Phytochemicals Screening, Minerals, Antimicrobial and Antioxidant Activities of Bitter Melon Seed Extracts and Characterization of Isolated Momordicin I

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Abstract

The present study was conducted to evaluate the phytochemicals, minerals, antimicrobial and antioxidant activities of bitter melon seeds (Momordica charantia Linn.). Firstly, phytochemical screening of bitter melon seeds was performed by employing the standard procedure and the results revealed the presence of alkaloid, phenolic and polyphenol compound, terpene and terpenoid, steroid, glycoside and saponin. According to the elemental analysis by Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer, phosphorus is the highest content in the sample followed by potassium, aluminium, sulphur, calcium, chlorine, iron, zinc, manganese, vanadium and copper. The antimicrobial assay was performed by agar-well diffusion method on six test microorganisms. Additionally, the ethanolic extract was studied for antioxidant property using DPPH free radicals scavenging assay. Ethanolic extract showed radical scavenging activity on DPPH with IC_{50} of 143.49 μg/mL. Moreover, the bitter principle of bitter melon, momordicin I was isolated from ethyl acetate extract by chromatographic methods and characterized by FT IR spectroscopic technique.

Keywords*—antimicrobial, antioxidant, DPPH, FT IR, phytochemical*.

1. Introduction

Fruits and vegetables are excellent source of bioactive compounds such as phenolics, flavonoids, terpenoids, alkaloids, etc. These naturally occurring compounds exhibit various biological properties including antioxidant and antimicrobial activity. Thus, these compounds and their properties have attracted the great attention from the scientific community.

Antioxidants are defined as those substances which significantly delay or inhibit the oxidation reaction thereby preventing cell damage or death [1]. Antioxidant prevents chain reactions or activation of oxygen into highly reactive products before they affect the cells [2]. Free radical or highly reactive oxygen attack healthy cells leading to loss of their structure and function [3].

Momordica charantia Linn. which is commonly known as bitter melon, bitter gourd, karela, belongs to the Cucurbitaceae family. It grows in the humid and subtropical regions of the world. Bitter melon has been intensively investigated for biologically active compounds and for their medicinal properties [4,5]. In 2008, Kubola and Siriamornpun investigated the phenolic contents and antioxidant properties of leaf, stem, and fruit extracts. They found that the fruit extract showed the highest antioxidant activity and gallic acid was the predominant phenolic compound in the fruit extract. The seeds of bitter melon are supposed to contain active components for anticancer, antidiabetic and gastrointestinal diseases [6].

The present research deals with the investigation of phytochemicals, minerals, antimicrobial and antioxidant properties of bitter melon seeds. Furthermore, the isolation and identification of momorcidin I from ethyl acetate extract of bitter melon seed was carried out by column chromatographic technique and FT IR spectroscopic method respectively.

1.1. Botanical Classification

Family – Cucurbitaceae
Botanical name – *Momordica charantia* Linn.
English name – Bitter melon, Bitter gourd
Myanmar name – Kyat-hinn-khar

Figure 1. Fruits of bitter melon

2. Materials and Methods

2.1. General Experimental Procedures

Column chromatography was carried out on MN silica gel 60, 0.05-0.2 mm; TLC was performed on Polygram SIL G/UV_{254}. All silica gel materials were purchased from Macherey-Nagel, Düren, Germany. Analytical grade reagents and solvents were purchased from Super Shell Co. Ltd, Yangon. PerkinElmer C93927 was used for FT-IR spectrum measurement. The absorbance was recorded by UV-spectrophotometer (UV-1800, Shimadzu). Stuart SMP 30 melting point apparatus was used for melting point determination. The antimicrobial activities of plant extracts were measured at Pharmaceutical Research Department, Insein, Yangon.

2.2. Plant Materials

Bitter melon was collected from Pyin Oo Lwin township, Mandalay region. The peel and pulp were removed and the seeds were air-dried and ground.

2.3. Phytochemical Analysis

Phytochemical tests were done according to standard procedures of Harborne [7]. The various phytochemicals in bitter melon seed were qualitatively determined by using Wagner's and Dragendorff's tests (alkaloid), ferric chloride test (phenol & tannin), foam test (saponin), mixture of ferric chloride and potassium ferric cyanide (polyphenol), Fehling's test (reducing sugar), Shinoda's test (flavonoid), Liebermann-Burchard test (steroid), lead acetate test (glycoside) and Salkowski's test (terpenoid).

2.4. Minerals

Mineral contents were measured by applying Energy Dispersive X-ray Fluorescence (EDXRF). The results were tabulated in Table 1.

2.5. Antimicrobial Assays

Antimicrobial assays were performed by agar-well diffusion method on six test microorganisms such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.* The test was carried out using agar-well with a diameter of 10 mm under standard conditions. The crude extract solutions were delivered into the well on an agar plates inoculated with test microorganisms. Then, the culture plates were incubated at 37 °C for bacteria (12 hours) and 27 °C for fungi (24 hours). After incubation, the diameter of the inhibition zones was measured in mm. If the zone of inhibition is over 20 mm, the sample was considered as highly active, from 15 to 19 mm was regarded as medium active and from 10 to 15 mm was designated as weakly active. The results were described in Table 2.

2.6. Antioxidant Activity of Ethanolic Extract of Bitter Melon Seeds

Radical scavenging activity of ethanolic extract on DPPH was determined by using the method of Kittasin et al. (2016). This method measured the hydrogen donating activity of the sample to DPPH radical. The stable free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl) (deep violet) reacted with antioxidants (hydrogen donors) and reduced to 2,2-diphenyl-l-picryl hydrazine (colorless) [8,9].

2.6.1. Preparation of 20 µg/mL DPPH solution.

2 mg of DPPH powder and 100 mL of 95% ethanol were thoroughly mixed by vortex mixer. This solution was freshly prepared in the brown coloured flask. Then, it was stored in the fridge for no longer than 24 hours.

2.6.2. Preparation of 400 µg/mL sample solution.

4 mg of test sample and 10 mL of 95% ethanol were thoroughly mixed by vortex mixer. The mixture solution was filtered and the obtained stock solution was diluted with 95% ethanol to get desired concentrations (25.0, 50.0, 100, 200, 400 µg/mL).

2.6.3. Preparation of 100 µg/mL standard solution.

1 mg of standard ascorbic acid and 10 mL of 95% ethanol were thoroughly mixed by vortex mixer. The standard stock solution was two-fold serially diluted with ethanol to obtain the solutions with the concentrations of 50, 25, 12.5, 6.25 and 3.125 µg/mL.

2.6.4. Measurement of DPPH radical scavenging activity by UV spectrophotometry.

The control solution was prepared by mixing 1.5 mL of 20 µg/mL DPPH solution and 1.5 mL of 95% ethanol. Similarly, the blank solution was prepared by mixing 1.5 mL of test sample solution and 1.5 mL of 95% ethanol. The test sample solution was also prepared by mixing 1.5 mL of 20 µg/mL DPPH solution and 1.5 mL of test sample solution in various concentrations. The solutions were kept in the dark for 30 min. Then, the absorbance of each solution was measured at 517 nm using UV-Vis spectrophotometer. Experiment was done in triplicate and the mean absorbance values were noted. The obtained absorbance values were used to calculate percentage of inhibition by the formula:

$$\text{Inhibition (\%)} = [Abs_{control}-(Abs_{sample}-Abs_{blank})/Abs_{control}]x100$$

Where $Abs_{control}$ is the absorbance of control solution, Abs_{sample} is the absorbance of sample solution and Abs_{blank} is the absorbance of blank solution. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using inhibition curve.

2.7. Extraction and Isolation of Momordicin I

The air-dried sample (600 g) was percolated with ethanol for one month. Then, it was filtered and evaporated the solvent. The residue was extracted with ethyl acetate and concentrated to obtain 2.13 g of ethyl acetate extract. The obtained extract was subjected to silica gel column using stepwise gradient of n-hexane and ethyl acetate. Each fraction was checked by TLC and visualized under UV and iodine vapor for purity. Momordicin I was isolated as white crystals and they melted at 125-128 °C. The structure of momordicin I was further confirmed by FT IR spectroscopic technique.

3. Results and Discussion

3.1. Preliminary Phytochemical Screening

According to phytochemical test, bitter melon seeds contained important secondary metabolites such as alkaloid, phenolic and polyphenol compounds, terpenoid, steroid, glycoside and saponin.

3.2. Determination of Mineral Content

According to the results of EDXRF method, the bitter melon seeds contained phosphorus, potassium, aluminum, sulphur, calcium, chlorine, iron, zinc, manganese, vanadium and copper. Phosphorus is the highest content in the sample.

Table 1. Elemental analysis of bitter melon seed

No.	Elements	Symbols	Concentration (%)
1	Phosphorous	P	0.7496
2	Potassium	K	0.5919
3	Aluminum	Al	0.1268
4	Sulphur	S	0.1134
5	Calcium	Ca	0.05189
6	Chlorine	Cl	0.03090
7	Iron	Fe	0.02101
8	Zinc	Zn	0.00878
9	Manganese	Mn	0.00273
10	Vanadium	V	0.00244
11	Copper	Cu	0.00148

Phosphorus is essential component in the structure of cell membrane. Together with calcium, phosphorus plays an important role in the building of healthy bone and tooth structure. Potassium is one of the important minerals in the body and it is necessary for the heart, kidneys and other organs to function properly. Potassium helps to reduce blood pressure and to regulate fluid balance, muscle contractions and nerve impulse. Calcium plays a vital role in muscle contraction, regulating heartbeat, formation healthy bones and teeth, blood clotting, nerve impulse and fluid balance within cells [10]. The sample also contained other trace elements such as sulphur, iron, copper, zinc and manganese. These trace elements have specific biochemical function in the human body. For example, iron is crucial component in many enzyme reactions and it is essential for the formation of haemoglobin. It has an important role in the immune system. Copper is also a component of many enzymes and zinc serves as cofactor in many enzymatic reactions [11].

3.3. Antimicrobial Activities of Various Extracts of Bitter Melon Seeds

According to antimicrobial activity test, ethyl acetate extract showed medium activity on *Bacillus subtilis*, low activities on *Staphylococcus aureus*, *Bacillus pumilus*, *Candida albicans* and *E. coli* and did not respond activity against *Pseudomonas aeruginosa*. Ethanol extract revealed low activities for all test microorganisms except *Pseudomonas aeruginosa*. However, n-hexane extract did not inhibit the growth of all test microorganisms.

Table 2. Antimicrobial activities of various extracts of bitter melon seed

Sample	Solvent	Inhibition Zone (mm)					
		I	II	III	IV	V	VI
Bitter Melon Seed	n-hexane	-	-	-	-	-	-
	EtOAc	16	13	-	12	13	13
	EtOH	12	11	-	11	11	11

I = *Bacillus subtilis* II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa* IV = *Bacillus pumilus*
V = *Candida albican* VI = *E. coli*

3.4. Antioxidant Activity of Ethanolic Extract

The antioxidant activity of ethanolic extract of bitter melon seed was evaluated by DPPH assay. The mean absorbance in five different concentrations of standard ascorbic acid and ethanolic extract of bitter melon seed were measured by UV-visible spectrometer at 517 nm. The inhibition (%) and IC_{50} value of standard ascorbic acid and ethanolic extract of bitter melon seed were tabulated in Table 3 and 4 respectively.

Table 3. Inhibition (%) in different concentration and IC_{50} Value of ascorbic acid

Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
50	68.50	17.97
25	61.61	
12.5	48.78	
6.25	40.41	
3.125	35.52	

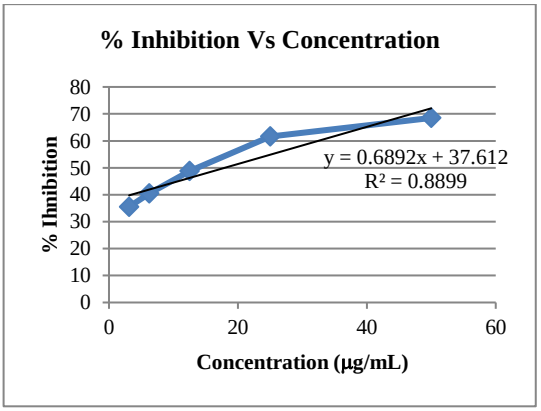

Figure 2. Plot of %inhibition in different concentration of ascorbic acid

Table 4. Inhibition (%) in different concentration and IC_{50} value of ethanolic extract of bitter melon seed

Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
400	72.55	143.49
200	58.19	
100	48.78	
50	40.41	
25	35.52	

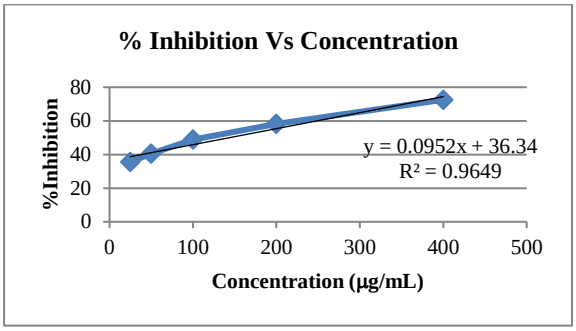

Figure 3. Plot of inhibition (%) in different concentration of ethanolic extract of bitter melon seed

According to DPPH assay, IC_{50} value of ethanolic extract of bitter melon seed was found to be 143.49 µg/mL. Meanwhile, IC_{50} value of standard ascorbic acid was 17.97 µg/mL. Therefore, ethanolic extract of bitter melon seed showed antioxidant activity but less than that of ascorbic acid solution, a standard antioxidant.

3.5. FT IR Assignment of Momordicin I

Momordicin I was isolated as white crystals and they melted at 125-128 °C. In the FT IR spectrum (Figure. 5), the broad band observed at 3379.40 cm^{-1} attributed to O-H stretching vibration of alcohol group. The peak appeared at 3041.48 cm^{-1} was assigned to C-H stretching vibration of sp^2 hydrocarbons. Asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbons and aldehydic C-H stretching absorption were observed at 2926.11 and 2854.74 cm^{-1}. The band at 1739.85 cm^{-1} represented C=O stretching vibration of aldehyde. In addition, the spectrum showed C=C stretching vibrations at 1618.33 and 1514.77 cm^{-1}, aldehydic C-H bending vibration at 1375.29 cm^{-1} and C-O stretching vibration 2°-alcohol at 1168.90 cm^{-1}. Therefore, the FT IR spectrum displayed the presence of aldehyde group, 2°-alcohol group, sp^2 and sp^3 hydrocarbon group. By the analysis of absorption bands in FT IR spectrum, the isolated compound could be assigned as momordicin I. It was further confirmed by comparing with literature data as well as melting point [12]. Momordicin I is a cucurbitane-type triterpenoid and a bitter principle of *Momordica charantia.*

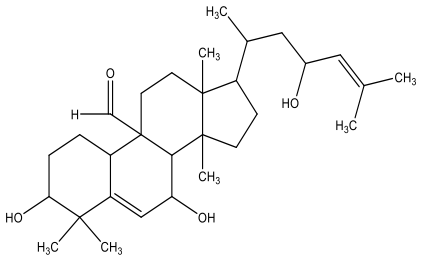

Figure 4. Structure of momordicin I

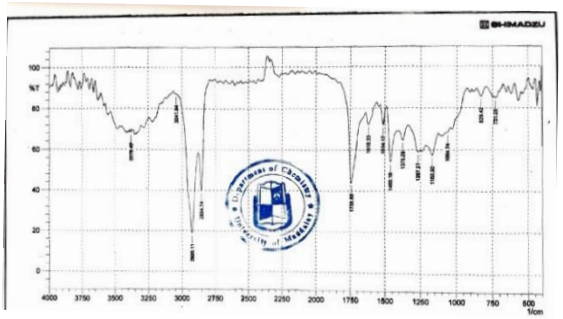

Figure 5. FT IR spectrum of momordicin I

4. Conclusion

The study on phytochemicals, minerals, antimicribial and antixoidant properties of bitter melon seed has been carried out by analysing the sample collected from Pyin Oo Lwin township, Mandalay. The phytochemical screening of the selected sample showed the presence of alkaloid, phenol, polyphenol, steroid, glycoside, terpenoid and saponin. The results of mineral content revealed the presence of phosphorus, potassium, aluminium, sulphur, calcium, chlorine, iron, zinc, manganese, vanadium and copper. The antimicrobial activities of three extracts of bitter melon seed were also investigated. According to radical scavenging assay, the ethanolic extract of bitter melon seed showed antioxidant activity with IC_{50} value of 143.49 µg/mL. Finally, the bitter principle of this plant, Momordicin I was isolated and confirmed by FT IR spectroscopic technique.

Bitter melon seed extract can be used as an antibacterial agent to fight off the infections caused by *E. coli* and *Staphylococcus aureus*. The seed is also rich in mineral such as potassium, calcium, iron, zinc and phosphorus. The bitter melon seed extract can also be used to prevent oxidative cell damage due to its antioxidant properties. Therefore, the finding of the present study suggested that bitter melon is an important source of antimicrobial and antioxidant compounds as well as mineral.

References

[1] L. C. Chapple, and J. B. Matthews, “The Role of Reactive Oxygen and Antioxidant Species in Periodontal Tissue Destruction,” *Periodontal*. vol. 43, pp.160-232, 2000.

[2] D. Venkat Ratnam, Ankola, D. D., Bhardwaj, V., Shana, D. k. “Role of Antioxidants in Prophylaxis and Therapy,” *A pharmaceutical perspective. J Control Release.* vol. 113, pp.189-207, 2006.

[3] M. Percival, “Antioxidants. Clinical Nutrition Insights.” Advanced Nutrition Publications. 1998.

[4] F. Majekodunmi, Y. Takeda, H. Yamashit, H. Okabe and T. Yamauchi. "New cucurbitane triterpenoids from *Momordica charantia*," *J. Nat. Prod*., vol. 53, pp. 1491-1497, 1990.

[5] S. Begum, M. Ahmed, B. S. Siddiqui, A. Khan, Z. S. Saify, and M. Ari. "Triterpenes, a sterol, and a monocyclic alcohol from *Momordica charantia*," *Phytochem*., vol. 44, pp. 1313-1320, 1997.

[6] N. Beloin, M. Gbeassor, K. Akpagana, J. Hudson, K. De Soussa, K. Koumaglo, and J. T. Arnason. "Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity," *J.Ethnopharmacol*., vol. 96, pp. 49-55, 2005.

[7] J. B. Harbone, Phytochemical Method: A Guide to Modern Techniques of Plant Analysis, 1973.

[8] K. Kiattsin, T. Nantarat, and P. Leelapornpisid, "Evaluation of antioxidant and anti-tyrosinase activities as well as stability of green and roasted coffee bean extracts from *Coffea arabica* and *Coffea canephora* grown in Thailand," *Journal of Pharmacognosy and Phytotherapy*, vol. 8, no. 10, pp. 182-192, 2016.

[9] P. Doungsaard, S. Chansakaow, J. Sirithunyalug, L. Shang-Chian, L. Wei-Chao, L. Chia-Hua, L. Kuan-Ha and P. Leelapornpisid, "*In vitro* Biological Activities of the Anti-aging Potential of *Dimocarpus longan* Leaf Extracts," *CMU J. Nat. Sci.*, vol. 19, no. 2, pp. 235-251, 2020.

[10] P. Pravina, D. Sayaji, and M. Avinash, "Calcium and its Role in Human Body," *International Journal of Research in Pharmaceutical and Biomedical Sciences*., vol. 4, pp. 659-668, 2013.

[11] C. D. Berdanier, J. T. Dwyer and D. Heber, "*Handbook of Nutrition and Food*," 3rd Edition, CRC Press. pp. 211-224, 2016.

[12] N. M. Puspawati, "Isolation and Identification of Momordicin I from Leaves Extract of *Momordica charantia* L," *Jurnal Kimia*., vol. 2. pp. 53-56, 2008.