Research Article

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QUALITATIVE ESTIMATION OF SEEDS OF *MOMORDICA CHARANTIA* LINN. (CUCURBITACEAE) AND CHARACTERIZATION OF GALLIC ACID ISOLATED FROM ITS ALCOHOLIC EXTRACT

Deepti Katiyar ^{1*}, Vijender Singh ² and Mohd. Ali ³¹Department of Pharmacognosy, KIET School of Pharmacy, Ghaziabad, UP, India²School of Pharmacy, Sharda University, Greater Noida, UP, India³Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

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***Corresponding author**

E-mail: katiyar_deepti@yahoo.co.in

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ABSTRACT

The current investigation engrosses the qualitative estimation of the seeds of *Momordica charantia* Linn. (Cucurbitaceae) and characterization of gallic acid isolated from its alcoholic extract. The seeds were successively extracted in petroleum ether, chloroform, ethanol and water in a Soxhlet Apparatus. Each of the extract was subjected to the preliminary phytochemical screening using the standard qualitative identification tests. The extract showing the presence of maximum phytoconstituents was utilized for the isolation of compounds by column chromatography and characterization was performed by using various spectral data analysis i.e., IR, ¹H NMR, ¹³C NMR and DART-MS. The alcoholic extract exhibited the presence of maximum number of phytoconstituents. Elution of the column with 0.2% Methanol in Chloroform gave a pale yellow, crystalline compound - Gallic Acid (3, 4, 5-trihydroxy benzoic acid). The diverse nature of chemical constituents present in the seeds of *Momordica charantia* Linn. (Cucurbitaceae) and the isolated compound-Gallic Acid can be further explored for their pharmacological potential which might be of immense value to mankind. This compound might act as the chromatographic marker and shall be beneficial in identifying and assuring the quality of the crude drug.

Keywords: *Momordica charantia*, Alcoholic Extract, Gallic Acid**INTRODUCTION**

Momordica charantia Linn., (Family: Cucurbitaceae) commonly known as karavella in Sanskrit, karela in Hindi, bittermelon, bitter cucumber, balsam pear or bitter gourd in English¹, is a monoecious annual climber upto 5m in height with branched, slender, grooved and angled stem; alternate, orbicular, petiolate, glabrous, 5-7 lobed leaves with jagged margins (due to which the plant has been named *momordica* meaning 'to bite'); pale yellow or orange, unisexual, solitary flowers; dark green to whitish, oblong, 5-25 cm long pepo fruit ribbed with many tubercles; brownish, compressed 12-16mm long seeds embedded in red pulp² mostly found in East Africa, South America and Asia-cultivated in India upto an altitude of 1500³. Charantin also known as momocharin (1:1 mixture of β sitosterol β -D-glucoside and stigmastadienol β -D-glucoside)⁴, a steroidal saponin agent possessing insulin like characteristics is the main phytoconstituent of *Momordica charantia*⁵.

Numerous phytoconstituents such as momordicins, cucurbitacins, momordenol, momordin, cucurbitanes, cucurbitins, momordicilin, momordolol, charine, momordicinin, cryptoxanthin, cycloartenols, elaeostearic acids, galacturonic acids, diosgenin, goyaglycosides, goyasaponins, gentisic acid, erythrodilol and multiflorenol⁶ have been isolated from all parts of the plant⁷.

Momordica charantia exhibits diverse pharmacological potential due to various phytoconstituents. The plant is a very well known anti-diabetic agent and the constituents responsible for this activity include charantin (Hypoglycemic effect)⁸; p-insulin, v-insulin, polypeptide-p (Insulin like effect)⁹; Momordicoside S & T (Increased glucose clearance and basal metabolic rate and β -oxidation)¹⁰; Karavilagenine E and

oleanolic acid (Intestinal GLP1 release)¹¹; Trehalose (Lowered postprandial blood glucose levels)¹²; Momordin (PPAR β/δ activation)¹³ and vicine (Hypoglycemic effect)¹⁴. It has also shown beneficial effects in diabetes related complications- diabetic nephropathy¹⁵, neuropathy¹⁶, cataract¹⁷, diabetic enteropathy¹⁸, insulin resistance¹⁹ and diabetic foot infection²⁰.

Various research investigations have reported the utility of *Momordica charantia* as abortifacient²¹, analgesic, anti-inflammatory & antipyretic²², anthelmintic²³, anti-allergic²⁴, anti-bacterial²⁵, anti-cancer²⁶, anti-malarial²⁷, anti-oxidant²⁸, anti-ulcer²⁹, anti-HIV³⁰, anti-herpes³¹, hepatoprotective³², hypocholesterolemic³³ and immunomodulatory³⁴ agents.

MATERIALS AND METHODS

The solvents used were of Fisher Scientific. Silica gel (Qualigens 60-120 μ m mesh) was used for column chromatography. TLC was performed on aluminium sheets coated with silica gel G 60 F 254 (Merck, Germany). All melting points were determined in Centigrade scale in one-end open capillary on the melting point apparatus. The IR spectra were recorded on IR Affinity-1 Fourier transform Infrared Spectroscopy model (Schimadzu). The DART-MS was recorded on a JEOL-Accu TOF JMS-T100LC Mass spectrometer having a DART (Direct Analysis in Real Time) source. The given samples were subjected as such in front of DART source. Dry Helium was used with 4 LPM (Litre per minute) flow rate for ionization at 350°C. The orifice 1 was set at 28V and spectra were collected and print outs are averaged spectra of 6-8 scan. The m/z values of the more intense peaks are mentioned and the figures in bracket attached to each m/z values indicated relative intensities with respect to the base peak. ¹H and ¹³C-NMR spectra were scanned on Bruker AvIII HD-300 MHz FT NMR

instrument instrument in CDCl_3 and D_2O utilizing Tetramethylsilane (TMS) and CDCl_3 as the internal standard. The coupling constants (J values) are expressed in hertz (Hz).

Collection of Plant Material

The plant material was procured from Khari Baoli, Delhi and it was authenticated as the seeds of *Momordica charantia* Linn. (Reference Number: NISCAIR/RHMD/Consult/2015/2911/104-1) by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR. The voucher specimen is preserved in the herbarium section of Department of

Pharmacognosy, KIET School of Pharmacy, Ghaziabad, Uttar Pradesh.

Extraction of Plant Material

The seeds of *Momordica charantia* were crushed to smaller pieces; re dried, coarsely powdered and successively extracted in petroleum ether, chloroform, ethanol and water using Soxhlet Apparatus for 72 hours. The scheme for successive extraction is depicted in Figure 1. Each of the extract was subjected to qualitative analysis of the phytoconstituents.

Figure 1: Schematic Representation of successive extraction of seeds of *Momordica charantia*

Qualitative Estimation

The crude petroleum ether, chloroform, ethanol and aqueous extracts were tested for the presence of alkaloids, steroids, tannins, saponins, glycosides, carbohydrates, flavonoids, proteins, phenolic and acidic compounds using the standard procedures for preliminary phytochemical screening^{35, 36}. The colour intensity, change in colour and appearance of solids in reaction mixture was observed³⁷. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Column Chromatography

Preparation of slurry

The concentrated alcoholic extract was taken in a china dish and placed on water bath and methanol was added in fractions to attain the desired consistency. Silica gel for column chromatography (60-120 mesh) was added gradually with constant mixing with a stainless steel spatula to obtain the slurry required to be packed into the column. Then, it was dried in air and the larger lumps were broken up. Finally, the uniform particle size was obtained by passing it through a sieve (No. 8).

Packing of column

The adsorbent cotton was plugged in the lower end of a dry column (5 feet high, 2.5 inches diameter) and then it was filled with petroleum ether upto half of its height. The required length of the packed column was obtained by adding silica gel in small

proportions and allowing it to settle down. The blank column was run thrice with solvent to allow the bubbles to escape. Then the column was packed with dried silica gel slurry of the extract and the column was plugged with adsorbent cotton. Different solvents (2.5 litres each) with increasing polarity were used to elute the column. The column was developed and eluted with solvents of increasing polarity in various combinations, viz., petroleum ether, chloroform in petroleum ether (0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%) chloroform (100%), and methanol in chloroform (0.1%, 0.2%, 0.3%, 0.4%, 0.5%). The Thin layer Chromatography was performed for the fractions (250ml each) thus collected. The fractions exhibiting the similar chromatographic profile were combined and concentrated.

Physico-chemical characterization

Elution of column with chloroform: methanol (49:1) afforded a pale yellow crystalline compound.

Yield: 95mg (0.21%)

R_f Value: 0.40 (Mobile Phase: Chloroform: Methanol (10:0.5))

Melting point: 159-160°C

IR ν_{max} (KBr): 3420, 3377, 3185, 1702, 1616, 1541, 1450, 1342, 1307, 1203, 1027, 868 cm^{-1} (Figure 2)

¹H NMR (MeOD): δ 7.08(2H, s, H-2, H-6) (Figure 3)

¹³C NMR (MeOD): δ 170.60 (C-7), 146.39 (C-3, C-4, C-5), 139.67 (C-1), 121.95 (C-2), 110.45 (C-6) (Figure 4)

DART MS m/z (relative intensity): 170[M]⁺ (C₇H₆O₂) (100) (Figure 5)

Table 1: Qualitative Estimation of *Momordica charantia* seeds

Qualitative Tests	Petroleum Ether Extract	Chloroform Extract	Ethanollic Extract	Aqueous Extract
Alkaloids				
Dragendorff's Test	-	-	+	+
Mayer's Test	-	-	+	+
Wagner's Test	-	-	+	+
Hager's Test	-	-	+	+
Steroids & Terpenoids				
Salkowski Reaction	-	-	+	+
Liebermann-Burchard	-	-	+	+
Tannins				
Ferric Chloride Test	-	+	+	+
Saponins				
Frothing Test	-	-	+	+
Glycosides				
Anthraquinones	-	-	+	+
Cardiac glycosides	-	-	+	+
Carbohydrates				
Molisch's Test	-	-	+	+
Fehling's Test	-	-	+	+
Phenolic Compounds				
Ferric chloride Test	-	-	+	+
Lead Acetate Test	-	-	+	+
Gelatin Test	-	-	+	+
Flavonoids				
Ammonia Test	-	-	+	-
Shinoda/Pew Test	-	-	+	-
Proteins and Free amino acids				
Millon's Test	-	-	+	+
Xanthoprotein Test	-	-	+	+
Biuret Test	-	-	+	+
Acidic Compounds				
Sodium Bicarbonate Test	-	-	+	+
Litmus Test	-	-	+	+

Figure 2: IR Spectrum of Gallic Acid

Figure 3: ¹H NMR spectrum of Gallic Acid

Figure 4: ¹³C NMR spectrum of Gallic Acid

Figure 5: Mass spectrum of Gallic Acid

Figure 6: Gallic Acid

RESULT AND DISCUSSION

The qualitative estimation of petroleum ether, chloroform, ethanol and aqueous extracts of the seeds of *Momordica charantia* Linn. (Cucurbitaceae) exhibited the diverse phytochemical profile of the plant material. The observations of various identification tests are shown in Table 1 which revealed the presence of maximum number of phytoconstituents in alcoholic extract, as it gave a positive response with all the qualitative identification tests. Thus, the alcoholic extract was further screened for the presence of its chemical compounds.

The compound- Gallic Acid was obtained as a pale yellow crystalline compound from chloroform: methanol (49:1) eluents. Its IR spectrum exhibited characteristic absorption bands for the crystal water and H-bridge or the H-bridge-OH (3420, 3377, 3185 cm^{-1}). The absorption bands at 1702 and 1616 cm^{-1} indicate the presence of $\text{C}_6\text{H}_6\text{-COOH}$ group while that at 1616 cm^{-1} designate the occurrence of conjugated or cyclic (aryl) structure. The distinct bands at 1450, 1342, 1307, 1203, 1027, 868 cm^{-1} respectively correspond to CH_2 , -R-COO , -OH , substituted aromatic, =C-O and isolated aromatic groups. The ^1H NMR spectrum showed two proton singlet at δ 7.08 attributed to H-2 and H-6 indicated the presence of 1-benzene ring. The shifts at δ 8.73 and 9.48 were ascribed to the occurrence of aromatic C-OH groups while that at δ 12.74 to the carboxylic acid group. The ^{13}C NMR spectrum exhibited the shift at δ 170.60 attributed to C-7 showed the presence of 1-carboxyl while the shifts at δ 146.39, 139.67, 121.95 and 110.45 designated the occurrence of 1-benzene ring. The mass spectrum exhibited a molecular ion peak at m/z 170 corresponding to the molecular formula $\text{C}_7\text{H}_6\text{O}_5$. The presence of $[\text{M}-\text{Me}]^+$ ion peak indicated the ring nature of the molecule. Thus, after the characterization of various spectra obtained for the compound, its structure was established as Gallic Acid (3, 4, 5-trihydroxybenzoic acid)-Figure 6.

CONCLUSION

The diverse nature of chemical constituents present in the seeds of *Momordica charantia* Linn. (Cucurbitaceae) and the isolated compound-Gallic Acid can be further explored for their pharmacological potential which might be of immense value to mankind. This compound might act as the chromatographic marker and shall be beneficial in identifying and assuring the quality of the crude drug.

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REFERENCES

1. Dasgupta AA, Mukherjee AA, Mitra AA. Phytopharmacology of *Momordica charantia* Linn. A review. JGPT 2009; 3: 7-14.
2. Kumar S, Ashish J, Satish N. *Momordica charantia* Linn.: a mini review. IJBR 2011; 2(11): 579-87.
3. Abascal K, Yarnell E. Using bitter melon to treat diabetes. J Altern Complement Med 2005; 1: 179-184.
4. Pitiphanpong J, Chitprasert S, Goto M, Jiratchariyakul W, Sasaki M, Shotipruk A. New approach for extraction from *Momordica charantia* with pressurized liquid extraction. Purif Technol 2007; 52: 416-422.
5. Krawinkel MB, Keding GB, Bitter Gourd (*Momordica charantia*): A dietary approach to hyperglycemia. Nutr Rev 2000; 64: 331-337.
6. Parkash A, Ng TB, Tso WW. Purification and characterization of charantin, a napin-like ribosome-inactivating peptide from bitter gourd (*Momordica charantia*) seeds. Journal of Peptide Research 2002; 59: 197-202.
7. Murakami T, Emoto A, Matsuda H, Yoshikawa M. Medicinal foodstuffs Part XXI Structures of new cucurbitane-type triterpene glycosides, goyaglycosides-a, -b, -c, -d, -e, -f, -g, and -h, and new oleanane-type triterpene saponins, goyasaponins I, II, and III, from the fresh fruit of Japanese *Momordica charantia* L. Chem Pharma Bull (Tokyo) 2001; 49: 54-63.
8. Lotlikar MM, Rao MR. Pharmacology of a hypoglycemic principle isolated from the fruits of *Momordica charantia* Linn. Indian Journal of Pharmacy 1966; 28: 129.
9. Dans A, Villarruz M, Jimeno C, Javelosa M, Chua J, Bautista R, Velez GG. The effect of *Momordica charantia* capsule preparation on glycemic control in type 2 diabetes mellitus needs further studies. J Clin Epidemiol 2007; 60(6): 554-559.
10. Kochhar A, Nagi M. Effect of Supplementation of traditional medicinal plants on blood glucose in non-insulin-dependent diabetics: A pilot study. J Med Food 2011; 8(4): 545-549.
11. Huang T, Lu K-N, Pai Y-P, Hsu C, Huang CJ. Role of GLP-1 in the hypoglycemic effects of wild bitter gourd. Evid Based Complement Alternat Med 2013; doi: 10.1155/2013/625892.
12. Matsuura H, Asakawa C, Kurimoto M, Mizutani J. α -Glucosidase inhibitor from the seeds of balsam pear (*Momordica charantia*) and the fruit bodies of *Grifola frondosa*. Biosci Biotechnol Biochem 2002; 66(7): 1576-1578.
13. Sasa M, Inoue I, Shinoda Y, Takahashi S, Seo M, Komoda T, Awata T, Katayama S. Activating effect of momordin, extract of bitter melon (*Momordica charantia* L.), on the promoter of human PPAR δ . J Atheroscler Thromb 2009; 16(6): 888-892.
14. Handa G, Singh J, Sharma ML, Kaul A, Zafar R. Hypoglycemic principle of *Momordica charantia* seeds. Indian J Nat Prod 1990; 6: 16-19.

15. Mardani S, Nasri H, Hajian S, Ahmadi A, Kazemi R, Kopaei MR. Impact of *Momordica charantia* extract on kidney function and structure in mice. J Nephropatho 2014; 13(1): 35–40.
16. Abas R, Othman F, Thent ZC. Protective Effect of *Momordica charantia* Fruit Extract on Hyperglycaemia-Induced Cardiac Fibrosis. Oxid Med Cell Long 2014; 8 doi .org /10 .1155 /2014 / 429060.
17. Rath SS, Grover JK, Vikrant V, Biswas NR. Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. Phytotherapy Research 2002; 16: 774–777.
18. Grover JK, Rath SS, Vats V. Amelioration of experimental diabetic neuropathy and gastropathy in rats following oral administration of plant (*Eugenia jambolana*, *Mucuna pruriens* and *Tinospora cordifolia*) extracts. Indian Journal of Experimental Biology 2002; 40: 273–276.
19. Ojewole JA, Adewole SO, Olayiwola G. Hypoglycaemic and hypotensive effects of *Momordica charantia* Linn (Cucurbitaceae) whole-plant aqueous extract in rats. Cardiovasc J S Afr 2006; 17(5): 227-232.
20. Viswasanthi T, Lakshmi SM, Thamizhvanan K, Rajesh J. Formulation and evaluation of poly herbal extract against diabetic foot infection. J Glob Tr Pharma Sci 2013; 4(4): 1285-1290.
21. Adewale OO, Oduyemi OI, Ayokunle O. Oral administration of leaf extracts of *Momordica charantia* affect reproductive hormones of adult female Wistar rats. Asian Pac J Trop Biomed 2014; 4(1): 521–524.
22. Patel R, Mahobia N, Upwar N, Waseem N, Talaviya H, Patel Z. Analgesic and antipyretic activities of *Momordica charantia* Linn. fruits. J Adv Pharm Technol Res 2010; 1(4): 415–418.
23. Vadamurthy AB, Rampurawala J, Paarekh PM, Jogaiah S, Hoskeri J. Evaluation of anthelmintic activity of *Momordica charantia* L. seeds. Ind J Nat Prod & Res 2015; 6(2): 153-155.
24. Sagkan RI. An in vitro study on the risk of non-allergic type-I like hypersensitivity to *Momordica charantia*. BMC Complementary and Alternative Medicine 2013; 13: 284.
25. Sen A, Dhavan P, Shukla KK, Singh S, Tejovathi G. Analysis of IR, NMR and Antimicrobial Activity of β -Sitosterol Isolated from *Momordica charantia*. Science Secure Journals 2012; 1(1): 9-13.
26. Pitchakarn P, Ogawa K, Suzuki S, Takahashi S, Asamoto M, Chewonarin T. *Momordica charantia* leaf extract suppresses rat prostate cancer progression in vitro and in vivo. Cancer Sci 2010; 101: 2234-40.
27. Subramaniam J, Murugan K, Kovendan K. Larvicidal and pupicidal efficacy of *Momordica charantia* leaf extract and bacterial insecticide, *Bacillus thuringiensis* against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). J Biopest 2012; 5: 163-169.
28. Patel SS, Shah RS, Goyal RK. Antihyperglycemic, antihyperlipidemic, antioxidant effect of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetes rats. Indian J Exp Biol 2009; 47: 564–570.
29. Alam S, Asad M, Asdaq SM, Prasad VS. Antiulcer activity of methanolic extract of *Momordica charantia* L. in rats. J Ethnopharmacol 2009; 123(3): 464-9.
30. Au TK, Collins RA, Lam TL, Ng TB, Fong WP, Wan DC. The plant ribosome inactivating proteins luffin and saporin are potent inhibitors of HIV-1 integrase. FEBS Letters 2000; 471: 169–17.
31. Bourinbaiar AS, Lee-Huang S. The activity of plant-derived antiretroviral proteins MAP30 and GAP31 against herpes simplex virus in vitro. Biochem and Biophy Res Comm 1996; 219: 923–929.
32. Tennekoon KH, Jeevathayaparan S, Angunawala P, Karunanayake EH, Jayasinghe KS. Effect of *Momordica charantia* on key hepatic enzymes. J Ethnopharmacol 1994; 44(2): 93-7.
33. Matsui S, Yamane T, Takita T, Oishi Y, Kobayashi-Hattori K. The hypocholesterolemic activity of *Momordica charantia* fruit is mediated by the altered cholesterol- and bile acid-regulating gene expression in rat liver. Nutr Res. 2013; 33(7): 580-585.
34. Pongnikorn S, Fongmoon D, Kasinerk W, Limtrakul PN. Effect of bitter melon (*Momordica charantia* Linn.) on level and function of natural killer cells in cervical cancer patients with radiotherapy. J Med Assoc 2003; 8661-68.
35. Harborne JB, Phytochemical Methods, A guide to Modern Techniques of Plant analysis, second ed. Chapman and Hall, London 1998; 54–84.
36. Iqbal E, Salim KA, Lim LBL. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy shaw) from Brunei Darussalam. Journal of King Saud University-Science 2015; 27: 224-232.
37. Archana M Navale, Komal S Patel, Archana N Paranjape. Phytochemical screening and HPTLC fingerprinting of *Anogeissus acuminata* extracts. Int. J. Res. Ayurveda Pharm. Mar - Apr 2016;7(Suppl 2):207-209 <http://dx.doi.org/10.7897/2277-4343.07288>

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