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Medicinal Uses and Molecular Identification of Two *Momordica charantia* Varieties – a review

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Abstract

Momordica charantia is a tendril bearing medicinally important vine. Medicinal properties of the plant include antimicrobial, antihelminthic, anticancerous, antimutagenic, antitumourous, abortifacient, antifertility, antidiabetic. Amongst the various medicinal properties, antidiabetic property of *M. charantia* is of utmost importance to human beings and animals**.** Mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids are the hypoglycemic constituents of *M. charantia* and these constituents are concentrated in fruits of *Momordica charantia*. *Momordica charantia* can be considered as an alternative therapy for lowering blood glucose levels in patients with diabetes. Although *Momordica charantia* has hypoglycemic effects, but available scientific data is not sufficient to recommend its use for treating diabetes, in the absence of careful supervision and monitoring. Investigation of the traditional uses of *Momordica charantia* in India revealed that it is one of the most important plant for ethnobotanical practices. Ethnobotanical uses of this plant in India suggest that it is capable of lowering blood glucose level in diabetic patients.

Furthermore, RAPD markers have been used to analyze the genetic diversity among 12 different accessions of *M. charantia*, collected from different districts of West Bengal. The clustering pattern based on RAPD markers was not in accordance with the grouping based on morphological characters. The presence of SCAR markers in the two varieties of *M. charantia* namely var. *muricata* and var. *charantia* has been determined so that nutritional and medicinal properties could be exploited judiciously.

Keywords: Antidiabetic; hypoglycaemic; phytochemic-als; RAPD.

1. Introduction

Momordica charantia L. commonly known as bittergourd is an economically important medicinal plant belonging to the family cucurbitaceae. Two varieties of this plant are cultivated in India 1. *M. charantia* var. *charantia* with large fruits which are fusiform in shape and 2. *M. charantia* var. *muricata*, which are identified by small, round fruit [1]. The immature fruits are eaten as vegetables and are a good source of vitamin C, vitamin A and phosphorus and iron [2, 3]. The bitter flavour of both the varieties is due to the alkaloid momordicine produced in fruits and leaves.

Fruits and seeds of bittergourd possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial, anti-tumor and last but not the least the important antidiabetic property [4].

The present investigation is being aimed in studying the medicinal and, ethnobotanical properties of *Momordica charantia* with specific importance to antidiabetic property of the plant. Furthermore estimation of genetic diversity among 12 different accessions of *M. charantia* growing in different districts of West Bengal, using RAPD markers has also been perfomed. A SCAR marker has been developed by the present investigators to distinguish the two varieties of *M. charantia.*

2. Phytochemicals related to anti-diabetic activity

It is evident from literature reviewed that diabetes is a killer disease that affects human subjects of different ages according to its type and the recurrence is specially noted in Indian population [5]. Mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids are the hypoglycemic chemicals of *Momordica charantia* [6] and these chemicals are concentrated in fruits of *Momordica charantia*, therefore fruit of *M. charantia* has shown most effective hypoglycemic property (Table 1) [7].

Table 1. Phytochemicals and constituents of *Momordica charantia.*

Source	Phytochemicals	Reference
Plant body	Momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, multiflorenol,	[13-17]
Plant body	Glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids	[6]
Fruit	Momordicine, charantin, polypeptide- p insulin, ascorbigen, Amino acids – aspartic acid, serine, glutamic acid, threonine, glutamic acid, threonine, alanine, g-amino butyric acid and pipecolic acid, luteolin, Fatty acids – Lauric, myristic, palmitic, palmitoleic, stearic,oleic,linoleic, linolenic acid	[8] [18] http://www.tropilab.com
Seeds	Urease, Amino acids – valine, threonine methionine, isoleucine, leucine, phenylalanine, glutamic acid	[19] http://www.tropilab.com

2.1 Steroids, Charantin

Charantin (Figure1) is one of the hypoglycemic compounds, which can be isolated from *Momordica charantia* fruit. It is a mixture of two compounds (1:1) sitosteryl glucoside ($C_{35}H_{60}O_6$) and stigmasteryl glucoside ($C_{35}H_{58}O_6$), both of which are steroidal saponins. Lolitkar and Rao [8] have shown charantin when taken taken either orally or intravenously in rabbits, it produces hypoglycemic effects.

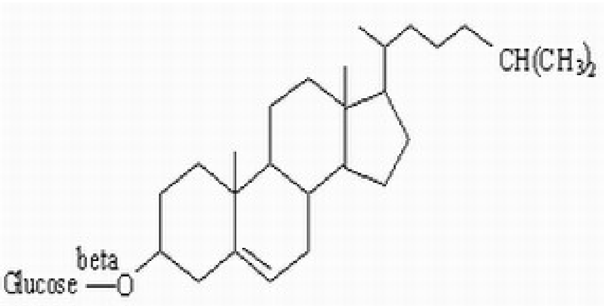

Figure 1. Structure of charantin.

2.2 Protein, P-insulin

Protein P- insulin is a polypeptide with molecular weight of about 11,000 Dalton and consists of 166 amino acids. Clinical study study revealed that the polypeptide-p-$ZnCl_2$ produced blood sugar lowering effect. Khanna and Mohan [9] reported that besides the fruits, p insulin was also found in seeds and tissue cultures of *Momordica charantia.*

2.3 Alkaloids

According to [10,11], seeds of bittergourd contain pyrimidine nucleoside vicine. Vicine has been found to induce hypoglycemia in rats, when administered intraperitoneally.

3. Medicinal uses

Medicinal properties include antimicrobial, anti-helminthic, anti-cancerous, anti-mutagenic, anti-tumourous, abortifacient, anti-fertility, anti-diabetic. Medicinal properties of *M. Charantia* are summarized in Table 2.

Table 2. Medicinal properties of *M. charantia.*

Property	Active phytochemicals / extract administered	Test system	Mechanism of action/ Targeted against an organism	References
Anti-viral	MAP 30 (Momordica Anti HIV Protein) in seed and fruit extracts	Viral cell line, H9	Anti -HIV1 (Human Immunodeficiency Virus) activity	[20]
	Anti-HIV proteins MAP30	Human lung fibroblasts	Anti -HSV (Herplex Simplex Virus) activity	[21]
	Alpha and beta momorcharin from seeds, fruits and leaf extracts	Rabbit reticulosyte lysate	Anti- HIV activity	[22]
	Methanol extract	Shrimp	White spot syndrome virus	[23]
Anti-bacterial	Water, ethanol and methanol extracts from	-	Broad spectrum antimicrobial activity	[24]

	leaves			
	Leaf extract	-	*Eshcherichi coli*, *Salmonella paratyphi and Shigella dysenterae*	[25]
	Essential oil	-	*Klebsiella pneumoniae, Bacillus megaterium, Bacillus subtilis, Proteus mirabilis, Aspergillus niger, Aspergillus flavus and Escherichia coli*	[26]
Anti-malarial	Aerial part extract	Rat	Plasmodium v*inckei petteri* 279BY (rodent malarial parasite)	[27]
	Leaf extract	Rat	*Plasmodium falciparum*	[28]
Anti-helminthic	Lyophilised plant extract	-	*Caenorhabditis elegans*	[29]
	Plant extract	-	Free living nematodes	[30]
Anti-cancerous	Crude plant extract, MAP 30	-	Lymphoid leukemia,lymphoma, squamous carcinoma of tongue, larynx, human bladder carcinoma, Hodgkin's disease	[31, 32, 33, 34, 35, 36]
	Fruit and seed extract	Mice	Extract activates natural killer cells in mice	[37]
Anti-mutagenic	Green fruits containing acylglucosylsterols	Mice	80% reduction in number of micronucleated polychromatic erythrocytes induced by mitomycin C	[38]
Anti-tumour	Crude extract	Mice	Tumour formation inhibition in CBA/DI tumour cell line	[39]
Abortifacient	Alpha and beta momorcharin in seeds extract	Mice	Induction of mid term abortion	[40]
Anti-fertility	Ether, benzene and ethanol extracts	Male rat	Antispermatogenic activity	[41]
Anti-diabetic	Green fruits	Human	Hypoglycemic activity detected	[9]
	Subcutaneously administered p- insulin	Human	Decrease in blood glucose level in IDDM (Insulin Dependent Diabetes Mellitus) patients	[42]
	Fruit powder	Human	Increase in glucose tolerance and fasting glucose levels in NIDDM (Non Insulin Dependent Diabetes Mellitus) patients	[43]
	Seed powder	Human	Decrease in post prandial glucose levels	[44]
	Charantin administered orally or intravenously	Rabbit	Fall in blood glucose level	[8]
	Chloroform extract administered intravenously	Alloxan induced rabbit	Fall in blood glucose level	[45]
	Pulp juice	Rats	Fall in blood glucose level	[7]
	Alcoholic fruit extract	Rat (1 hour after feeding glucose to the normal	Fall in plasma glucose level	[46]

4. Ethnobotanical uses

Chakravarty [12], documented about the ethnobotanical uses of the plant in India. Since both the varieties grow abundantly throughout India, *M. charantia* is a very important so far as ethnomedical practices are concerned. Ethnobotanical uses of *M. charantia* in India are enumerated in Table 3.

Table 3. Ethnobotanical uses of *M. charantia* in India.

Serial No.	Plant Part	Ethnobotanical uses	Type of extract	Used By	References
1.	Leaf	Purgative in children	Leaves or Oral	Human child	[47]
2.	Leaf	Anti-helmintic	Hot water extract or Oral	Human adult	[48]
3.	Leaf	Treatment of leprosy, piles and jaundice	Hot water extract or oral	Human adult	[49]
4.	Leaf and	Used regularly each month to avoid childbirth	Hot water	Human	[50]

	flower	through early abortion	extract or oral	female	
5.	Leaf	Leaf juice rubbed on the affected parts for treatment of ringworm.	Juice	Human adult	[51]
6.	Leaf	Five to six drops of leaf juice extracted from half-fried leaves is administered to infants during breast feeding for bowel movement	Juice	Human child	[51]
7.	Leaf	One teaspoon leaf juice plus few drops of honey administered thrice a day for cough, congestion and chest pain in children	Juice	Human child	[51]
8.	Vine	Emmenagogue	Hot water extract or oral	Human female	[52]
9.	Root	Used for abortions upto fifth month of pregnancy	Hot water extract or oral	Human-pregnant	[53]
10.	Root	Used as an abortifacient	Hot water extract or oral	Human-pregnant	[54, 48]
11.	Root	Used as an abortifacient	Decoction or oral	Human-pregnant	[55]
12.	Root	Root paste administered in milk to reduce the scars in small pox	Paste	Human adult	[51]
13.	Fruit	Treat diabetes	Decoction or oral	Human adult	[56]
14.	Fruit	Treat diabetes	Hot water extract or oral	Human adult	[57]
15.	Fruit	Used for treatment of hydrophobia	Powder or oral extract	Human adult	[58]
16.	Fruit	Abortifacient	Fruit or oral	Human-pregnant	[59]
17.	Fruit	Remedy for diabetes mellitus	Fruit or juice	Human adult	[8]
18.	Fruit	Used as antivenin	Oil extract or external or juice	Human adult	[60]
19.	Fruit	Anti-helminthic	Not stated or oral	Human adult	[61]
20.	Fruit	Used for jaundice, piles, leprosy, rheumatism and gout	Not stated or oral	Human adult	[49]
21.	Fruit juice	Used for treatment of malarial fevers	Fruit juice or oral	Human adult	[47]
22.	Fruit juice	Used to treat diabetes	Fruit juice or oral	Human adult	[62]
23.	Seeds	Anti-helminthic	Seeds or oral	Human adult	[48]
24.	Seeds	Seeds are boiled and the extremely bitter effusion is said to produce instantaneous vomiting.	Hot water extract or oral	Human adult	[63]
25.	Seeds	Used for treatment of diabetes, diabetes insipidus and used to reduce fat	Seeds or oral	Human adult	[64]
26.	Shoots	Used to treat pneumonia and leucorrhagia.	Shoots or oral	Human adult	[47]

5. Molecular markers in M. charantia

Genetic diversity among populations can be determined using molecular markers. Different types of molecular markers which has been used to assess the genetic diversity of *M. charantia* are mentioned be in Table 4.

Table 4. Molecular markers in *M. charantia.*

Molecular marker	Collection site	Genotypes analysed	Remarks	References
RAPD (Randomly Amplified Polymorphic DNA)	Different parts of South East Asia	45 cultivars	Cluster analysis divided 45 cultivars into two groups- striped protuberances and granular (thorny) protuberances.	[65]
	Different states of India	38 genotypes	The clustering pattern based on morphological character and molecular variation was different. 29 RAPD primers produced 76 polymorphic bands out of 208 reproducible bands.	[66]
	Different parts of India	20 genotypes	Dendrogram grouped the genotype into 2 clusters – A, B. Cluster A consisted of only one variety "Arka Harit" characterized by plants that are highly susceptible to fruit fly and downy mildew infestations. Cluster B consisted of 19 genotypes.	[67]
AFLP (Amplified Fragment Length Polymorphism)	Different parts of India	38 cultigens	Jaccard's genetic similarities ranges between 0.44 and 0.88, indicating a genetically diverse group.	[68]
	Different states of India	38 genotypes	404 polymorphic bands were produced by six AFLP primer pairs.	[69]
	6 countries	22 genotypes	High genetic divergence	[70]
ISSR (Inter Simple Sequence Repeats)	Different states of India	38 genotypes	Jaccard's similarities ranges between 0.48 and 0.91. Cluster analysis divided 38 genotypes into two groups genotypes of group 1 (2 genotypes) had a similarity of 0.56 and group 2 (36 genotypes) had a similarity ranging from >0.56 to 0.91.	[71]

6. APD Analysis

Two varieties of this plant are cultivated in India. One is *M. charantia* var. *charantia* with large fruits which are fusiform in shape and other is *M. charantia* var. *muricata*, which is identified by small, round fruits. The present investigation has been carried out to determine genetic diversity using RAPD marker and developing a SCAR marker to distinguish between the varieties of *M. charantia* as stated above.

RAPD markers have been used extensively in bittergourd to classify accessions identify cultivars and analyze genetic diversity. Changyuan et al. (2005) have employed RAPD markers in order to detect genetic relationship in 45 bittergourd cultivars, collected from different parts of South East Asia. Dendrogram based on UPGMA cluster analysis divided 45 cultivars into two groups- striped protuberances and granular (thorny) protuberances. Striped protuberances type included two groups, which came from South-eastern Asia and China. Granular protuberances type included two groups, which came from Hongkong and China mainland.

Dey et al. (2006) have analyzed the diversity of 38 genotypes of *M. charantia*, collected from different parts of India, both at morphological (agronomic traits) and molecular (RAPD) level. RAPD analysis revealed 76 polymorphic bands out of total 208 bands. The clustering pattern based on yield related traits and molecular variation was different.

Rathod et al. (2008) have used morphological characters along with RAPD markers to access genetic diversity and relationships among 20 genotypes of bittergourd collected from different parts of India. 69 polymorphic bands were obtained out of total 143 bands. Dendrogram grouped the genotype into 2 clusters – A, B. Cluster A consisted of only one variety "Arka Harit" characterized by plants that are highly susceptible to fruit fly and downy mildew infestations. Cluster B consisted of 19 genotypes.

In our laboratory, RAPD markers have been used to analyze the genetic diversity among 12 different accessions of *M. charantia*, collected from different districts of West Bengal. Genetic variation patterns of 12 accessions (V1, V2, V3, V4, V5, V6 and V7 belonging to variety *muricata and* V8, V9, V10, V11, V12 belonging to variety *charantia*) of two bittergourd varieties were examined using 23 selected RAPD primers. Amongst 23 primers used, 17 primers produced clear and reproducible bands. Among 17 primers, 16 primers produced polymorphic bands and only 1 primer produced monomorphic band. The highest number of 5'GACCGCTTGT3' (Figure 2) (NC-Negative Control). The similarity coefficients based on 81 RAPD markers ranged from 0.562 – 0.881. A dendrogram was constructed by cluster analysis to establish the affinity and relationship between the 12 accessions of *Momordica charantia*, using average linkage between the groups.

The dendrogram divided 12 accessions of *M. charantia* into 2 major clusters. It was observed that, varieties - V4, V6 and V7 belonging to variety *muricata* (based on morphology) grouped along with the varieties - V8, V9, V10, V11, V12 belonging to variety *charantia* (based on morphology) to form a single cluster. Another cluster comprised of the varieties - V1, V2, V3, V5 belonging to variety *muricata.* Thus, the clustering pattern based on RAPD markers was not in accordance with the grouping based on morphological characters. The similarity coefficients based on 81 RAPD markers ranged from 0.562 – 0.881. A dendrogram was constructed by cluster analysis to establish the affinity and relationship between the 12 accessions of *Momordica charantia*, using average linkage between the groups. The dendrogram divided 12 accessions of *M. charantia* into 2 major clusters. It was observed that, varieties - V4, V6 and V7 belonging to variety *muricata* (based on morphology) grouped along with the varieties - V8, V9, V10, V11, V12 belonging to variety *charantia* (based on morphology) to form a single cluster. Another cluster comprised of the varieties - V1, V2, V3, V5 belonging to variety *muricata.* Thus, the clustering pattern based on RAPD markers was not in accordance with the grouping based on morphological characters.

Due to the sensitivity of RAPD technique to PCR conditions, there is less reproducibility of RAPD results. This problem was solved by Paran and Michelmore (1993) who first documented the use of SCAR (Sequence Characterised Amplified Regions) marker in lettuce, where the marker was related to downey mildew resistance genes. SCAR markers can be developed by producing primers from unique polymorphic RAPD band. The primer is then allowed to amplify the genomic DNA of any particular genotype and not of other genotypes. Thus, PCR conditions of RAPD technique can be made more reliable by using SCAR markers.

Thus, we can take the help of SCAR markers in studying the genetic relatedness amongst intra and inter varieties and species of plants. Reproducible SCAR markers for studying inter and intra specific genetic diversity have already been successfully obtained from RAPD fragments in *Lactuca*, *Triticum*, and *Agrostis* [72,73,74].

In the present investigation, one polymorphic band was identified using RAPD primer 5'CAAACGTCGG3'. A SCAR marker was developed from the RAPD data [75]. This marker will be useful for future studies to identify the polymorphisms (6) was observed with primer no. 5, molecular differences between the two varieties used in agricultural practices.

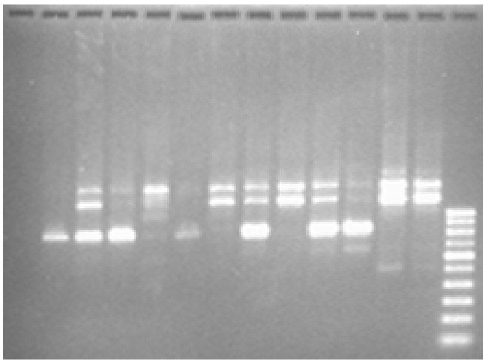

Figure 2. RAPD profile.

7. Conclusion

Two varieties of *M. charantia* namely var. *muricata* and var. *charantia*, grown in India are used for various medicinal properties like antimicrobial, antihelminthic, anticancerous, antimutagenic, antitumourous, abortifacient, antifertility, antidiabetic. *In vitro* and *in vivo* uses of the compounds isolated from extracts of *M. charantia* have been documented in this review with retrospective as well as recent publications. Ethnobotanical uses of this plant in India suggest that it is capable of lowering blood glucose level in diabetic patients. Thus, numerous medicinal and ethnobotanical uses of nearly all parts of the plant indicate a long association of the plant with people, especially in India. In the present study several plants were collected from different districts of West Bengal and subjected to DNA amplification. A polymorphic band was identified and used for developing a SCAR marker. Molecular markers such as RAPD and SCAR could be used to explore the genes associated with medicinal properties of *M. charantia*.

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