

# A Review on Extraction, Isolation Separation Technique of Bitter Gourd

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**Abstract:** Bitter gourd (*Momordica charantia L.*) is a vegetable and also an important medicinal plant with economic importance. There is a rapidly increasing demand for this plant in Turkey and its cultivation has started. However, no production status has entered the statistics yet. At the same time, this plant is not known enough in Turkey. There is a need for more scientific studies that reveal the importance of this plant for the production of bitter melon in Turkey in Turkey, theses, research articles, review articles, and book chapters have been written, and congress presentations and projects have been made so far. A total of 50 studies on the subject have been reached. Studies on bitter gourd began in 1993 and have continued to increase until today. These studies were mostly focused on antioxidant and antimicrobial activity. The most recent studies have been studies on industrial use and Covid19 treatment. Bio- technological and nanotechnological studies on bitter gourd are not yet sufficient and no studies on gene transformation have been found. This review aims to bring together all the studies on the potency of bitter gourd in Turkey and to reveal a future perspective. As a result, it was concluded that more scientific studies should be done in Turkey.

**Keywords:** Bitter Gourd: *Momordica Charantia* L; Turkey, Research: Medicinal Plant

## I. INTRODUCTION

Bitter gourd is an important plant of the Cucurbitaceae family with high nutritional content. It is also used by people in the treatment of many diseases. It is also known as bitter gourd, African cucumber, balsam apple, balsam pear, papilla, and karela. Bitter melon is a tropical plant that likes moist and warm areas. Its home land is India. It is grown in Yalova, Düzce, Bursa, Balıkesir, Istanbul, Antalya, Hatay, Rize, Mersin, Tokat, Adana and Urfa in Turkey. It is grown in open areas in temperate regions of Turkey and green- house environments in cold regions.

This plant is used both for treatment and food in many regions where it grows and is grown [1]. The bitter melon plant is grown in Turkey by sowing from its seed in May and the ripe fruits are harvested in August. The bitter melon is an annual, herbaceous, and climbing plant, and its delicate stem can be up to 1-2 m in length. The leaves come out of the armpits, and the body extensions called leeches extend from the same area. The leaves are hand-shaped.

### Chemical Structure and Properties of bitter gourd

It is hollow in cross- section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large, flat seeds and pith. The fruit is most often eaten green, or as it is beginning to turn yellow.

### Chemical Structure

**IUPAC Name:** 3,7,23-trihydroxycucurbitan-5,24-dien-19-ol

**Chemical Formula:** C<sub>30</sub>H<sub>48</sub>O<sub>4</sub>

**Molar mass:** 472.710 g-mol<sup>-1</sup>

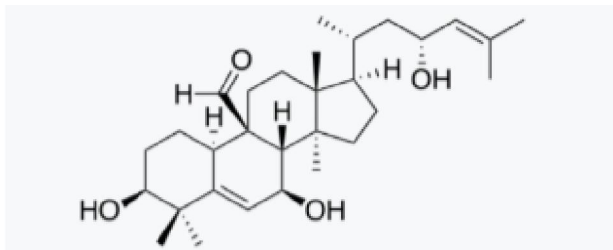


Figure : Molecular Structure of bitter melon

**Properties:**

Nutrients: Bitter gourd is rich in vitamins A, C, and B vitamins, as well as minerals like potassium, calcium, and iron.  
 Antioxidants: Bitter gourd contains compounds that help fight harmful free radicals in the body.  
 Blood sugar control: Bitter gourd can lower blood sugar levels and improve insulin sensitivity.  
 Anti-inflammatory: Bitter gourd can reduce inflammation in the body.  
 Digestive health: Bitter gourd is rich in fiber, which promotes digestion and prevents constipation.

**Occurrence:**

Bitter gourd (*Momordica charantia*) is one of the most popular vegetables in Kerala. The fruits are used in a variety of culinary preparations and possess high nutritive and medicinal value.

**II. GENERAL SIGNIFICANCE OF BITTER GOURD IN FOOD HEALTH AND INDUSTRY**

Bitter gourd, also known as *Momordica charantia* or karela, is a vegetable that has many uses in the food industry and in medicine:

**1.Food-**

Bitter gourd is a rich source of vitamins, minerals, and other nutrients, and is used in many dishes after cooking. It can be stuffed, fried, or sliced and dried for later use.

**2.Medicine-**

Bitter gourd is used in traditional Indian medicine to treat a variety of diseases, including diabetes, asthma, blood diseases, and rheumatism. It contains medicinal compounds such as charantin, vicine, and polypeptide-p, which can help lower blood glucose levels.

**3.Functional foods and beverages-**

Bioactive compounds from bitter gourd can be incorporated into functional foods and beverages.

**4.Forage for rabbits-**

Bitter gourd leaves and fruits can be used as a forage for rabbits, but should not be fed to breeding does.

**III. OBJECTIVES OF THE REVIEW**

The most popular cucurbitaceous vegetable, commonly cultivated in many countries.

It is also known as bitter melon, balsam pear

It is extensively grown in China, Japan, SE Asia, Tropical Africa and South America.

In India it is grown both as rainy season and spring-summer season crop.

Bitter gourd (*M. charantia*) is also grown as an ornamental crop in the USA, Japan and some other countries.

The immature fruits are rich source of vitamin and minerals and contain 0.61 mg iron, 20 mg calcium, 70 mg phosphorus & 88 mg vitamin C.

#### **IV. GINGER COMPOSITION:BITTER GOURD VS OTHER BIOACTIVE CHEMICAL**

Bitter gourd, also known as *Momordica charantia*, is a tropical vine that contains a variety of nutrients, including vitamins, minerals, and amino acids:

Vitamins: Vitamin A, vitamin C, thiamine (B1), riboflavin (B2), niacin (B3), folate (B9), and vitamin E

Minerals: Potassium, iron, calcium, magnesium, phosphorus, zinc, and sodium

Amino acids: Glutamine, asparagine, glycine, lysine, alanine, leucine, valine, arginine, proline, serine, isoleucine, phenylalanine, tryptophan, histidine, threonine, and methionine

Other compounds: Antioxidants, flavonoids, and polyphenol compounds .

Bitter gourd also contains triterpenoid saponins, which are a class of glycosides.

The nutritional value of bitter gourd can vary depending on the type of bitter gourd. For example, light green big bitter gourd has the highest protein content, while light green small bitter gourd has the highest carbohydrate content.

Bitter gourd is also known for its nutraceutical properties, including anti-diabetic, anti-cancer, anti-oxidative, and anti-dementia properties.

#### **Variation in concentration across different bitter gourd variety and growing condition**

Punjab Komal: This variety matures early, producing the first fruit about 70 days after sowing.

Punjab Long: This variety has vigorous plants with cylindrical, light green fruits that are suitable for long-distance marketing.

Kashi Bahar: This hybrid variety has long fruits, green vines, and vigorous growth. The fruits are straight, light green, and weigh 780-850 g on average.

Pusa Summer Prolific Long: This is a variety of bottle gourd.

Pusa Summer Prolific Round: This is a variety of bottle gourd.

Pusa Manjari: This is a variety of bottle gourd.

#### **Growing condition-**

Bottle gourd grows best in sandy loamy soil with good drainage and a pH of 6.5 to 7.5. It prefers moderate warm temperatures, with an optimum temperature of 20 to 30°C for better plant growth, flowering, and fruiting. Bottle gourd cannot withstand frost

#### **Example of Concentration Range:**

There are several varieties of bitter gourd, but the two most common are Chinese bitter gourd and Indian bitter gourd. The Chinese variety more closely resembles a pale green cucumber with crimped, bumpy skin. The Indian variety has narrow, tapered ends and sharp, angled ridges all over its surface..

#### **V. FACTOR INFLUENCING BITTER GOURD YIELD**

Fertilizer-

Using a combination of organic and inorganic fertilizers, such as 50% poultry manure and 50% NPK, can increase the yield of bitter gourd.

Plant growth regulators-

Applying plant growth regulators like MH, CCC, Ethrel, silver nitrate, and boron at the 2-leaf and 4-leaf stages can increase the number of female flowers and yield.

Foliar sprays-

Foliar sprays of Trp, Gln, and Phe can increase plant length, fruit number per plant, and total fruit yield per hectare.

Spacing-

Wider spacing can increase the individual fruit weight.

Micronutrients-

The application of boron and calcium can affect fruit weight.

Drought stress-

Drought stress can increase the ascorbic acid of bitter gourd fruit, but it can also decrease RWC and chlorophyll synthesis.

AMF-

AMF can moderate drought stress by increasing antioxidant enzymes activity and phenol.

Varieties -

Some high yielding varieties of bitter melon include Priya, Preethi, Priyanka, and Arka Harit. .

## 2. Conditions for Growth

a) Climate:

Bitter melons require a very hot and humid climate to grow well. Plant bitter melon seeds in a location that gets 6 to 8 hours of sunlight everyday. Seeds will take 8 to 10 days to germinate in soil temperatures ranging from 15 to 20°C. Bitter melons will take almost 55-60 days to mature.

b) Rainfall and Watering:

Water bitter melon plants regularly, especially during the initial stages of growth when you should water every 3–4 days. During flowering and fruiting, water every other day. You can check if the soil is dry by sticking your finger two inches into the soil.

## 3. Fertility and Soil Type:

Soil type: Bitter melon grows best in well-drained, sandy or sandy loam soil. It can also grow in medium black soils rich in organic matter or alluvial soil along river beds.

pH: The ideal pH range for bitter melon is 6.0–7.0.

Nutrients: If the soil is lacking nutrients, you can add compost to make the plant fertile.

## 4. Agricultural Practices:-

Seed treatment: Before sowing, soak seeds in a solution of 25-50 ppm Gibberelic acid and 25 ppm boron for 24 hours. You can also treat seeds with Thiram at a rate of 3 g/kg of seed.

Sowing time: The best time to sow bitter melon seeds is February to March or June to July.

Sowing depth: Sow seeds 2.5–3 cm deep.

Sowing spacing: Sow seeds on both sides of 1.5 m wide beds, with a plant-to-plant spacing of 45 cm.

Seed rate: Use a seed rate of 2.0 kg per acre.

Soil preparation: Prepare the land well and make pits that are 60 cm in diameter and 30–45 cm deep.

Fertilizer: Apply 28:10:10 g N:P2O5:K2O of fertilizer per pit.

Watering: Water the plants every 3–4 days during the initial stages of growth, and alternate days during flowering and fruiting.

Support: Bitter melon is a weak climber, so it needs support for its growth. You can use a bower system to provide support.

Roguing: During the fruit formation phase, remove rogues based on the fruit's length, size, shape, and color.

Disease control: Spray Benlate (0.1%), Karathane (0.05%), or Bavistin at 2.0 g/liter of water to control disease. .

## 5. Extraction Method

1. Ultrasound-assisted extraction-

This method can reduce the time and temperature needed to extract bitter melon, while producing an extract with higher antioxidant activity and total phenolic content.

2. Aqueous extraction

This method can be used to extract phenolic compounds from bitter melon, and is considered the safest and easiest extraction method.

3. Ethanol extraction

This method can be used to extract bitter melon saponin. The extract is then destained with activated carbon and impurities are removed using an aluminum oxide column.

4. Chloroform- or alcohol-based extraction

These methods are efficient, but the extracts are not suitable for human consumption. .

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## 6. Environmental Stress and Plant Health

### a) Drought

Drought stress can increase the amount of coumaric acid in bitter gourd fruit, but it can also decrease chlorophyll synthesis and reduce relative water content (RWC). A combination of phosphorus fertilizer and AMF can help improve fruit yield under drought stress.

### b) Cold

Bitter gourd plants can adjust their carbohydrate metabolism to tolerate cold stress. They accumulate sucrose and raffinose, which help stabilize cellular membranes and maintain turgor.

### c) High humidity

High humidity during vegetative growth can make bitter gourd plants susceptible to fungal diseases.

### d) Heat

High temperatures can impair photosynthetic activity.

## VI. LITERATURE REVIEW

### 1. Bitter gourd

The plant *Momordica charantia* is commonly known as bitter melon or bitter gourd. It is widely used in Asia, Africa, and the Caribbean as a vegetable as well as medicinal product. Again, it has a long history of use in TCM, ayurveda, and in other traditional systems.



Fig:- bitter gourd

*Momordica charantia* is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. Its many varieties differ substantially in the shape and bitterness of the fruit.

### 2. Main Chemical Compound in bitter gourd

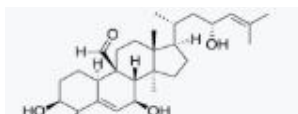


Fig- Molecular Structure of bitter melon (adapted from wikimedia.org, 2010)

Primary metabolites: Common sugars, proteins, and chlorophyll

Secondary metabolites: Phenolics, carotenoids, cucurbitane triterpenoids, alkaloids, and saponins

Fatty acids: Palmitic, stearic, myristic, pentadecanoic, and arachidic acid

Phenolic acids: Gallic, chlorogenic, caffeic, and ellagic acids

Flavonoids: Myricetin, quercetin, kaempferol, catechin, rutin, hesperidin, naringenin, biochanin a, and naringin



Peptide and protein: Ribosome-inactivating proteins (RIPs), peroxidase

Essential oils: Trans-nerolidol, apiole, cis-dihydrocarveol.

3,7-dihydroxy-17-(4-hydroxy-6-methylhept-5-en-2-yl)-4,4,13,14-tetramethyl-2,3,7,8,10,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene-9-carbaldehyde.

#### 4. Essential Oil

Bitter gourd essential oil is composed of various bioactive compounds, including:

1. Sesquiterpenes:  $\alpha$ -caryophyllene,  $\beta$ -caryophyllene, and humulene.
2. Monoterpenes: limonene,  $\beta$ -pinene, and  $\alpha$ -pinene.
3. Aldehydes: benzaldehyde and hexanal.
4. Phenolic compounds: phenol, catechol, and hydroquinone.

#### 5. Usage of Essential Oil

Bitter Gourd Oil is a great option for hot oil massages and pre-shampoo treatments for preventing dandruff, scalp flaking, and restoring your hair and scalp's natural moisture.

### VII. METHOD OF EXTRACTION

#### 1. Extraction of bitter gourd oil Soxhlet Distillation

Place a determined amount of n-hexane in the Soxhlet extractor

Put a weighed amount of bitter gourd seeds on filter paper in the thimble of the extractor

Set the heating mantle to a specified temperature

Run the extraction for a set amount of time

Dismantle the setup and pour the mixture of n-hexane and bitter gourd oil into a distillation flask

Set the heating mantle to 680°C, which is the boiling point of pure hexane

Distill and weigh the oil.

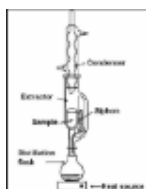


Figure. Soxhlet Extractor (adapted from technologylodging.com)

#### 2. Theory of Soxhlet Extraction

Soxhlet extraction is actually one of the methods to extract essential oils such as *Origanum Onite* and *Coriandrum sativum* L (Ozel and Kaymaz, 2004). This extraction method use chemical solvents to extract oils by repeated washing or percolation with an organic solvents. The extraction of the ginger essential oils began when steam contact to the ginger in the extraction tank. The steam carried out the essential oils from the ginger out of the rhizome and go through the condenser. Then, the steam with the essential oils will be condensed into liquid phase and will collected in the beaker. Soxhlet extraction shows more significant matrix compare to the ultrasonic method and that matrix effects depend heavily on the solvent used for the extraction (Schmeck and Wenclawiak, 2005). The Advantage of this method is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. Using stronger extraction conditions then the complete extraction is achieved (Schmeck and Wenclamiak, 2005). However, the Soxhlet extraction is about time consuming and labour intensive (Ozel and Kaymaz, 2004) where it is need large amount of solvents about up to 150 ml and large samples size which is up to 10 g (Saifuddin and Chua, 2003).

The most protruding advantages in using this Soxhlet method is when the sample phase is repeatedly bring into contact with fresh portion of the solvent, so that enhancing the displacement of the analyte from the matrix and no filtration is

required (Ayuso et.al., 1998). Nevertheless, the Soxhlet extraction is still the preferred method because of its comparative extraction results despite the nature of matrix sample. Besides, Soxhlet extraction yields similar results with methods such as the supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE) and ultrasonic methods. Some more the results also show small variations with low relative standard deviations (Lau et.al., 2010).

However, the soxhlet extraction is still gave higher yield for certain components or materials to be extracted. It is proven in the Figure 2.4 when comparative studies were carried out, it was found that the highest efficiency of Petroleum Ether Extracts (PEE) from tobacco was obtained by Soxhlet extraction and this method is especially suited to sterols that cannot usually be extracted by Accelerated Solvent Extraction (ASE) or are seldom extracted by ultrasonically assisted extraction (Shen and Shao, 2005).

**3. Nature of Solvent**

Solvent can be a liquid, solid or gas that dissolves another solid, liquid or gas solute but usually the solvent is in a liquid form that capable to dissolves or dispersing one or more substances. Below Table shows that there is the best solvent to be used in the extraction which is depending on the materials to be extracted.

Nutrients	Nutritional value
Energy	19.35 kcal
Carbohydrate	2.29 gm
Protein	1.61 gm
Calcium	17.62 mg

Table 2.1 : Nutritional value of 2 hours Soxhlet extraction of bitter gourd in various solvents (Alfaro et.al., 2003).

**4. Extraction Time**

Time that consumes to run the extraction process is known as extraction time. Extraction time is one of the important parameter that need to be optimized even in order to minimized energy cost of the process (Spigno et.al., 2006). The optimum extraction time will give the optimum of extraction yield where it is proven in the Table as shown below.

Extraction Time (h)	The yield of the extract (%)
3	9.54
4	10.90
5	12.19
6	13.40
7	15.70
8	16.97
9	16.97
10	16.97

Table : Effect of extraction time on the yield of the extracts (Li et.al., 2009)

### 5. Rotary Evaporator

Rotary evaporator is a device which is used to separate or remove the solvent from the mixture by undergoes evaporation. It consists of heated rotating vessel which is maintained under a vacuum and connected to a condenser. Rotation of the vessel improved heat transfer to the contained liquid. The solvent vapor will leave the vessel through the connecting tube and then condensed in the condenser flask. It is a very efficient way of rapidly removing large quantities of the solvents.

### 6. Gas Chromatography- Mass Spectrometer (GC-MS)

The gas chromatography- mass spectrometer (GC-MS) is simply a gas chromatograph with a very large and very expensive detector but one that can give a definitive identification of the separated compounds. The combination of gas liquid chromatography (GC) for separation and mass spectrometry (MS) for detection and identification of the components of a mixture of compounds. GC-MS system is used for both qualitative and quantitative analyses (Shen and Shao, 2005) and it is stated that the volatile compounds of all extracts will be analyzed by GC-MS (Natta et.al., 2008).

## VIII. METHODOLOGY

### 1. Materials

The homogenized bitter gourd extract is obtained by high pressure homogenizer at 300 psi and it is spray dried using wall material such as maltodextrin and gum acacia in varied core to wall ratios (1:2 & 1:3) and different drying inlet air temperatures 140°C and 160°C.

### 2. Apparatus

The primary tools utilized in the bitter gourd extraction process. The Soxhlet extractor is officinale. To separate the materials, a rotary evaporator was utilized. solvent as well as the oil that the Soxhlet extractor produces. The GC-MS was then employed for identification and detection of the oil samples' constituent parts.

### 3. Experimental Procedure

There were 4 main steps in this experiment which is sample preparation, extraction of bitter gourd, separation of the solvent from the oil and the last one was sample analysis.

#### 3.1. Sample Preparation

The leaves of bitter gourd was sliced into small pieces and then that pieces has been dried in the oven for 24 hours to 48 hours. After the leaves was totally dried, then it was grinded into very small particles (powders) by using the blender.

#### 3.2 Extraction of bitter gourd by using Soxhlet Distillation

30 gram of bitter gourd powders was placed into the thimble and then the thimble has been inserted into the Soxhlet chamber. The solvent was poured into the volumetric flask at the bottom part of the Soxhlet extractor. The extraction was started once the heating was begun to heat the solvent with the constant temperature. Lastly, the product was collected in the flask.

#### 3.3 Separation of the mixture

In this step, the solvent has been separated from the extracted oil by using the rotary evaporator. The mixture was poured into the boiling flask and the temperature was setting above the boiling point of that solvent. The solvent was collected in the condenser flask and the oil was remained in the boiling flask. Then, the mass of oil was obtained in order to get the yield percentage.

#### 3.4 Sample Analysis

The bitter gourd oil was analyzed by GC-MS Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent Chem. Station software to determine qualitative analysis of the volatiles. Firstly,



every 0.1 ml of oil sample has been diluted by hexane with ratio of 1:10 of hexane. Then, the diluted oil sample was inserted in each vial and they were analyzed by using GC-MS system.



Figure : Gas Chromatography- Mass Spectrometer.

The column temperature was at 50 C for injection, maintained for 2 minute then heated to 230 C at 10 C/min where it held for 15 minutes. Split injection(1 $\mu$ l) was conducted with a split ratio of 1:10 and helium was used as the carrier gas of 1 l/hr flow rate. Temperature of injector was maintained at 250 C. A solvent delay time of 2 min was used.

### IX. CONCLUSION

Bitter melon is a good source of phenolic compounds which possess potent antioxidant activity. The extracts obtained by SCWE had higher total phenolic contents and antioxidant activity than that obtained by solvent extraction and soxhlet extraction. Extraction is essential for isolation of different chemical constituent from crude drug material. Extraction depends on properties of material to be extracted.

### X. RECOMMENDATION

To improve this research, instead of using alternative Soxhlet Distillation, it will be much better if the method is changing to the Focused microwave-assisted The bitter is momordicine lowers blood glucose content in humans; this observation has aroused considerable pharmacological interest in the crop.

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