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Study of antioxidant activity and phytochemical analysis of fruits of *Momordica charantia var.muricata* using LC-MS

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Abstract

Momordica species are vegetable crops, belonging to the family of Cucurbitaceae. Cucurbitaceae crops are known to reservoir of several essential nutrients, minerals, vitamins, dietary fibers and a number of nutraceuticals and phytomedicinal compounds. *Momordica charantia* var. *muricata* small fruited variety mentioned in Horthus Malabaricus as Pavel or Pandipavel. They are reported to cultivated extensively in the past. Now its cultivation restricted to a few home gardens due to the entering of large fruited variety. The whole plant possesses a significant therapeutic value in the traditional system of medicine. The present study aimed to conduct phytochemical screening and antioxidant analysis. The pyto chemicals are those chemicals that are not established as a food nutrients but acts as a healing agent for different diseases in human beings. Phytochemical analysis of fruit extract through LC-MS analysis reveals the presence of primary metabolites like carbohydrates, Proteins, fatty acids, amino acids and secondary metabolites like alkaloids, terpenoids, coumarins, plant sterols, phenolic acids, flavonoids etc.,. DPPH assay and Nitric oxide assay is used to test antioxidant activity. In conclusion that the fruits of *Momordica charantia* var. *muricata* possess potential antioxidant activity, and could be used as a good source of natural antioxidants.

Keywords: *Momordica charantia* var .*muricata*; Pandi pavel; LC-MS; Phytochemicals; DPPH Assay; Antioxidant activity

1. Introduction

Momordica charantia L. Cucurbitaceae is commonly known as bitter gourd, bitter melon or balsam pear. The *Momordica* species have been used in indigenous medical systems in various countries in Asia and Africa. Based on the indigenous knowledge, wild plant foods play a vital role in the complex cultural system of tribal people for reducing various disorders. Cucurbitaceae species are valued for nutritional and medicinal purposes. Cucurbits are an excellent fruit in nature having composition of all the essential constituents required for good health of humans [1]. The genus *Momordica* species has sixty identified species [2] among them 7 species are in existence in India [3]. The *Momordica* genus is native to tropical areas of Africa and Asia [4], so consumption of these species as vegetables and their use as indigenous medicine is mainly concentrated in these countries. Even though, *Momordica* species are valuable, i.e. they are diverse and rich sources of phytochemicals and essential nutrients. Bitter gourd has been used as a traditional medicine for diabetes[5]. Many epidemiological results indicate an association between people who have a diet rich in fresh fruits and vegetables and a decrease in the risk of cardiovascular diseases and certain forms of cancer[6]. *Momordica* is a monophyletic genus that originated in tropical Africa and Asian species are considered the result of one long distance dispersal event that occurred about 19 million years ago. Chakravarthy has made a distinction between large and small fruited forms, the former being *Momordica charantia* var. *charantia* (cultivated type) and the latter *Momordica charantia* var. *muricata* (wild type). The wild variety *Momordica charantia* var. *muricata* is considered as the progenitor of cultivated *Momordica charantia* var. *charantia.* The cultivated form is generally large fruited. The small fruited type occur in wild, they are much resembling cultivated material except for miniature fruit size, and fruit surface, seed

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size[7].The small fruited forms are variously described as *Momordica charantia* var.*abbreviat*a Ser., *Momordica muricata* Willd. and *Momordica charantia* var. *muricata* (Willd.) Chakrav. by various workers.

Phytochemicals with antioxidant capacity naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration [7]. Numerous evidences have shown that increased consumption of fruits and vegetables reduce the risk of various pathological events such as cancer, cardiovascular diseases and cerebrovascular diseases [8,9]. This is often attributed to the antioxidants in the fruits and vegetables such as vitamin C, E, carotenoids, lycopenes and flavanoids that prevent damages caused by free radicals [10]. Free radicals play an important role in the development of tissue damage and pathological events in living organisms.

The objective of the study is to identify the different compounds present in the fruit pulp of *Momordica charantia var.muricata* using LC-MS technique and also list out the various phytochemically important compounds and evaluate total phenol content, flavonoid content and antioxidant activity of Fruit pulp.

2. Material and methods

2.1. Plant selected for the study

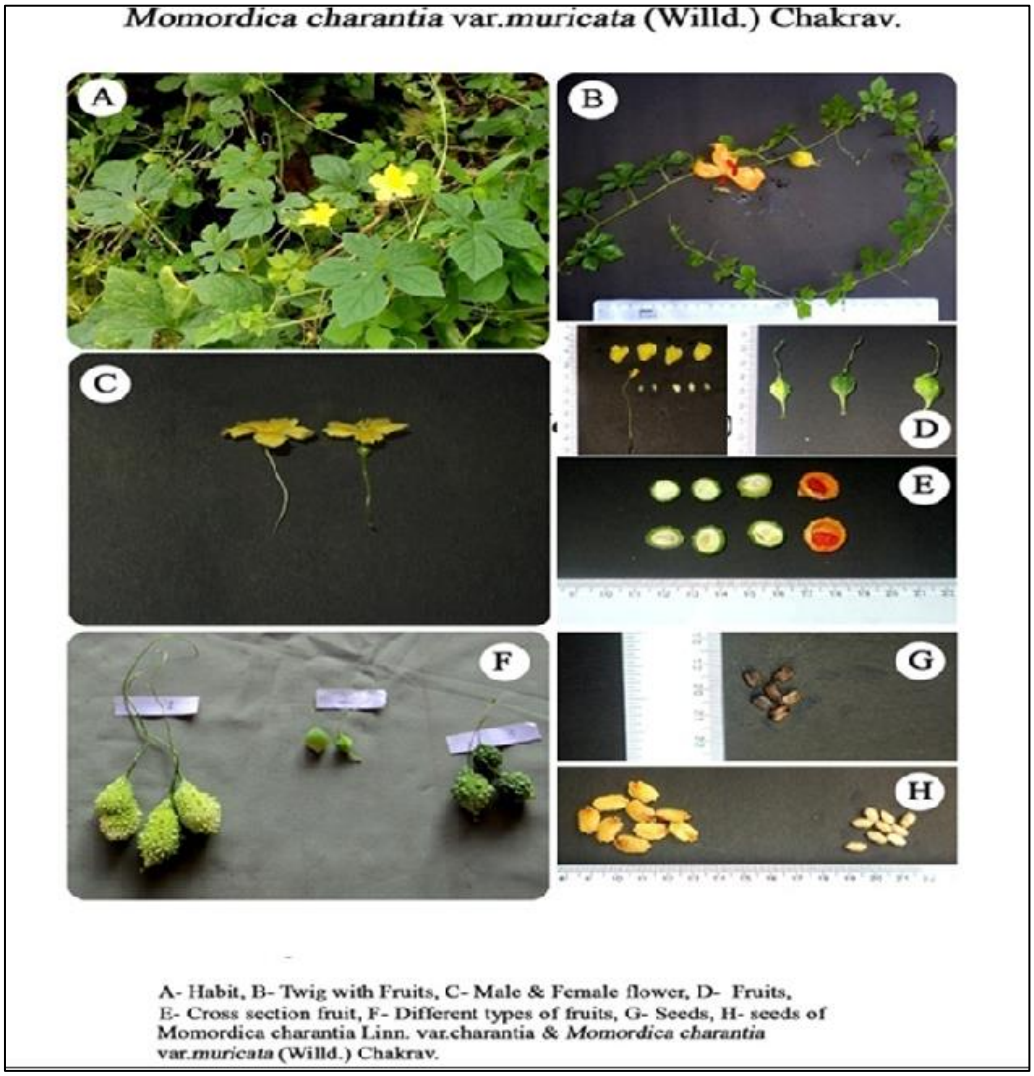

Figure 1 Momordica charantia var. muricata

2.1.1. Momordica charantia var. muricata

Annual, slender climber, 2–4 m high, scarcely to densely pubescent (tender parts wooly), monoecious. Flowers solitary in the axils, pubescent; petals yellow. Male flowers: solitary, stalks slender with bract midway or towards base ;

peduncles2-4 cm long, glabrous ; bract reniform, 5-10 mm diameter, green, mucronate at apex , margins entire ; pedicel 2-5 cm long ; Receptacle tube cup shaped ,2-4 mm long and 2-3 mm wide ; sepals ovate –elliptic, 4-6X2-3 mm, pale green – touching each other and protecting the corolla tube; petals obovate, 10-20X 7-15 mm, mucronate at apex, scales 2; filaments 1.5 - 2 mm long , inserted in the throat of the receptacle tube ; anthers coherent ; disc shortly cup shaped 1.5 mm diameter. Female flowers ; peduncle 1-6 mm long ; bract 1-9 mm diameter ; pedicel 1-8 cm long ; sepals narrow, oblong – lanceolate, 2-5 mm long ; petals smaller than or equal to that in male ,7-10 mm long ; stamens modes ovary fusiform , narrowly rostrate , 5-11 X 2-3 mm, muricate, tuberculate or longitudinally ridged ; style. 2mm long. Fruits small 3-9 cm, weighing 5- 35 g, tapering at both ends, seeds small to medium, flat, sub tridentate, brown, black, senescence late, wild rarely cultivated.

2.1.2. Collection and Authentication

The fruits of *Momordica charantia* var. *muricata* were collected from different localities and are authenticated from the Regional Herbarium of S.B. College (RHK), Changanacherry, Kerala. The voucher specimens of the plant sample were deposited in the Herbarium of S.B.College Changanacherry. Collected fruits were manually washed with distilled water and residual matter evaporated at room temperature.

2.1.3. LC-MS Analysis

10µl of the filtered sample was then injected to the manual injector using a micro syringe (1-20µl Shimadzu). The mobile phase used was water: methanol(50:50) in an isocratic mode. The column used was RP-C18(phenomenex). The separated compounds were then ionized using APC method using the split mode (50:50). The flow rate was maintained to 2 ml/mn with temperature 25±2° C. The class VP integration software were used for the data analysis. The Library used for the analysis was Metwin–LS. The version of the library was version 1.0-52.09.

2.1.4. Estimation of Flavanoids[11]

Procedure : 0.5 ml of aqueous extracts of sample is diluted with 3.5 ml of distilled water at zero time and 0.3 ml of 5% sodium nitrate was passed to the tubes. At the 5 minutes, 2ml of sodium hydroxide (1M) was added to the mixture. Immediately, the contents of the reaction mixture were diluted with 2.4 ml of distilled water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm versus a prepared blank immediately. Gallic acid was used as the standard compound for quantification of total flavanoids as mg/100 g.

2.2. Estimation of phenol [12]

2.2.1. Procedure

The volumes of control solution used ranged from 5 µl up to 400µl. Then, 300 µl of 0.2 M CH_3COONa was added to each one in order to keep the solution within a range from 3 to 4 pH values. 200 µl of 0.1 M $FeCl_3$ and 200 µl of 0.5% O-phenanthroline solution were added until a 10 ml final volume was obtained. Absorbance was mesured after 24 hours in the dark at 500 nm using UV-Spectrophotometer(UV- Spectrophotometer, Elico India Ltd, Model SL 160). Using standard calibration curve the concentration of the phenol was calculated.

2.3. Antioxidant Activity

2.3.1. DPPH Scavenging Assay[13]

Procedure

The fruit extracts (20µl) were added to 0.5 ml of methanolic solution of DPPH (0.3 mm in methanol) and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methnol served as the blank and DPPH in methnol, without the plant extracts, served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer (Elico INDIA Ltd, model SL 160). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity } \% = \frac{100 - A518(\text{sample}) - A\ 518(\text{blank}) \times 100}{A518(\text{blank})}$$

2.3.2. Nitric oxide assay[14]

Procedure

The reaction was initiated by adding 2.0 ml of sodium nitroprusside, 0.5 ml of PBS, 0.5 ml of fruit extract (50 mg) and incubated at 25^0C for 30 minutes. Griess reagent (0.5 ml) was added and incubated for another 30 minutes. Control tubes were prepared without extracts. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer and percentage of scavenging activity was measured with reference to ascorbic acid as standard.

3. Results and discussion

3.1. Phytochemical Analysis

Phytochemical screening is of paramount importance in identifying new source of compound having medicinal significance, to make the best and judicious use of available natural wealth [15]. Plant synthesize a vast range of organic compounds that are majorly classified in to Primary and Secondary metabolites. Importance of plant lies in their biological active compounds. Primary metabolites such as sugars, amino acids, proteins, fats, chlorophylls etc. are involved in the growth and development, respiration and photosynthesis, hormone and protein synthesis. Plant produce diverse compounds that have no direct participation in the role of growth and development are secondary metabolites. Phytochemical analysis of the extract revealed the presence of carbohydrate, fatty acids, amino acids, carotenoids, phytosterols, coumarins, alkaloids, flavonoids, phenolic acids, terpenoids etc.

Table 1 The phytochemicals present in the fruit pulp of M.charantia var. m uricata

Serial number	**Phytochemicals**	***Momordica charantia* var. *muricata***
1	Alkaloid	+
2	Carbohydrates	+
3	Coumarins	+
4	Lipids and fatty acids	+
5	Flavonoids	+
6	Carboxylic acid	+
7	Steroids	_
9	Terpenoids	+
10	Polyphenols	+

Presence or absence of certain important bioactive compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act like dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation.

Table 2 The presence of different flavonoids, terpenoids, Lipids and fatty acids, Carboxylic acid, Alkaloids, Polyphenols present in fruits of *Momordica charantia* var. *muricata.*

Plant Name	**Flavonoids**	**Terpenoids**	**Lipids and Fatty acids**	**Carboxylic acids**	**Alkaloids**	**Polyphenols**
Momordica charantia var *muricata*	Quercitrin Deoxy kaempherol	Momordicin Cucurbitacin D Cucurbitacin Q Ursolic acid Echinocystic acid Asiatic acid	Steric acid Oleic acid Linoleic acid Myristic acid	Pipecolic acid Rosmarinic acid Glucoronic acid	Echitamine	Caffeic acid Syringic acid

In *Momordica charantia* var. *muricata* Asiatic acid, Echinocystic acid, Ursolic acid Momordicin, Cucurbitacin D, Cucurbitacin Q *etc* are present. The Coumarins are of great attention due to their therapeutic property. Coumarins like Rutamarin present in *Momordica charantia* var. *muricata*. Cucurbitacin glucosides are reported to possess antioxidant and free radical scavenging activities [16]. Most of the people avoid the bitter gourd due to its bitter taste. The bitterness of most cucurbits is mainly due to cucurbitacins. Cucurbitacins constitute a group of diverse triterpenoids substances. A number of compound of this group have been investigated for their cytotoxic, hepatoprotective, anti-inflammatory and cardiovascular effects [17,18] .From LCMS Analysis Cucurbitacin D, Deoxycucurbitacin I, Cucurbitacin Q, Momordicin etc are obtained. Phytochemicals with antioxidant capacity naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration.

Antioxidants are the compounds that inhibit or delay the oxidation of molecules by inhibiting the initiation or propagation of oxidizing chain reaction [19,20]. Plant secondary metabolites such as polyphenols, play an important role in the defense against free radicals. Medicinal plant parts (roots, leaves, stems, flowers and fruits) are commonly rich in phenolic compounds, such as flavonoids, tannins, stilbenes, coumarins, lignans [21]. The antioxidant properties of polyphenols are due to their redox properties, which allow them to act as reducing agents, hydrogen donators, metal chela-tors and single oxygen quenchers.

Plant produce a very impressive array of antioxidant compounds that includes carotenoids, flavanoids, cinnamic acids, benzoic acids, folic acids, ascorbic acids, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate[22]. Phenolics have biological and pharmacological properties such as anti-inflammatory, antioxidant, and antimutagenic and anticarcinogenic activities [23].

Various types of diseases especially diabetes, atherosclerosis, cancer, aging, and inflammations are caused due to oxidative damage that reduced by the action of antioxidants. In this scenario the role of vegetable containing these anti-oxidants are very important. No single method is capable of providing a comprehensive view of the oxidative profile of a sample. Therefore a multi-method approach is necessary to assess antioxidant activity. In this study we use different methods. Such as DPPH assay and Nitric oxide assay. Percentage of antioxidant activity of *Momordica charantia* var. *muricata* fruit obtained after DPPH assay 46.09 ±0.004., and Nitric oxide assay 37.004±0.0089.

The results obtained shows that *Momordica charantia var.muricata* have higher antioxidant activity the extract of wild bitter gourd grown in Taiwan, possessed higher antioxidant and free radical scavenging activities that the normal ones[24].Previous studies agreement with this result. The antioxidant activity of gourd family vegetables is well known and studied by various researchers. The antioxidant property of the plant material is due to the presence of many active phytochemicals including vitamins, flavonoids, terpenoids, carotenoids, coumarins, curcumins, lignin, saponin, plant sterol. *etc*., [25].Flavonoids are found in vegetables, fruits, nuts, seeds, stem, flowers, tea, etc. These are an integral part of our a daily diet[26,27,28].

Previous studies have found that phenolic compounds are major antioxidant constituents in selected herbs, vegetables and fruits, and are direct relationships between their antioxidant activity and total phenolic content[29].Total phenol content in *Momordica charantia* var. *muricata* fruit is 0.316 ±0.008.

Flavonoids are considered favored bio compounds as chemotaxonomic markers in plants because they show large structural diversity and are chemically stable. Plant produce a very impressive array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acids, ascorbic acids, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate. Total flavonoid content obtained in the fruit of *Momordica charantia* var. *muricata* 0.314 ±0.005.

The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing several diseases through free radical scavenging activity [30]. *Momordica charantia* var. *muricata* faces a medium level of threat across its geographic range. Habitat loss and fragmentation brought about by population pressure and developmental activities, poor distribution and low population density of *Momordica* species coupled with in adequate in situ conservation efforts, and acculturation of the forest dwelling communities are the major factors attributed to their heightened threat status affecting their long-term survival in the wild [31].

4. Conclusion

Cucurbitaceous vegetables are well known for their medicinal and nutritional properties. Traditional knowledge leads to the development of new therapeutics. The present analyses suggest that *Momordica charantia* var. *muricata* contains

potentially health-protective phytochemical compounds with a potent source of natural antioxidants that may be clinically promising. Thus, it is also adding new compounds to the ever-increasing canvas of secondary metabolites acting as fountains of health. Medicinal use of different plants varies from place to place and community to community but some medicinal use of plants use common for all community and tradition. All most all the part of the people used bitter gourd against diabetes.

Momordica charantia var. *muricata* grown in India are used for various medicinal properties like antimicrobial, anti-helminthic, anti-cancerous, antimutagenic, anti-tumorous, abortifacient, antifertility, antidiabetic. Numerous medicinal and ethnobotanical uses of nearly all parts of the plant indicate along association of the plant with people, especially in India. Plants having vitamins, flavanoids, polyphenols possess remarkable antioxidant activity. So the plants discussed in this paper exhibited significant clinical and pharmacological activity. So the conservation of these plants and also give an awareness to the people about its traditional knowledge is very important. In our homested garden we can easily cultivate these nutraceuticaliy important vegetables. They are the treasure of biologically active compounds, they have different physiological functions.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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