International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-2, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Qualitative Analysis of Flavonoids and Phenols in *Momordica Charantia* Callus

Ujjwala Supe*, Prarthna Daniel, M. G. Roymon

Plant Tissue Culture Laboratory, St. Thomas College, Bhilai, District -Durg, Chattisgarh, India.

Manuscript No: IJPRS/V3/I2/00227, Received On: 23/04/2014, Accepted On: 03/04/2014

ABSTRACT

Momordica charantia commonly known as bitter melon/gourd which is a member of Cucarbitaceae and is a slender, tendril climbing, annual vine. 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 photochemicals like flavanoids, saponins, terpenoids, alkaloids, proteins, cardiac glycosides, anthraquinones, anthocyanins, steroids etc. The explants of *Momordica charantia* were cultured on MS medium. Callus was obtained on MS medium supplemented with BAP, IAA, 2, 4-D, kinetin and IBA respectively. Thin layer chromatography was carried to separate phenols and flavonoids from leaf extracts of bitter gourd. It showed different R*f* values with their respective chromatography solvents.

KEYWORDS

Callus, PGR's, TLC, solvent system, Quercitin

INTRODUCTION

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 functions as a hypoglycemic agent by regulating carbohydrate metabolism. Being a tropical crop, it does not find any organized cultivation in temperate areas. Bitter melon plants contains high levels of iron, beta carotene, calcium, potassium, vitamins, phosphorus, and good dietary fiber[5,6].

The plant secondary metabolites are important for the human consumption as food and as medicinal compounds used in the pharmaceutical industry require special attention[7]. If the naturally occurring compounds of medicinal importance are produced in *in vitro* cultures; the problem of provision of raw materials for the extraction of bioactive compounds can be solved at door step. Plant parts containing important compounds are the main source of natural antioxidants and can be cultured on artificial medium as renewable source of required chemical compounds[8]. This study was undertaken to analyze Phenols and flavonoids in *in vitro* cultures of *Momordica charantia*.

***Address for Correspondence:**
Dr. Ujjwala Supe
Plant Tissue Culture Laboratory, St. Thomas College, Bhilai, District -Durg, PIN-49006, Chattisgarh, India.
E-Mail Id: ujsupe@gmail.com

MATERIALS AND METHOD

Callus Induction

Leaf explants were taken from aseptically grown seedling. Many types of media along with various combinations of growth hormones were used to induce calli. The desirable callus cultures were maintained on Murashige and Skoog[9] (MS) medium supplemented with 30 g/l sucrose as a sole source of carbon, various combination of auxins and cytokinins and 1% agar. The initial pH of the medium was adjusted to 5.8 before autoclaving. The cultures were

© Copyright reserved by IJPRS

incubated at 22 ± 2^0C with 16 hours light and 8 hours dark. The callus cultures were subcultured after every 30 days.

Preparation of Leaf and Callus Extract

The leaves were air dried and 10 gm crushed leaves were soaked in methanol and distilled water for 72 hrs before extraction. Then methanol extract was concentrated to dryness in rotary evaporator and then was stored in 4ºC until use. Same method is used for preparation of callus extract.

Preparation of Quercitin Standard

A stock solution of quercetin was prepared by dissolving 10 mg of accurately weighed quercetin in methanol and making up the volume to 10 ml with methanol to get the final concentration of 1 mg/ml.

Phytochemical Analysis

The extract of leaf and callus were screened for phytochemical analysis for phytoconstituents using standard procedure of analysis[10,11,12].

Qualitative Analysis of Phenols and flavonoids

The qualitative analysis of flavonoids and phenols was done by thin layer chromatography technique on silica gel plates. Exactly 10 µl of each of the standard along with samples were applied in triplicate on TLC plates. The plates were developed in a solvent system of toluene: ethyl acetate: formic acid (4.5: 3: 0.2) at 25 ± 2^0C temperature and 40% relative humidity and allowed to travel up to a distance of 8 cm. After development the plates were dried in air and the spots were visualised by exposure of the plates to iodine vapour.

RESULTS AND DISCUSSION

For the growth of *Momordica charantia* callus culture different composition and combination of growth regulators were tested. Kin, BAP, 2, 4-D, IAA and IBA were tested (Table 1). 2, 4-D alone shows proliferation and friable callus which turn into more friable callus after sub culturing, but growth was very slow. 2, 4-D and Kin are known to trigger the stimulation of embryogenic determined cell to undergo cell division in many plant species[13].

Table1: Effect of growth regulator on callus induction of *Momordica charantia*

PGR 's	Concentration	Callus growth
BAP	0.5 mg/ml	+ +
IAA	3mg/ml	+ + +
BAP+IAA	0.5 +3 mg/ml	+ + +
IBA + BAP	1+2 mg/ml	+
BAP +2,4-D	1+ 1.5mg/ml	+ + +
BAP+IAA	2+2 mg/ml	+ + +
BAP + IAA	4+4mg/ml	+ + + +
2,4-D+ kinetin	3+1mg/ml	+ +
IAA+IBA +kinetin	2+2+1mg/ml	+ + + +

The effect of 2, 4-D and Kin on callus initiation and maintenance so observed will be helpful and attempts can be made to assay the concentration of bioactive molecules as this plant is rich in bioactive molecules. Desired secondary metabolites can be manipulated as conditions required from the callus. It can be precisely controlled and cell culture and organ culture can be done. Callus started proliferating from seed explants cultured on MS medium supplemented with 2, 4-D within 1 week of inoculation. Morphology and growth of callus varied with different concentrations of 2, 4-D used[14]. The callus was fast growing soft and greenish yellow, when explant cultured on MS medium supplemented with different concentrations of IAA, IBA and kinetin (Figure1 A-B).

At different concentrations of BAP and kin green, compact and hard calluses were produced. It was reported by[15] that combination of BAP and 2,4-D are most suitable for callus induction. The preliminary analysis was done by some standard chemical test. This revealed the presence of anthraquinone, flavonoids and phenols tannins and steroids in leaf and callus

© Copyright reserved by IJPRS

cultures. The result of the Phytochemical screening of *Momordica charantia* was presented in Table 2.

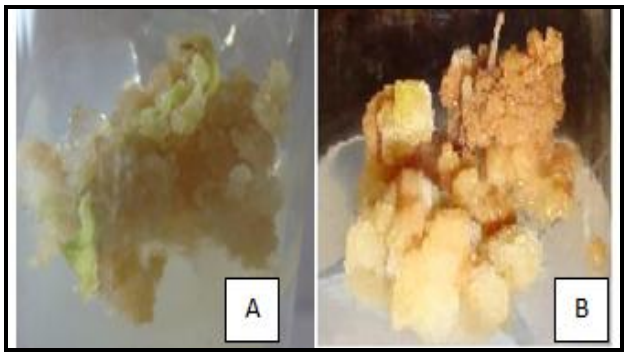

Figure 1: A-B Induction of callus in IAA, IBA and Kin (A) BAP with IAA (B)

Table 2: Components of leaf and callus of Bitter guord *(Momordica charantia)* based on the preliminary phytochemical screening

Secondary Metabolites	Leaf		Callus	
	Aqu. extract	Methanol extract	Aqu. extract	Methanol extract
Alkaloids				
Hager's Reagent	+	-	+	-
Mayer's reagent	+	+	+	+
Anthraquinones	+	+	+	+
Flavonoids	+	+	+	+
Phenolics	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+

The aqueous extract of plant was found to contain Alkaloids, anthraquinone flavonoids, phenols, sterols and tannins. The methanol extract of plant was showed the presence of alkaloids, anthraquinone flavonoids, phenols, sterols. Tannins was absent in methanolic extract. The assorted phytochemicals are common compounds to give pharmacological benefit. However, there were certain compounds present in this plant are likely to be different from the other plants. Therefore, they can be recommended to be used as therapeutic agent to certain illnesses Still, there are primary factors influencing the variability of phytochemicals in plants comprising genotype, size and maturity, soil conditions, fertilization, irrigation, pesticide utilization, disease and pests, location and climate, and season[16]. Thus, these factors can be applied to improve and enhance phytochemical content in plants.

However there are possibilities for the presence of other compounds in Bitterguord. Sugar and carbohydrates may as well presence in Bitterguord due to the sweet odour released during concentrating the filtrate. However a tests need to be carrying out to prove the presence of sugar and carbohydrates. Plants are conceived as sources of antioxidants due to presence of polyphenols and flavonoids which possess wide biological properties[17]. Recent studies showed that many flavonoids & related polyphenols contribute significantly to the total antioxidant activity of many plants[18]. The qualitative analysis of flavonoid was done by TLC. The leaf extract and callus extract sample showed pink colour spot indicating the presence of Phenols and flavonoids in the leaf and callus extract sample (Figure C-D). No marked variability was observed in flavonoids and phenolics production The Rf value was found to be 1 for leaf sample and Rf value for callus extract was found to be 0.5.

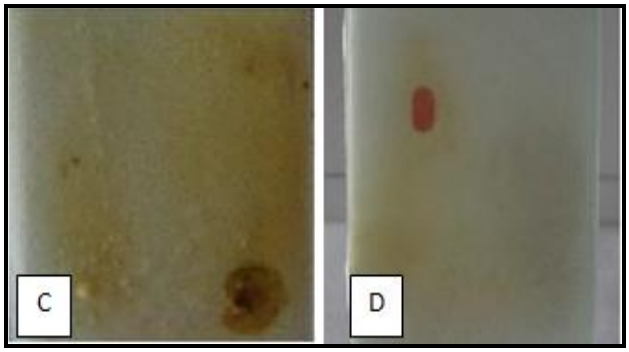

Figure 1: C-D TLC of flavonoids and phenols of leaf and callus with standard

© Copyright reserved by IJPRS

CONCLUSION

Through this work an attempt was made to do *in vitro* callugenesis of *Momordica charantia* with the different plant growth regulators which can produce genetic variability and plant can be grown in short breeding period. It enables to production of secondary metabolites in large quantity and biochemical assays. The TLC methods were found to be simple, precise, specific, sensitive and accurate and can be used for the quantification in plant materials.

ANKNOWLEDGEMENT

The authors are grateful to the Principal, St. Thomas College, and Bhilai) for the provision of research tools. Authors are thankful Dr. M. G. Roymon, Professor, and Head. Department of Microbiology and Biotechnology for suggesting valuable suggestion and their moral support to smooth conducting the work.

REFERENCES

1. Cefalu, W.T., Ye. J., & Wang. Z.Q. (2008) Efficiency of dietary supplementation with botanicals on carbohydrate metabolism in humans. Endocrine. *Metabolic & Immune disorders-Drug Targets*, *8*, 78-81.
2. Leung, L., Birtwhistle, R., Kotecha, J., Hannah, S., & Cuthbertson, S. (2009), Anti-diabetic and hypoglycaemic effects of *Momordica charantia* (bitter melon): A mini review, *British Journal of Nutrition*, (in press).
3. Modak, M., Dixit, P., Londhe, J., & Ghaskadbi, S, (2007). Indian herbs and herbal druga used for the treatment of diabetes. *J. Clin. Biochemistry Nutri*, *40*, 163- 173.
4. Nahas, R, Moher. (2009). Complementary and alternative medicine for the treatment of type 2 diabetes. *Can Fam Physician*, *55*, 591-596.
5. Paul, A., & Raychaudhuri, S. S. (2010). Medicinal uses and molecular identification of two *Momordica charantia* varieties – a review. *Electronic Journal of Biology*, *6(2)*, 43-51.
6. Sultana, R. S., & Bari, Miah, M. A. (2003). In *vitro* propagation of karela (*Momordica charantia* L). *J Biol Sci*, *3*, 1134–1139.
7. Hill, A. F. (1952). Economic botany. A text book of useful plants and plant products. 2nd edn. Mcgraw-Hill book company Inc, NY.
8. Agarwal, M. & Kamal, R. (2004). *In vitro* clonal propagation of *Momordica charantia* L. *Indian J.Biotechnol*, *3*, 426-430.
9. Murashige, T. & Skoog, F. A. (1962). revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiology plantarum*, 15, 473-479.
10. Odebiyi, A., & Sofovora, E. A. (1978). Phytochemical screening of Nigeria medicinal plants, Part 2 *Lisyadia*, *403*, 234-246.
11. Banso, A., & Ngbede, J. E. (2006). Phytochemical screening and *in vitro* antifungal properties of *Fagara zanthozyloides*. *J Food Agric Env*, *4*, 8-9.
12. Williamson, E. M., Okpako, D. G, & Evan, F. J. (1996). Pharmacological methods in phytotherapy research. Vol.1 Selection, preparation and pharmacological evaluation of plant material. *Wiley*, Chichester, England, 9-13.
13. Supe, U. (2013) *In vitro* antibacterial activity and calluogenesis of *bryonia laciniosa*. *International Journal of Pharmaceutical Sciences and Research*, *4(4)*, 1556-1560.
14. Kim, S. G, Chang, J. R, Cha, H. C, & Lee, K. W. (1988) Callus growth and plant regeneration from cotyledons in diverse cultures of Cucumber (*Cucumis sativus* L.), *Plant Cell, Tissue Org. Cult*, *12*, 67-74.
15. Cheng, T. Y., & Voqui, T. H., (1977). Regeneration of Douglas fir plantlets through tissue culture, *Science*, *98*, 306.
16. Xin, Z., Edward, E. C., Weiqun, W. & Rajeshekar, C. B. (2006). Does organic production enhance phytochemical content

© Copyright reserved by IJPRS

of fruit and vegetables? Current knowledge and prospects for research. *Hort. Technology*, *16(3)*, 449-456.

17. Durgas, Jr A. J., Castaneda A. J., Bonin, G. C., Prince, K. L., Fischer, N. H. & Winston G. W. (2006). Evaluation of total peroxyl radical scavenging capacity of flavonoids, structure activity relationships, *J. Nat. Prod*, *63(3)*, 327 – 331.
18. Luo, X. D., Basile M. J., & Kennelly, E .J. (2002). Polyphenolic antioxidants from fruits of chrysophylolum cainito L. (Star apple), *J Agric Food Chem*, *50*, 1379-1382.

© Copyright reserved by IJPRS