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Research Article

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Characterization of antihyperglycemic and antiretroviral components of *Momordica charantia* (Bitter melon)

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ABSTRACT

Bitter melon (Momordica charantia) is chosen for the study due to its diversified nutritional and medicinal properties (antihyperglycemic and antiretroviral). It contains artificial insulin and also acts as antitumor agent and inhibits HIV-1 infection. Various plant parts (seed, leaf, stem, and fruit), in vitro formed callus and suspension cultures of Momordica charantia were used for the isolation of polypeptide-P, MAP-30 protein aqueous and detergent soluble fraction,. Polypeptide – P was found utmost in leaves, seeds and fruits. The antiretroviral protein (MAP 30) was recovered in the highest amount from leaves followed by fruit and seed. Aqueous soluble fraction I was highest in leaves followed by seed and fruit. Whereas, detergent soluble fraction II was found maximum in suspension culture followed by fruit and callus. Popularity of Momordica charantia *in various systems of traditional medicine for several ailments focused the investigator's attention on this plant. Constituents of bitter melon can be utilized for preparing many herbal formulations which can cure with no adverse effects.*

Key words: antihyperglycemic, antiretroviral, *Momordica charantia*, *in vitro* culture

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INTRODUCTION

Momordica charantia commonly known as bitter melon is chosen for the study as it consists of number of constituents which contribute to the nutritional value of the plant. Bitter melon is rich in iron, has twice the beta-carotene of broccoli, twice the calcium of spinach, twice the potassium of bananas, and contains vitamin C and vitamin B1 to B3, phosphorus, iron and good dietary fiber. It is believed to be good for the liver and contain insulin and act as antitumor agent and inhibits HIV-1 infection. About 32 active constituents have been identified in bitter melon, including GABA, citrulline, lycopene and zeaxanthin. [14]

It contains saponin, charantin, polypeptide - p and vicine act as antidiabetic; flavanoids, methanolic fraction of plant have shown antihyperlipidemic effect; α – and β – momorcharin, lectin and MAP-30 has been reported to exhibit antiviral activity; methanolic extracts of leaves have also been reported to exhibit antibacterial properties; triterpenes and momordicin I and II are reported to show antihelmintic effect; momordin I, α and β - momorcharin and cucurbitacin B exhibit anticancer activity and anti-HIV activity and methanolic extracts of bitter melon seeds show analgesic and anti pyretic.[5]

Among these various pharmacologically important components, Osmotin (aqueous soluble fraction & detergent soluble fraction), Polypeptide p & MAP30 are the most extensively worked upon protein fractions of bitter melon.

Osmotin (24 kDa) is a basic pathogenesis-related protein of group 5 (PR-5) that displays antifungal activity in vitro and in vivo. Also it has been found that Osmotin & osmotin-like proteins have also been shown to be induced in several plant species in response to various types of biotic and abiotic challenges. The protein is generally believed to be involved in protecting the plant against these stresses. [13]

Polypeptide – P (11kDa), is a plant insulin (i.e. an insulin like polypeptide), which lowers blood sugar levels when injected subcutaneously into Type I diabetic patients. It has been isolated from fruit, seeds, and tissue of *Momordica charantia* Linn (bitter gourd). Also, it has been found to be very effective hypoglycaemic agent when administered subcutaneously to gerbils, langurs, and humans. [7]

Molecular studies disclosed the contribution of both caspase-8 regulated extrinsic and caspase-9 regulated intrinsic caspase cascades in MAP30-induced cell apoptosis. The antitumor potential was also effective in Hep G2-bearing nude mice. Since bitter gourd is a staple in many Asian countries, MAP30 would serve as a novel and relatively safe agent for prophylaxis and treatment of liver cancer. [3]

Therefore, the present work aims in isolation of various protein fractions associated with bitter melon such as aqueous soluble fraction, detergent soluble fraction, poly peptide-P (antihyperglycemic) and MAP 30 (antiretroviral) protein associated with various parts of the plant.

EXPERIMENTAL SECTION

Collection of plant material

The plant material was collected from the local market to isolate various associated protein fractions. The plant parts used under the study subjects were leaf, fruit, seed, seed coat suspension culture and callus.

In vitro culture of bitter melon

The seeds of *Momordica charantia* were germinated under sterile conditions on medium Murashige-Skoog (1962) without growth substances. [11] When the plants reached 2-3 cm seed-lobe, leaves, and meristem were inoculated, as explants of size 0.5cm on MS medium supplemented with different combinations of growth substances, to induce callus and bud regeneration. Hypocotyls harvested from field were rinsed with a little 20% (v/v) Tween 80, and washed in running water for 1h. Then they were surface sterilized with 75% (v/v) ethanol for 1 minute and immersed in 0.1% (w/v) mercuric chloride with periodic agitation for 6 minutes in a laminar air-flow cabinet. After surface-sterilization stems were finally washed 5 times with sterile distilled water. The callus induction media consisted of MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg et al., 1968) plus 3% sucrose and solidified with 0.8% agar; supplemented with different concentrations of 1.0 to 7.0 µM of naphthaleneacetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D) separately and in combination with 1.0 - 4.0 µM benzyl amino purine (BAP) were tested for callus induction. The medium was adjusted to pH 5.8 prior to autoclaving at 121°C for 15 min. The cultures were maintained at 25 ± 2°C under 16 h light and 8 h dark photoperiod with a light intensity of 150 µmol $m^{-2}s^{-1}$. Two transfers were made at an interval of 11 days in the same induction medium. [4,11,14,15,16]

Isolation of Polypeptide – p from various plant parts and cultures raised of bitter melon.

Leaf, fruit, seed, seed coat suspension culture and callus (100g each) were frozen. The frozen samples were crushed in 10ml of distilled water 45ml of 95% ethanol and 3.6ml of H_2SO_4 was added and stirred vigorously for 15-20 mins. at 25-28°C, homogenized by adding 60ml distilled water. Than 20 ml of 95% ethanol was added separately, filtered and the pH was adjusted to 3.0 using Ammonium hydroxide (28%v/v). To the flask 1.5 L of acetone was added and kept at 5°C for 8-10hr. [7]

Isolation of MAP 30 from various plant parts and cultures raised of bitter melon.

The plant samples were crushed in sterile normal saline (pH 3.6 – 4.0). The samples were centrifuge at 12,000rpm for 30mins. and the supernatant was collected. 500 µl of 50mM Phosphate buffer (pH 6.3) and 500 µl 50% Ammonium sulphate were added to the supernatant. The vial was centrifuged at 12,000rpm for 10mins. and the pellet was resuspended into 100µl of Phosphate buffer (50mM). [3]

Isolation of Aqueous soluble and Detergent soluble fractions of proteins (i.e. Fraction I and Fraction II) from various plant parts and cultures raised of bitter melon.

Samples were crushed in extraction buffer I [20M Pot. Phosphate Buffer (pH 6.5), 5mM EDTA, 1mM PMSF (100mg/ml)] homogenized using vortex mixer. The sample vials were centrifuge at 10,000rpm for 10mins. The supernatant was collected in a separate vial and label as Fraction I which was aqueous soluble. The pellet was resuspended in extraction buffer I and recentrifuge. Pool supernatants obtained in the previous steps in the vial labeled as Fraction I. The pellet was resuspended in extraction Buffer II [20mM Pot. Phosphate buffer, 2mM PMSF, 4M urea, 0.2% NP40 or Triton 100X] and homogenized. The vials were centrifuge at 10,000rpm for 10mins. The supernatant was collected separately and labeled as Fraction II which is detergent soluble. Protein precipitation/purification using ammonium sulphate salt fractionation: Fraction I which is aqueous soluble was purified using 40% and 80% ammonium sulphate salt solution simultaneously and resuspended the pellet in 100 µl phosphate buffer solution. Fraction II which is detergent soluble was purified using 80% ammonium sulphate salt solution and resuspended the pellet in 100 µl phosphate buffer solution.[12]

Quantitative analysis of the protein by Lowry's method

The protein by the method of Folin-Lowry method by Lowry *et al*. (1951). [9]

RESULTS AND DISCUSSION

The callusing ability of internodal explants derived from 30 day old *in vivo* plants of bitter melon was evaluated on MS medium supplemented with individual treatment of different auxins (NAA and 2,4-D) or their combination, either with BAP or TDZ. Proliferation of bitter melon explants on MS media supplemented with 2.0 mg /L BAP+0.2 mg /L NAA concentration under *in vitro* response responded best. Explants of nodal and root segments of bitter gourd were cultured on MS supplemented with various concentrations of BAP in combination with either 2,4-D or NAA. Nodal segments produced the highest percentage (93.75) of callus in MS supplemented with 1.0 mg/l 2,4-D and 1.0 mg/l BAP. No sign of regeneration of shoot was found from root segments in any of the combinations. However, some combinations produced only roots. [14] The quality of the callus was assessed after 3 weeks of culture. Selvaraj et al. (2006) obtained nodular, greenish compact and organogenic callus in the presence of 2,4-D and BAP for hypocotyl explants of cucumber. [15] Study reported callus formation in cucumber cultivars in the combination of NAA and BAP for petiole, leaf and cotyledon explants, respectively.[14,16]

Different kinds of proteins (Polypeptide – P (antihyperglycemic), MAP 30 (antiretroviral), Aqueous and detergent soluble fractions) associated with various plant parts and *in-vitro* cultures of *Momordica charantia* were isolated. Different kinds of proteins as, Aqueous and detergent soluble fraction) associated with various plant parts and *in-vitro* cultures of *Momordica charantia* were isolated. Research carried out in last few decades has certified several such claims of use of several plants of traditional medicine.

Polypeptide – P acting as artificial insulin was found utmost in leaves (9.868 mg/gm), seeds (5.895 mg/gm) and fruits (4.596 mg/gm). (Table 1) A hypoglycemic peptide, polypeptide-p, has been isolated from fruit, seeds, and tissue of *Momordica charantia* Linn (bitter gourd). Amino acid analysis indicates a minimum molecular weight of approximately 11,000 (166 residues). Polypeptide-p is a very effective hypoglycemic agent when administered subcutaneously to gerbils, langurs, and humans. [7] Abundant pre-clinical studies have documented in the anti-diabetic and hypoglycaemic effects of *M. charantia* through various postulated mechanisms. However, clinical trial data with human subjects are limited and flawed by poor study design and low statistical power. The present study highlighted the antidiabetic activity as well as phytochemical and pharmacological reports on *M. charantia* and calls for better-designed clinical trials to further elucidate its possible therapeutic effects on diabetes. [2]

The antiretroviral protein was recovered in the highest amount from leaves (4.158 mg/gm) followed by fruit (2.868 mg/gm) and seed (2.491 mg/gm). (Table 1) MAP 30 is protein isolated from bitter melon which has shown anti HIV and anti cancer activities. MAP30 (Momordica anti-HIV protein of 30 kDa) and GAP31 (Gelonium anti-HIV protein of 31 kDa) are anti-HIV plant proteins that we have identified, purified, and cloned from the medicinal plants Momordica charantia and Gelonium multiflorum. [6] Our results suggest for the first time that oral administration of BME inhibits prostate cancer progression in TRAMP mice by interfering cell-cycle progression and proliferation. [10]

During the isolation and quantitative analysis of the protein the recovery of the aqueous soluble fraction I was highest in leaves (3.096 mg/gm) of the plant followed by seed (1.184 mg/gm) and fruit (1.123 mg/gm). Whereas, detergent soluble fraction II was found maximum in suspension culture (20.211 mg/gm) followed by fruit (17.386 mg/gm) and callus (13.649 mg/gm). (Table 1) Osmotin is a basic pathogenesis-related protein of group 5 (PR-5) that displays antifungal activity in vitro and in vivo. Basic, vacuolar forms of PR-5 proteins have a C-terminal extension compared to the acidic forms which are secreted extracellularly. Plants over expressing an osmotin gene with a complete open reading frame accumulated osmotin mostly in an intracellular compartment, probably the vacuole. In contrast, in plants over expressing a C-terminal 20 amino acid truncated osmotin gene, osmotin was totally secreted into the extracellular matrix. Truncated osmotin purified from transgenic tobacco plants retained antifungal activity. Potato plants that over expressed the truncated osmotin protein exhibited resistance to *Phytophthora infestans*. [8]

The study indicated that the recovery of the protein is higher in the plant material with respect to *in vitro* cultures respectively. Study also suggests that the leaves serves as a better source of the antihyperglycemic and antiretroviral protein Bitter melon (*Momordica charantia*) is an alternative therapy that has primarily been used for lowering blood glucose levels in patients with Diabetes mellitus. Components of bitter melon extract appear to have structural similarities to animal insulin. Antiviral and antineoplastic activities have also been reported *in vitro*. Four clinical trials found bitter melon juice, fruit, and dried powder to have a moderate hypoglycemic effect. Bitter melon may have additive effects when taken with other glucose-lowering agents. Bitter melon may have hypoglycemic effects, but data are not sufficient to recommend its use in the absence of careful supervision and monitoring. [1]

Table 1: Amount of isolated various proteins from different plant parts of Bitter melon

Sample (Bitter Melon)	Concentration (in mg/gm)			
	Aqueous soluble fraction I	**Detergent soluble fraction II**	**Polypeptide – P**	**MAP – 30**
Leaf	3.096	2.939	9.868	4.158
Callus	0.929	13.649	1.877	0.728
Fruit	1.123	17.386	4.596	2.868
Suspension culture	0.851	20.211	2.035	2.351
Seed	1.184	Nil	5.895	2.491
Seed Coat	0.947	Nil	4.570	0.518

CONCLUSION

Bitter melon (*Momordica charantia*) is an alternative therapy that has primarily been used for lowering blood glucose levels in patients with diabetes mellitus. Components of bitter melon extract appear to have structural similarities to animal insulin and act as artificial insulin. Antiviral and antineoplastic activities have also been reported *in vitro*. Various clinical trials found bitter melon juice, fruit, and dried powder to have a moderate hypoglycemic effect. The present study inferred that the amount of proteins was higher in plants part especially leaves in comparison to various *in vitro* cultured explants. Bitter melon may have antihyperglycemic and antiretroviral effects, but the study conduct recommends its use in the presence of careful supervision and monitoring

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