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Review Article

A review on Phytochemical analysis of *Momordica charantia*

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ABSTRACT

Momordica charantia commonly known as bitter melon/gourd, a member of Cucarbitaceae, is a slender, tendril climbing, annual vine. Bitter melon is a common food in tropics and is widely used as medicinal plants in countries like India, Brazil, China, Africa etc. In *M. charantia* primary metabolites are common sugars, proteins and chlorophyll while secondary metabolites are alkaloids, flavonoids, tanins, saponins, disogenin, proteins, calcium, copper etc. Secondary metabolites are responsible for medicinal activity of *Momordica charantia*. The medicinal values of Bitter melon lies in the bioactive phytochemical constituents that are non nutritive chemicals that produce definite physiological effects on human body and protect them from various diseases. Qualitative phytochemical analysis of *Momordica charantia* confirms the presence of phytochemicals like flavanoids, saponins, terpenoids, coumarins, emodins, alkaloids, proteins, cardiac glycosides, anthraquinones, anthocyanins, steroids etc.

Keywords : *Momordica charantia*, Regeneration, Phytochemical, callus.

INTRODUCTION

Momordica charantia L. commonly known as bitter melon/gourd, a member of Cucarbitaceae, is a slender, tendril climbing, annual vine. Bitter melon is a common food item of the tropics and is used for the treatment of cancer, diabetes and many ailments[1-4]. It is a potent hypoglycemic agent [5, 6] and hypoglycaemic actions for potential benefit in diabetes mellitus are possible due to at least three different groups of constituents in bitter melon. These include alkaloids, insulin like peptides, and a mixture of steroidal sapogenins known as charantin. Clinical studies with multiple controls have confirmed the benefit of bitter melon for diabetes [7]. Alpha and beta momarcharin are two proteins found in bitter melon, which are known to inhibit the AIDS virus [8]. *M. charantia* plant has not been much investigated for its *in vitro* culture response. However, formation of callus is reported[9]. *Momordica charantia* Linn. (bitter melon) belongs to the family of Cucurbitaceae. It is a climbing vine which is commonly seen growing on walls and shrubs in the tropics. The textured leaves look as a bite that is why the plant is given name Momordica which means to bite. The orange fruits are soft when ripe and have black seeds with a red covering.

MEDICINAL PROPERTIES OF *MOMORDICA CHARANTIA*

Popularity of *Momordica charantia* in various system of traditional medicines for several ailments (antidiabetic, contraceptive, jaundice, abdominal pain, kidney (stone), piles , *pneumonia*, fever etc.) focused the investigator's attention on this plant [10]. Guanylate cyclase is thought to be linked to the pathogenesis and replication of not only psoriasis, but leukemia and cancer as well [11] .

The anticancerous and antileukemic activity of bitter melon against numerous cell lines including liver cancer, human leukemia melanoma and solid sarcomas have also been documented [12]. Saponins inhibit Na^+ efflux leading to higher Na^+ concentrations in cells , there by activating a Na^+, Ca^{2+} antiport. This effect produces elevated cytosolic Ca^{2+} which strengthens the contraction of the heart muscles and thereby reducing congestive heart failure [13]. Other uses include to expel intestinal gas, for tumors wound treatment, rheumatism , malaria ,vaginal discharge and the seeds are used to induced abortion [14]. Bitter gourd has also been used as a traditional medicine for several other ailments, including dysentery, colic, fever, burns, painful menstruation, scabies and

other skin problems [15]. The presence of phenolic compounds in the plants indicates that these plants might have antimicrobial agent. Bittter gourd might be used in the treatment of the placenta and navel of newborn baby which not only heals fast but also prevent the formation of infections [16, 17] . The extract of crude bitter gourd is used for different disease such as disease of liver and pancreas, anti-inflamatory, analgesic,reduces cholestrol level ,promotes appetite and supports blood sugar management for diabetes of the people with high risk of developing diabetes[18] .

The plant extracts and juices have been found suitable for different diseases / problems [19]. Beside these stem and leaf of bitter gourd is used in cancer treatment, in vital infections (HIV, herpes, Epstein Barr, hepatitis, influenza, and measles), in bacterial infections (Staphylococcus, Streptococcus, and Salmonella), as a bitter digestive aid (for dyspepsia and sluggish digestion) and in diabetes. The Bitter gourds have many health benefits and medicinal properties. These are such as kills bacteria, reduce inflammation, kill viruses, fights free radicals, kills cancer cells, kills leukemia cells, prevents tumors, cleanses blood, reduces blood sugar and balance hormones[14] .

Phytochemical studies revealed plant to contain lutein and lycopene which are responsible for its antibiotic antitumor activities, charatin , momordicine and other alkaloids, saponins, phenolic constituents, glycosides and 5-hydroxytryptamine [20].

[21] Plattel and Srinivasan, reported the hypoglycemic effect of the leaf extract of the plant. Antibacterial, antineoplastic, antiviral and antimutagenic activities of the plant have also been reported [22, 23.] Sofowora ., 1979 reported the purgative effect and the contractions of the guinea pig ileum of the plant extract. They also contain an array of biologically active proteins, namely, momordin , a- and b-momorcharin , cucurbitacin, and MAP30, that have shown to have highly effective anti-human immunodeficiency (HIV), anti-tumor anti-diabetic, and anti-rheumatic properties and to function as febrifuge medicine for jaundice, hepatitis, leprosy, hemorrhoids, psoriasis, snakebite, and vaginal discharge [25,26 ,27] .

PHYTOCHEMICAL PROPERTIES:

The medicinal values of Bitter melon lies in the bioactive phytochemical constituents that are non nutritive chemicals that produce definite physiological effects on human body and protect them from various diseases. In *M.charantia* primary metabolites are common sugars, proteins and chlorophyll while secondary metabolites are alkaloids, flavonoids, tannins and so on (Table 1).

Plant tissue culture have been investigated for industrial production of several useful secondary metabolites [28, 29.] Phenolic compounds, flavinoids are one of the widespread groups also acting as chemotaxonomic markers [30, 31.] Phytosteroids are pharmologically important for human life [32.] Diosgenin is synthesised in all plant parts with higher concentrations in fruit of M.charantia [5, 33.] Exposure to salicylic acid frequently induces the synthesis of secondary metabolites in plant [34, 35, 36.] Diosgenin is produced from different species of Dioscoria [37, 38.] Flavonoids and steroids were carried out by using the methods of [39, 40, 41, 42] respectively. Sterols are ubiquitous in higher plants and probably also in plant tissue cultures. *B*-sitosterol has been reported from *Nicotiana tabacum* [43], *M.charantia* [9]and stigma sterol is reported from *Dioscorea tokoro* [42] and *M.charantia* [9] . In plant pathogen interaction jasmonic acid and salicylic acid functions as endogenous signal compounds, thereby increasing the production of secondary metabolites by inducing the expression of defence related gene [44]. Bitter gourd has important role as a source of carbohydrate, protein, vitamins minerals and other nutrients in human diet [45.] The presence of total sugar and starch was reported [46], reducing sugar [47], water soluble protein [48] and total protein by Micro – Kjeldahl method of AOAC [49]. Vitamin C by the standard method of AOAC [50], lipid content [51]. Alkaloid with R.f.0.098 and 5- hydroxytryotamine is also reported [52.]

The potein content of bitter gourd was reported [53,] and protein of unpeeled bitter gourd [54.] The high amount of calcium and copper was reported in bitter gourd [53, 55] .charantin ,[56] pure D-galacturoic acid[57,] sterol glucoside[58] isolated a polypeptide –P all the phytochemicals posseses hypoglycaemic action. Alkaloids and saponins are present in Momordica and volatile components are released during cooking which enhance the flavour [59, 60.]

Bitter melon plants contains high levels of iron, beta carotene, calcium, potassium, vitamins, phosphorus, and good dietary fiber [61, 62]. *Xiao et al* found that the seed oil of M.charantia contained saturated fatty acids mainly steric acids, monounsaturated fatty acids like linoliec acid and polyunsaturated fatty acids.

In vitro* regeneration of *Momordica charantia

There are two ways to regenerate plants through the initiation of adventitious buds or through somatic embryos [64]. In the present study, shoot and leaf explants were taken from aseptically grown seedling. tip, nodal and Internodal explants. A final treatment with 0.1% $HgCl_2$ was given for 5 mins after which explants were washed with distilled water. The explants were then inoculated on 15 ml aliquots of 0.8% agar containing MS medium [65] and incubated in dark for 20 days. When the radical emerged from the seeds, they were

transferred to incubation at 25 ± 20^{0}C with a 16 h photoperiod provided by cool fluorescent light (50 µ Em-2s-1) (Phillips, India). Approximately 2000-3000 lux artificial light intensity is needed. Cool white fluroscent light lamps are generally used for providing light. Generally 55 -60 % relative humidity is maintained in the culture room. After 5 weeks, from 15 cm long seedlings, 5-6mm shoot tip explants, 20-25 mm long nodal and internodal explants were dissected and inoculated on MS medium with various concentrations and combinations of growth regulators .

Cytokinin concentration of 0.5 mg/l to 6 mg/l produced shoots after 20 days in culture and the best response was observed on media containing 0.5 mg/l and 2 mg/l BAP, while IBA/NAA were suitable for rooting with best response at 4 mg/l IBA and 2mg/l NAA, the root formation was observed after 22 days in culture. Callus was formed on 2,4-D, with profuse callusing at 2mg/l of 2,4-D.A combination of NAA+BAP+2,4-D was most effective for callus formation with best response in 2mg/ l NAA + 0.5 mg / l BAP + 2 mg/l+2,4-D . This is in agreement that [66,67, 68] the tissue organ used as a source of explant can also be a determinant for the success of a plant tissue culture. Our results support the observation [69] that organogenesis is determined by auxins and cytokinins. When tissues in *vitro* do not appear to require exogenous supply of auxins and cytokinin, it may be that sufficient endogenous levels of hormones exist in the culture system for organogenesis. MS medium without hormones also shows medium multiple shoots as well as roots formed.

The secondary metabolites are of immense importance for use as commercially as well as biologically active compounds. Flavonoids are naturally occurring phenolic compounds, which have a widespread distribution in intact plants and have been found in a number of tissue cultures. In callus cultures, the maximum amount of total steroid content was observed in six-week-old callus (27.34mg/gdw) and minimum was in 2 weeks old callus cultures (12.12mg/gdw) . The steroidal sapogenin, diosgenin is found in many plants but it is obtained principally from Dioscorea roots (4 to 6%dw) for conversion to commercially useful drugs. The presence of diosgenin was observed by thin layer chromatography coinciding with its standard sample in Rf values (Diosgenin-0.50). The color reaction test when sprayed with 50% H_2SO_4 fluorescent spot coinciding with their respective standard marker (Diosgenin-Green).

The liquid MS medium with NAA (2mg/l) + BAP (0.5mg/l) +2, 4-D (2mg/l) was prepared. Callus was transferred to each flask and liquid cultures were grown on reciprocal shakers (125rpm; 5cm/stroke) for 6 days, on the 6th day liquid MS media was supplied with three concentration (0.1mM, 0.05mM, 0.025mM) of salicylic acid and the cultures were again grown on same shakers for exactly 24h.The tissue grown on different media were harvested and weighted again and dried at room temperature. The callus samples were powdered and subjected to qualitative and quantitative estimation of diosgenin.

In vitro clonal propagation of *M.charantia* has been done [70] leading to shoot and root differentiation at different level of cytokinin (BAP, Kin) and auxin (IBA, IAA, NAA) in MS medium. Leaf explants showed maximum callus percentage and callogenic response than other two explants stem and cotyledons. At different concentration of BAP and Kin green, compact and hard calluses produced. Best response towards multiple shoot regeneration was obtained from the nodal segments of *M.charantia* on MS medium supplemented with BAP and NAA .It was suggested by [71] that the combination of 2,4-D and BAP is most suitable for callus induction from shoots.

The shoots regenerated from callus, seedling explants and from multiple shoots were separated and transferred on MS medium containing different concentrations of rooting hormones, best response was on 3mg/l IBA, and the roots were developed roots in ten days . The complete plantlets thus formed, were hardened in green house and transferred to pots where 40% plants survived successfully. This is in agreement with previous report that multiple shoots can be formed on MS medium without hormones.

This study concludes that endogenous and exogenous level of growth regulators is also important for callogenesis and for differentiation.

Significance of *in-vitro* regeneration of *Momordica charantia*

The production of adventitious root and shoot from the cells of the tissue culture is called ‘organogenesis’. If any wound or cut in plant is caused by any reason such as mechanical, chemical or by any infective agent they will form an unorganised proliferated mass of cell known as callus. When callus is grown aseptically on artificial nutrient medium in the glass vials under controlled experimental condition Gentheret was first to successfull promote the development of cell tissue. It is difficult and taking too much time to breed using normal method, while tissue culture is an efficient method for plant breeding and can shorten breeding period. The auxins are used to promote shoot growth and cytokinins are involved for root growth.

Due to valuable factors of *Momordica charantia* it is utilized as food and medicine importance in countries like China, India, Africa and West indies since ancient times . Present study was conducted

for micropropagation using different explants through *in vitro* regeneration large number of plants can be produced through callus.

Tissue by manipulating hormonal and nutrient constituents in medium. The callus tissue from explants can provide genetic variability in *Momordica charantia*. Through callus culture of bitter gourd several secondary metabolites and biochemical assays can be produced[71]. All the varieties were found to contain tannin, flavonoids, terpenoids, cardiac glycosides, triterpin and sterol, resin, amino acids and phenolic compounds except coumarin and free anthraquinones .

Plant tissue culture has been found to have potentials as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites [72].

Table1: Phytochemical analysis of Leaf and Callus extract

TESTS	LEAF EXTRACT	CALLUS EXTRACT
TANINS	+	+
ANTHOCYANINS	+	-
LEUCOANTHOCYANINS	-	-
FATTY ACIDS	-	-
COUMARINS	+	+
EMODINS	+	+
QUINONES	-	+
CARBOHYDRATES	-	-
CHOLESTROL	-	-
AMINO ACIDS	-	-
CARDIAC GLYCOSIDES	+	+
PHLOBATININS	-	-
TEST FOR PHENOLS		
$FeCl_2$ TEST	+	-
LIEBERMANN's TEST	+	-
TEST FOR PROTEINS		
NINHYDRIN TEST (aqueous)	-	-
NINHYDRIN REAGENT	+	+
OTHER TESTS	-	-
TEST FOR ANTHRAQUINONES		
BORNTAGER's TEST	+	+
NINHYDRIN REAGENT	+	-
BIURET TEST	-	+
TEST FOR ALKALOIDS		
MAYER's TEST	+	+
HAGER's TEST	+	+
WAGNER's TEST	-	-
OTHER TEST	+	+
TEST FOR FLAVONOIDS		
NaOH TEST	+	-
H_2SO_4 TEST	-	+
OTHER TEST	+	+
TEST FOR STEROLS		
TERPENOIDS	+	+
SAPONINS	+	+

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