bitter melon plant have shown a hypoglycemic effect in various animal models [3]. Amira et al. [4] applied the bitter melon fruit extracts to male rats and determined that the plant was a good source for bioactif compounds. It was determined that fatty extract of the bitter melon exhibited a protective effect macroscopically on the generated ulcer model in the rat [26]. According to Ozusaglam and Karakoca [25] unripe fruit and ripe fruit extracts have a potential use as they show high antimicrobial and antioxidant activities, respectively.

However, *Momordica charantia* L. shows a principal toxicity on the liver and reproductive system in animals [28]. Nontheless these effects haven’t been reported in humans in spite of widespread use of the fruit medicinally and as vegetable.

The extracts of bitter melon have positive effects on human health. This study aim to determine the suitable solvent for extraction yield of different parts of bitter melon fruits.

MATERIALS and METHODS

In this research, the plants were grown by the research and practice field in Faculty of Agriculture and Natural Sciences in Pazar/Rize/Turkey. The used seeds were bitter melon (*Momordica charantia* L.) populations belonging to the faculty envantery. Ten fruits were harvested separately from each of ripe and unripe fruits.

Each fruit seperated from their parts is as the following:

1. Ripe fruit flesh
2. Seeds of ripe fruits
3. Seed coat of ripe fruits
4. Unripe fruit flesh
5. Seeds of unripe fruits
6. Seed coat of unripe fruits

After fruits were seperated from their parts, they were dried in an oven at 60 0C. The dried material was divided into small pieces in a mill, pounded in a mortar to pulverize and then, maintained at +4°C until extraction. The powdered fruits and seeds of bitter melon were separately extracted with methanol, ethanol and hexane.

Experiments were carried out in a randomized block design with 3 replications in 2014. Variance analysis was performed by using the software package JMP 5 [30] and LSD test was applied to test the significance of differences among the means. The principle component analysis was carried out using the software SPSS 20.0.

Extraction Procedure

Different parts of bitter melon fruits were extracted by using three solvents, methanol, ethanol and hexane. Suitable solvents were determined for each part of the fruit.

Providing the higher extract yield with high concentration of bioactive components is normally required for an efficient process. In addition, it is known that some phytochemicals are very sensible to oxygen and heat [18]. Therefore, the choice of suitable process for extraction should be made with care to prevent their oxidation and thermal degradation. In this study, this case was considered while deciding for extraction method, and so a shorter extraction period was preferred.

0.5 g of the dried sample was used in extraction. These samples were placed into falcon tube and then solvents (10 ml) were separetly added to the samples. The samples were extracted 7 times at 60 0C in ultrasonic water bath during 20 minutes. After each extraction, the extracts were centrifuged for 5 minutes, at 4.000 rpm and then solvent-extract mixture was filtrated by using filter paper (whatman 1) and the solvent was evaporated in a rotary evaporator under vacuum at 60°C. Extract values were determined in based on weight (g) and calculated as percentage.

RESULTS and DISCUSSION

Bitter melon is a healthy food with bitter flavor. It is also widely used in folk medicine. In addition, it deserves in-depth studies for various clinical applications in the [14].

Different parts of the plant which is a potential medicinal herb [12] are used in various forms for human healthy. However, the most suitable dose of bitter melon is not known exactly. Powders obtained from dried fruit, fresh juice, an aqueous decoction of the fruit and standardized extract are consumed in a range of 3-15 g/day, 50-100 mL/day, 100 to 200 mL/day, 100 to 200 mg (3 times in a day) respectively.

The evaluation of extracts obtained from the plant to utilise them more efficiently can bring many benefits. In particular, the positive effects on complementary medicine in the treatment of patients exhibit the need for the plant extract. The properties of extracts and bioactive compounds which they contain vary depending on the applied solvents. In the present study, ripe and unripe fruits were seperated to three parts as flesh fruit, seed and seed coat, and these different parts of fruits were extracted with solvents individually. Which solvent produces the highest extract yield was determined. The results from the present study have an importance in terms of bringing a new perspective in the future because of the limited investigation regarding this matter.

In this research, the effect of the used three solvents on extraction of various parts of fruits in bitter melon was determined. The effects of solvents on extract rate were found highly significant ($p<0.01$) in the parts of fruits for ripe and unripe fruit flesh, seed coats of ripe and unripe fruits and seeds of ripe and of unripe fruits ($p<0.05$) (Table-1).

The yield of extracts obtained from various plants is affected by many factors. These factors consist of extraction conditions such as extraction technique, solvents used, extraction period and level of heat during the extraction, besides genetic structure, growth stage and parts of plant, and environment conditions for plant [20, 35, 5, 11].

Selection of effective technique is also important to obtain maximum amounts of various extractable compounds from the plant material. The researchers reported several techniques used in extractions such as microwave-assisted extractions, soxhlet extraction, sonication, pressurized fluid extraction and super critical fluid extraction [21, 9]. Ultrasound assisted extraction is one of the extraction techniques and it has been studied for the extraction of different parts of various plant. Ashraf et al [7] determined that the application of sonication demonstrated significantly ($p<0.05$) higher antioxidant activities as compared to the extracts obtained from using magnetic stirrer and orbital shaker. In this study, the extraction was accrued by using ultrasonic sound bath due to its positive effects on extraction.

Several solvents like ethanol, methanol, acetone, hexane, ethyl acetate and their aqueous combinations have been used for the extraction of flavonoids and other bioactive components from bitter melon [35]. However, Tan et al [36] reported that acetone was the best of five solvents (methanol, ethanol, acetone, n-butanol and water) for extraction of flavonoids from bitter melon while Shan et al [33] stated that ethanol modified SC-CO2 extraction was a suitable method for the selective extraction of flavonoids from *M. charantia* L. Considering a lot of studies such as those above mentioned, it is possible to say that every researcher could chose the method according to the respective study. In the present study, a shorter extraction period and lower temperature application by using solvents as methanol, ethanol and hexane were prefered to prevent especially thermal degradation and their oxidation.

In this study, the highest extract rates were obtained from methanolic (73.7 %) and ethanolic extraction (68.5 %) of seed coat of ripe fruits while the lowest extract rate was calculated from hexane exraction (5.5 %). Extract yield of seed coat greatly decreased from ripe fruit to unripe one. The differences between the yields of extract might be based on the availability of different extractable components [24] and ripeness of the fruit.

The first solvent was methanol for extraction of ripe fruit flesh (41.3 %) and unripe (38.4 %) fruit flesh. The second one for the same parts was ethanol (12.9 and 15.3 % respectively) and the last one was hexane (4.6 and 6.4 %). However Ozusaglam and Karakoca [25] reported that the extractive yield of the ripe fruit, unripe fruit, ripe seed and unripe seed for ethanol extracts of *M. charantia* L. using soxhlet apparatus for 24 h were 63.22 %, 5.05 %, 30.92 % and 23.40 % respectively. In the present study, ethanolic extract rates obtained from ripe seed and unripe seed showed similarities with the results of this research but the ethanolic extract rates of both ripe and unripe fruits showed great differences. These differences were derived from high temperature differences and longer extraction period in soxhlet extraction method.

Chandrasekar et al [10] reported that ethanolic extract of *M. charantia* (250 mg/kg dose) affected significantly lowing blood sugar in fasted as well as glucose loaded non-diabetic rats.

In the present study, methanol was generally an more effective solvents than ethanol and hexane for all parts of fruits. Rakholiya et al. [27] calculated extractive yield of different solvent extracts on the different parts of *M. charantia*. They reported that methanol was much more effective than hexane in all of parts of the plants, aerial part, peel, pulp and seed. On the other hand, the peel and the pulp aqueous extract had considerably more extractive yield than 100 % MeOH. As the concentration (100, 75 and 25 % methanol) of methanol decreased, there was a slight increase in extractive yield. The extracts of bitter melon have a potential for the control the foodborne illness, resulting from consumption of food contaminated with pathogenic bacteria. The same researchers found that the best antibacterial activity was shown by 100 % MeOH extract for foodborne pathogens.

Kwatra et al [23] suggested that the methanolic extracts of bitter melon could be an effective preventive and also therapeutic agent for colon cancer. The methanolic extracts from fruits of *Momordica charantia* L. increases healing of gastric ulcer and also prevents development of gastric ulcers and duodenal ulcers in rats [2].

Among the used solvents for extraction of seeds of ripe fruits, the best one was ethanol with 35.1 % of extract rate followed by methanol (25.6 %) and hexane (20.1 %). On the other hand, in extraction of seeds of unripe fruits, extract rates calculated for each solvents, methanol, hexane and ethanol were 27.7 %, 21.3 %, 17.6 % respectively. Biswas et al [8] reported that methanolic extract from the seeds of *M. charantia* showed analgesic activity in mice in conjunction with dose. In additon, wound healing in a diabetic patient may be delayed because of high blood glucose level and pressure of free radicals, specially oxidative free redicals. Seed extracts of *Momordica charantia* L. significantly promote to heal wound in diabetic rats [1].

In Figure 1, the scatter plot analysis of the solvent used is presented. The calculated PC1 and PC2 were determined as 70.90 % and 29.01 % respectively. These three solvents could be clearly differentiated based on different fruit parts (Figure 2). In particular, seeds of ripe fruits and seed coat of ripe fruits were helpful to distinguish among these three solvents. Further, Figure 3 represents the differentiation based on different fruit parts. Seed coat of ripe fruits especially showed a different solvent extract compared to the rest. Based on the component analysis of fruit parts, all three solvents were effective for distinguishing the investigated fruit parts (Figure 4).

In conclusion, bitter melon is one of the most important medicinal plants. As stated in many literatures, the extracts of different parts of its fruits have a serious potential to fight against various diseases. The results obtained from the present study showed that extract quantities from different parts of the fruit varry widely depending on the solvents used. In addition, ethanol is preferable in extraction of especially ripe seed and methanol in extraction of ripe fruit flesh, unripe fruit flesh, seed coat of ripe and unripe fruits and seeds of unripe fruits for high extract yield. According to the previous researches, these extracts obtained from every parts of bitter melon could be used to be evaluated for different purposes. Therefore prospective studies could focus on the plant in terms of gaining new raw materials to Rize industry for the production of herbal drugs.

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Table 1. Comparison of solvents regard to extract rate (g/g) in extraction of various parts of fruits in bitter melon (%)

Parts of the fruit	Methanol	Ethanol	Hexane	Mean
Ripe fruit flesh	41.3a	12.9b	4.6c	19.6**
Seeds of ripe fruits	26.5b	35.1a	20.1c	27.2**
Seed coat of ripe fruits	73.7a	68.5a	5.5b	49.2**
Unripe fruit flesh	38.4a	15.3b	6.4c	20.0**
Seeds of unripe fruits	27.7a	17.6b	21.3ab	22.2*
Seed coat of unripe fruits	16.9a	3.9b	3.8b	8.2**
Mean	37.4a	25.6b	10.3c	24.4**
*, **Level of significance; p<0.05*, p<0.01**, means with the same letter are not statistically significant				

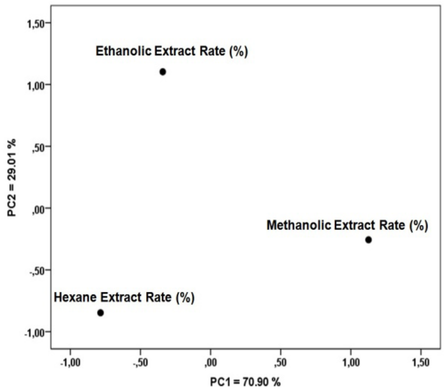

Figure 1. Scatter plot of different extraction solvents

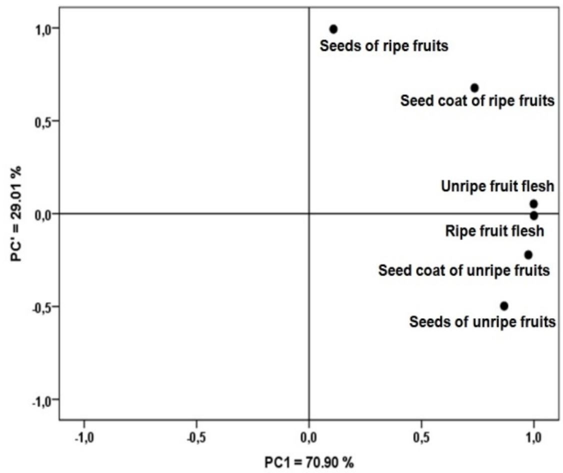

Figure 2. Component diagramme of different extraction solvents

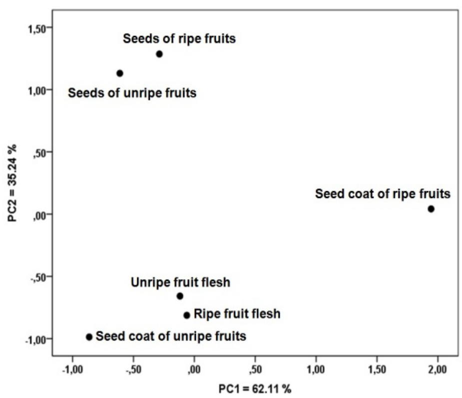

Figure 3. Scatter plot of different fruit parts

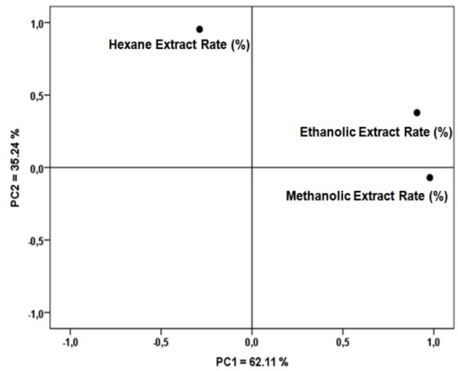

Figure 4. Component diagramme of different fruit parts