ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative Phytochemical Analysis of *Ex-Situ* and *In-Vitro* Grown Plants of *Murraya koenigii.*

R Katiyar, N Baisla, K Thakur, N Salaria, U Goutam, and S Kukreja*.

School of Biotechnology and Biosciences, Department of Biotechnology, Lovely Professional University, Phagwara, India-144402.

ABSTRACT

The aim of the present study was to perform and compare the phytochemical analysis taking methanolic, ethanolic and acetone extracts of leaves of *in vitro* and *ex-situ* grown plants of *Murraya koeinigii*. Different extracts showed the presence of alkaloids, flavonoids, tannins, terpenoids, cardiac glycosides, quinones and coumerin which are responsible for providing plant the medicinal importance. Further, conditions were also optimized for the *in vitro* culture of plants by using nodal segments as the explants. Results revealed that best shoot proliferation was obtained in MS media supplemented with BAP (4 mgl^{-1}) and NAA (0.4 mgl^{-1}). Further, it was observed that *in vitro* grown plants retained the potential to produce bioactive compounds. The importance of theses phytochemicals may be further assessed by evaluating the antimicrobial potential against human pathogenic microorganisms and this information will be helpful for their further utilization in pharmaceutical industries.

Keywords: Phytochemicals, in *vitro*, explants, *Murraya koenigii*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

World plant biodiversity is the largest source of herbal medicine and still about 60-80% world population rely on plant based medicines which are being used since the ages as traditional health care systems. It is now clear that, the medicinal values of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. Though the traditional Indian system of medicine has a long history of use, they lack adequate scientific documentation, particularly in light modern scientific knowledge [1].

These natural compounds formed the base of modern drugs as we use today. Phytochemicals may protect human from various diseases. They are non-nutritive plant chemicals that have protective or disease preventive properties. These phytochemicals may be either primary or secondary metabolites [2]. Out of these, it is the secondary metabolites which offer potential health benefits. The important bioactive compounds responsible for attributing the medicinal value to a plant are alkaloids, flavanoids, tannins and phenolic compounds [3]. Tannins, essential oils and aromatic compounds present in leaves of plants have antimicrobial principles [4, 5]. Moreover, plant tannins and flavanoids have been reported to exhibit antibacterial properties [6-8]. In addition, phenolic compounds protect the plant from microbial infection and deterioration [9]. Risk of cancer can be significantly reduced due to antioxidant and anti-inflammatory effects shown by polyphenols. Phytochemicals have also been reported to prevent colorectal and other cancers [10-12]. The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases. Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments.

Murraya koenigii commonly known as curry leaf belongs to the family Rutaceae. It is commonly found everywhere in India [13]. Its common occurrence is observed in foothills of Himalaya, Assam, Sikkim, Kerala, Tamil Naidu, Andhra Pradesh and Maharashtra [13]. Leaves of *Murraya koenigii* have been reported to contain alkaloids, volatile oil, Gycozoline, xanthotoxine and sesquiterpione [14]. Further reports suggest leaves to show antioxidant, hypoglycemic and antibacterial activity [15, 16].

The conventional method of propagation of *Murraya koenigii* plant is limited to seeds, which germinate under partial shade conditions. The seeds retain their viability only for a short period and whenever the seeds germinate, the young seedlings lead to death under natural conditions. A very sparse work regarding the micropropagation of this important aromatic and medicinal plant has been done by using immature seeds, intact seedlings and nodal segments [17, 18]. This work was done with the objective to standardize the protocol for the *in vitro* culture of this plant and to compare the phytochemicals present in the *in vitro* and *ex-situ* grown plants of *Murraya koenigii* so that the *in vitro* plants can be used as factories for the production of important phytochemicals.

MATERIALS AND METHODS

In vitro culture of plants

Fresh leaves and shoots of *Murraya koenigii* were collected from the botanical gardens of Lovely Professional University, Punjab. Actively growing shoot tips with one or two nodes and leaf segments were taken as an explant. The explants were surface sterilized with 0.05% bavistin followed by Tween-20, 70% ethanol and 0.1% $HgCl_2$ and were inoculated on Murashige and Skoog basal media with various concentrations and combinations of growth regulators (BAP, NAA, IBA, Kin and 2,4-D).

Plant extracts preparation and phytochemical analysis

Murraya koenigii leaves were shade dried for a period of 10 days. Dried leaves were blended and made into fine course powder. 20 gm of powdered leaf material was taken and 200 ml of ethanol, 200 ml of methanol and 200 ml of acetone were used as solvent separately. Extraction was run in Soxhlet apparatus continuously for 2 hours and extracts were collected [19]. These extracts were subjected to various biochemical tests to check the presence of different phytochemicals [19][20]. The same protocol was followed to determine the types of phytochemicals present in leaves of *in vitro* grown *Murraya koenigii* plants.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Various combinations of IAA, NAA, 2,4-D and BAP, Kin were used to establish *in vitro* culture of *Murraya koenigii*. Experiment was initiated by taking different explants but results revealed that out of them nodal explants proved to be the best in terms of shoot proliferation. Out of various growth regulators used, maximum shoot proliferation was observed by 4 mgl^{-1} BAP and 0.4 mg l^{-1}NAA. Further, comparative phytochemical analysis of leaves extracts of *in vitro* and *ex situ* grown plants of *Murraya koenigii* was performed to detect the presence of important bioactive compounds viz., alkaloids, flavonoids, cardiac glycosides, steroids, tannins, saponins, quinones, coumerin, etc. Methanolic extract of leaves collected from *ex situ* plants of *Murraya koenigii* showed presence of alkaloids, terpenoids, flavonoids, cardiac glycosides, tannins, quinones and coumerin, however, ethanolic extracts showed the presence of most of the phytochemicals present in methanolic extract except alkaloids and in addition showed the presence of steroids. Further, acetone extracts showed the presence of flavonoids, terpenoids, tannins, coumerin and alkaloids only (Table-1). *Ex situ* and *in vitro* comparison were studied with respect to methanolic and ethanolic extracts only. The *ex situ* and *in vitro* studied plants showed similar results with respect to methanolic extracts, however, ethanolic extracts of *in vitro* grown leaves showed the absence of steroids (Table-2). This may be because of the epigenetic effect brought about by the culture conditions. The presence of these phytochemicals in *in vitro* grown plants can be further exploited to use this system to micropropagate *Murraya koenigii* and to further assess the antimicrobial potential of the extracts of different plant parts so that it may be utilized in pharmaceutical industries.

Table 1: Comparison of phytochemicals present in methanolic, ethanolic and acetone extracts of leaves of *Murraya koenigii* obtained from *ex-situ* grown plants

S. No.	Name of phytochemical	Methanol	Ethanol	Acetone
1	Flavonoids	+	+	+
2	Terpenoids	+	+	+
3	Cardiac glycosides	+	+	-
4	Steroids	-	+	-
5	Tannins	+	+	+
6	Saponins	-	-	-
7	Phlobatinins	-	-	-
8	Gum	-	-	-
9	Amino acids	-	-	-
10	Quinones	+	+	-
11	Coumerin	+	+	+
12	Alkaloids	+	-	+

Table 2: Comparison of phytochemicals present in methanolic and ethanolic extract of leaves of *Murraya koenigii* obtained from *in vitro* grown plants

S.No.	Name of phytochemical	*Methanolic extract*	*Ethanolic extract*
1	Flavonoids	+	+
2	Terpenoids	+	+
3	Cardiac glycosides	+	+
4	Steroids	-	-
5	Tannins	+	+
6	Saponins	-	-
7	Phlobatinins	-	-
8	Gum	-	-
9	Amino acids	-	-
10	Quinones	+	-
11	Coumerin	+	+
12	Alkaloids	+	+

CONCLUSION

Plants can be used as factories for the production of bioactive compounds which contributes to pharmacological importance of these plants. *In vitro* grown plants were studied for their ability to produce these phytochemicals and were compared with those present in *ex-situ* plants. Results revealed that tissue culture raised plants retains the ability to produce the phytochemicals viz., alkaloids, tannins, flavonoids etc. This system can thus be exploited for micropropagation of large number of plants of the same type so as to optimize the production of bioactive compounds. The importance of theses phytochemicals may be further assessed by evaluating the antimicrobial potential against human pathogenic microorganisms and this information will be helpful for their further utilization in pharmaceutical industries.

REFERENCES

[1] Shrivastava S, Leelavati S. International Journal of Pharmaceutical Science Research and Review 2010; 3: 114-118.
[2] Kumar A, Ilavarsan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N, Krishanan MRV. Pakistan J Nutr 2009; 8: 83-85.
[3] Edego HO, Okwu DE, Mbaebie BO. African J Biotechnol 2005; 4: 685-688.
[4] Kumar R, Singh, M. J Agr Food Chem 1984; 32: 447-453.
[5] Kumar R, Singh, NP. Indian J Animal Sci 1984; 54; 881- 884.
[6] Haslam E. Plant polyphenols. In: Haslam, E. (eds). Vegetable tannins, Cambridge, England: Cambridge university press, 15-89.
[7] Scarlbert A. Phytochem 1981; 30: 3875-3883.
[8] Chung KT, Wong TY, Wei Y, Hung YW, Lin Y. Crit Rev Food Sci Nutr 1998; 8: 421-464.
[9] Cowan MM. Clin Microbiol Rev 1999; 12: 564-582.
[10] Michaud DS, Feskanich D, Rimm EB. American J Clin Nutr 2000; 72: 990-997.
[11] Greenberg ER, Baron JA, Tosteson TD. New England J Med 1994; 331: 141-147.
[12] Birt DF, Handrich S, Wang WQ. Pharmocol Ther 2001; 90: 157-171.
[13] Bhattacharjee SK. Handbook of medicinal plant. 3rd pointer publishers, jaipur, 2001, pp. 230.
[14] Pande MS, Gupta SPBN, Pathak A. Journal of Herbal Medicine and Toxicology 2009; 3(1).
[15] Zachariah SM, Muthumani P, Ramasehu K. Int J Pharmacol 2009; 6 (2): 18.
[16] Adebajo AC, Ayoola OF, Iwalewa EO, Akindahunsi AA, Omisore NO, Adewunmi C O, Adenowo TK. Phytomed 2006; 13: 246.
[17] Babu NK, Anu A, Remashree AB, Praveen K. Plant Cell Tissue Organ Culture 2000; 61: 199-203.
[18] Rani U, Sharma MM, Ismail N, Batra A. Indian J Biotechnol 2012; 11: 108-110.
[19] Salna KP, Sreejith K, Uthiralingam M, Mithu AP, John MMC, Albin TF. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3: 291-292.
[20] Santhi R, Lakshmi G, Priyadharshini AM, Anandaraj L. International Research Journal of Pharmacy 2011; 2: 131-135.