

COMPREHENSIVE REVIEW ON *IN VITRO* CULTURE AND ANTI CANCEROUS ACTIVITY OF *MOMORDICA DIOICA*

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ABSTRACT

Momordica dioica is a perpetual, dioecious, cucurbitaceous climbing creeper, generally recognized as kakrol, spiny gourd, and teasle gourd. The plants required artificial transfer of pollen from male to female flower. There is no natural pollinator, hand pollination is necessary. It is not only used as a protective and therapeutic agent for many diseases but also as a nutritious vegetable over thousands of years. The perpetual dioecious plant, *Momordica dioica* was having an abroad range of biological activities. In the present review, there is a compilation of the previous research work about medicinal and anticancer activities of the plant. Current review suggests that the extracts have shown a cytotoxic ability on various human-derived hematological and compact tumor cell lines. Due to problems associated with natural propagation of this species, a thorough review on the tissue culture aspect of this plant has been presented here.

Keywords: *Momordica dioica*, Tissue culture, anticancerous activity

INTRODUCTION

Momordica is a climber from *Cucurbitaceae* and is a native of Indian subcontinent, distributed throughout India. It is not only used as a self-protective and healing agent for various illnesses. *Momordica* has about 80 species (Bharathi *et al* 2011). According to the newest revision of Indian *Momordica*, there are six well-recognized types of which four are dioecious and two are monoecious (Joseph *et al* 2005). In India, we can see its various species such as *M. balsamina*, *M. charantia*, *M. cymbalaria* and *M. dioica*. Among them *M. dioica* is a perennial, dioecious climber and is commonly known as Kokrol/Kakoda (teasle gourd or spine gourd). It yields a nutritionally valuable vegetable. Its fruits are rich source of proteins (Maharana and Tripathy 1996). This plant has the phytochemical, pharmacological, phytotherapeutical, and ethnobotanical properties of kantola according to the view of traditional medicinal plant-based treatment including Ayurveda along with current scientific descriptions (Bawara *et al.*, 2010). Although this genus is created from the Indo-Malayan area, it is now starting to grow in India, Bangladesh, China, Japan, South East Asia, Polynesia, Srilanka, Myanmar, Tropical Africa, and South America. Its agricultural up to a height of 1500 meters in Assam and Garo hills of Meghalaya is described (Ram *et al.*, 2002). It is generally known as spine gourd, small bitter gourd, or teasel gourd worldwide since in Bangladesh it is known as kakrol and in India also as kankro, kantroli, kantola, kartoli ban karola, janglee karela. Plant of Kakrol is about 5–7 meters in length, a popular summer vegetable of which its fruit, young branches, and leaves are used as a vegetable (Singh *et al.*, 2008).

Agronomic Problems with *M. dioica*

Improvement of *Momordica dioica* has not been done adequately, due to its dioecious nature and its vegetative mode of propagation. Till today its propagation entirely depends on underground tuberous roots, which occupy and engage the farm land for a long period i.e. next year.

Maintaining tuber quality in farm condition as well as to conserve it in storage is a tough task. Micropropagation may help overcome these problems to a great extent. An attempt of *in vitro* propagation of teasle gourd was demonstrated by Hoque *et al.*, (1995).

The commercial development of farming of this vegetable crop has not been attempted, because of its dioecious wildlife and vegetative manner of propagation (Baratakke *et al.*, 2013). Generally, in cucurbits,

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the seed location and seed propagation are low, possibly impermeability to water and gases and making them latent for many days. In nature, these male, female plants are shown collected at a ratio of 1:15. Due to the above-declared reason, the natural population of the plant is regularly decreasing and hence conservation of this plant is compulsory (Ghosh *et al.*, 2005). Under such conditions, plant biotechnology plays an alternative tool for large-scale duplication, propagation, and conservation. The examination of the literature on the tissue culture aspect specified that few reports are available for conservation and propagation of *Momordica dioica*. The female plant is selected for *in vitro* regeneration.

Medicinal Potential of *M. dioica*

Momordica dioica is an important medicinal plant invented in the Indo-Malayan region. Fruits are used as a vegetable in India, Bangladesh, and other neighboring countries, as it is a rich base of vitamin C, carotenoids, and proteins. Preference for this vegetable is more uniform with the bitterness of this fruit. Traditionally it is being used for treating eye illnesses, poisoning, and fever. Fruits, leaves, and tuberous roots are used as a popular remedy for diabetes (Vaidya *et al.*, 2003). The plant was observed to have antidiabetic, Analgesic, postcoital anti-fertility, nematocidal, anti-allergic, anti-malarial, antifeedant, and anti-bacterial, anti-oxidants and hepatoprotective, jaundice, and bleeding pile possessions (Narayan *et al.*, 2014). *Momordica dioica* plants extract shows anticancer activity (Kumar *et al.*, 2017). Cancer normally arises in the breast, prostate, lung, liver, colorectal, cervix, uterus, and stomach. Factors such as age, lifestyle and food habits, ingesting of alcohol, tobacco, and lack of physical activities helped cancer development in human beings. In India, lung and ovarian cancers are leading diseases because of tobacco use in both sexes (Bhavana *et al.*, 2015).

According to Thiruvengadam *et al.*, (2007) *Momordica* species have been used in indigenous medical organizations among various countries in Asia and Africa. Based on the indigenous information, wild plant foods show an energetic role in the complex cultural system of tribal people for reducing many illnesses. Research has exposed that many wild plants are rich in specific elements, mentioned as phytochemicals, which may have health encouraging effects.

Cancer comprises of a group of diseases characterized by uninhibited development and the spread of anomalous cells and is among the important reasons of death in humans worldwide. Natural products from fruits and vegetables have been used to treat cancer problems. Certain agents such as taxanes, irinotecan, topotecan, vincristine, vinblastine, etc derived from many plants have been verified to have anticancer activity. Methanolic extracts from various plants were proven to have anti-cancer effects (Kumar *et al.*, 2017).



Figure 1: Fruits and plant of *Momordica dioica*

Momordica dioica contains lots of medicinal properties, every part of *Momordica* plant have different medicinal properties like fruit, leaves, root. The fruit is diuretic, alexiteric, stomachic, cathartic, hepatoprotective, and consummative no property (Ali and Srivastava *et al.*, 1998). It is also used to treat

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asthma, leprosy, prevent the inflammation caused by a lizard, excessive salivation, snake bite, elephantiasis, fever, digestive disorders, mental disorders, and troubles of the heart. Fresh fruit juice is set for hypertension. The fruit is cooked in a small amount of oil and used for treating diabetes. Tender fruits are cleansed over the skin for pimples and acne (Reddy *et al.*, 2005). Leaves of the plant used as an anti-helminthic and aphrodisiac medicine. It is also used as medication in *tridosha*, fever; and alters pitta, jaundice, asthma, bronchitis, piles, hepatic damages, mental digestive illnesses, bleeding piles bowel affection, and urinary complaints. Juice of *Momordica dioica* leaves mixed with coconut, pepper, red sandalwood, etc, to form a liniment and practical in the head to release pain in the head. Leaf paste is applied externally to the skin and orally two or three times daily for skin disease (Nadkarni *et al.*, 1976). Roots of the *Momordica dioica* is full of medicinal values. Juice of root is stimulating, astringent, antiseptic. The mucilaginous tubers are antihelminthic, spermicidal, antifertility abortificant, used in case of bleeding piles, similar bowel diseases and urinary complaints. Powder of root is useful to skin to make it soft and to reduce (Satyavati *et al.*, 1987).

Need for Tissue Culture

As the conservative methods of *Momordica dioica* proliferation execute several limits for the large-scale proliferation of sex-specific plants, a well-organized clonal propagation method is a must. An *in-vitro* propagation system offers limitless availability of planting material early in the planting season. The application of micropropagation is entrenched for fast large-scale propagation of many harvests and cucurbitaceous vegetables with *Momordica* species (Rai *et al.*, 2012). These have also been completed for *in-vitro* propagation of *M. dioica*, wherein shoots were renewed from callus cultures obtained from various explants, but not from the nodal segments. Callus cultures, however, transfers the risk of somaclonal variations which can tremendously limit the broader usefulness of micropropagation organizations. On the other hand, the axillary bud proliferation method ensures clonal regularity among the regenerants and, therefore, can subvert the limits rising from callus cultures and organogenesis (Rai *et al.*, 2012).

Nabi *et al.*, (2002) performed tissue culture study on *M. dioica*. Of the four types of explants of teasel gourd (*Momordica dioica* Roxb.) viz. node, shoot tip, leaf and the cotyledon, best performance was given by the cotyledon explants. 1.0 mg/l BAP and 0.1 mg/l NAA was found most suitable in callus induction followed by 0.2 mg/l NAA. The highest number of multiple and tallest shoots were obtained on MS medium along with 1.0 mg/l BAP and 0.1 mg/l NAA. For rooting, half strength MS supplemented with IBA proved better than IAA. On half strength MS medium supplemented with IAA tallest shoots were seen.

Shekhawat *et al.*, (2011) proposed an *in vitro* propagation protocol for female plants of *Momordica dioica* (Roxb.). The nodal segments were. Bud breaking occurred on MS agar-gelled medium + 2.0 mg L-1 6-Benzylaminopurine (BAP) + 0.1 mg L-1 Indole-3 acetic acid (IAA). The cultures were further amplified by passages on MS medium supplemented with 1.0 mg L-1 BAP + 0.1 mg L-1 IAA. Shoot amplification (29.2 shoots per vessel) was achieved by subculturing of *in vitro* regenerated shoot clump on MS medium + 0.5 mg L-1 BAP + 0.1 mg L-1 IAA. These shoots were then transferred for root formation on half-strength MS medium + 2.0mg IBA with 89% success rate. These *in vitro* regenerated shoots were then rooted *ex vitro* with 34% success. These plantlets were hardened in the greenhouse and transferred to the field. The established protocol is suitable for true to type cloning of mature female plant of *M. dioica*.

Patel and Ishnava (2015) developed a protocol for *in vitro* shoot multiplication and regenerations of spine gourd. The nodal segments were taken and cut end of the explants then surface sterilized and cultured. MS agar-gelled medium with optimum concentration of BAP+NAA (1.5+0.1 mg/l) and NAA+NB6 (0.5+0.5mg/l) had an effect on callus production. Shoot multiplication was seen best in NB6 + BAP (0.5+0.5mg/l). After 15 days, shoot length of 5.2 ± 0.37 cm and shoot numbers 10 ± 1.4 were seen.

Raju *et al.*, (2015) proposed a possible media for large scale production of spine gourd and result of the study revealed that highest percentage (85%) of embryogenic callus was obtained from MS medium supplemented with 2.0 mg/L each of 2, 4-D and BAP in leaf explants of spine gourd. Maximum shoots

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(12.15 ± 1.51 shoots) were seen on MS medium augmented with BAP (4.0 mg/L) in combination with L-glutamine (2.0 mg/L) from leaf derived embryogenic callus of spine gourd.

Mustafa *et al.*, (2013) cultured the nodal explants on MS medium supplemented with 2.0 mg/l 6-Benzylaminopurine BAP +2.0 mg/l L-Glutamic acid, the explants produced little amount of callus and shoot buds. The shoot buds on successive subcultures on the same medium produced multiple shoots. Shoot proliferation was further seen even after six months.

Role of *Momordica dioica* in Cancer Therapy

Maharudra *et al.*, (2012) reported, that the perennial dioecious plant, *Momordica dioica* was having a broad range of biological activities. In the investigation, they studied the antioxidant, antimicrobial and anticancer activities of the both male and female plant extracts by *in vitro* studies. The extracts have shown a cytotoxic ability on various human-derived hematological and solid tumor cell lines. The overall result provided evidence for the antioxidant, antimicrobial and anticancer activities of studied plant extracts. Kumar *et al* (2017), investigated the in-vitro anticancer activity of the methanolic seed extract of *Momordica dioica* using MTT assay on two human carcinoma cell lines i.e., MCF-7 breast and A549 lung on cancer cell lines, tracked by DNA fragmentation of MCF-7 cell line which presented a high growth inhibition on the addition of the seed extract.

According to Kumar vijay *et al.*, (2017), life saving drugs like aspirin, morphine, dioxin, and ephedrine were derived from medicinal plants and introduced into modern therapeutics numerous centuries ago. *Momordica dioica* plant shows anticancer properties. Bawara *et al.*, (2010) described that *Momordica dioica* roots and five isolated constituents presented anticancer activity in pharmacological testing on cancer cell line (L1210). kumar *et al.*, (2017) stated that Human cancer cell lines used in this study [Hep-G2 (liver cancer) and MCF-7 (breast cancer)] were grown in nominal important medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L₂ glutamine, and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% co₂ incubator. Luo *et al.*, (1998) showed that the CHCL₃ extract of root and five isolated elements have anticancer activity during pharmacological testing on cancer cell lines (L1210).

Ahirrao (2019) evaluated the anticancer activity of fruits of *Momordica dioica* using MTT assay. The aqueous extract of fruits of *Momordica dioica* was tested against ovarian carcinoma of PA-I cell lines and human cervical cancer of Hela cell line. The results showed that aqueous extract of fruits of *Momordica dioica* inhibited growth of PA-I and Hela cells. Alterations in the cell morphology were also observed after treatment of the cell lines with fruit extract. This work revealed that *Momordica dioica* contains some important chemical constituents extracted using aqueous as solvent that can be used further in the management of cancer treatment.

Madesh *et al.*, (2020) studied the anticancer efficacy of *Cucurbitacins* obtained from *Momordica dioica* Roxb.. Based on previous *in-vitro* studies performed, *in-vivo* studies were done on mice model. They inoculated Ehrlich ascites carcinoma (EAC) cells into swiss albino mice intraperitoneally to form a liquid tumor and then they treated the tumor by oral administration of 50, 100, 200mg/kg. Treatment with *Cucurbitacins* enriched fraction showed anti-tumor effects against liquid tumor as indicated by a significant ($P < 0.05$) reduction in body weight. It is observed that the enriched bio fraction restored the altered hematological parameters of tumor-bearing animals and significantly increased their life span. This work indicates the cytotoxic potential effects of MDRon tumor cells opening new opportunities for further studies on the anti-cancer effects of this agent.

Patil *et al.*, (2018) utilized the *Allium cepa* root tip meristem model to evaluate antimitotic activities of fruits of *Momordica dioica*. Antimitotic screening was done using *Allium cepa* root tip assay. The herbal powder obtained from plant part-dry fruits of *Momordica dioica* were extracted; the aqueous extract of fruits of *Momordica dioica* (10 mg/ml) and methotrexate showed significant concentration dependant inhibitory influence against the dividing cells of *Allium* roots and decreased root growth and mitotic index as compared to control distilled water.

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Revathy *et al.*, (2015) studied antitumor effect of various extracts of MDR fruits on DLA induced tumour models in mice. The crude chloroform and methanolic extract were prepared by soxhlet extraction. The extracts were evaluated for their in vitro antioxidant and in vitro antitumor activity to select the promising ones. Chloroform extract has shown maximum activity on in vitro anti cancer activity among all three extracts. The reason may be due to chloroform soluble phytochemical may possess good antitumor activity.

Gayathri *et al.*, (2016) studied the anticancer effects of the crude methanolic seed coat extract of *Momordica dioica* on human breast (MCF-7) and lung cancer (A549) cell line. They observed that the methanolic seed coat extract of *Momordica dioica* exhibited 50% growth inhibition in MCF-7 cancer cell line at 50 µg/mL and A549 cancer cell line at 70 µg/mL. The crude methanolic seed coat extract of *M. dioica* could be a potential source of natural anti-cancer agents.

In a recent study done by Naik and Devi (2020) the authors fabricated gold nanoparticles, AuNPs using aqueous root extract of *Momordica dioica* (*M. dioica*) and explored their anticancer application with mechanistic approaches. These AuNPs exhibited anticancer activity against different cancer cell lines like, human breast cancer cells, human cervical cancer cells (HeLa) and human lung cancer cells. Further, it was also noted that the pro-apoptotic genes such as Bcl₂ were down-regulated and BAX, Caspase-3, -8, and -9 were up-regulated in HeLa cells as compared to untreated cells. AuNPs induced apoptosis by accumulation of intracellular reactive oxygen species. This may open up new avenues in therapeutic applications.

Zuqiang *et al.*, (1999) isolated five anti-cancer compounds from the dried root of *M. dioica* Koxb. ex Willd. They were identified as bryonolic acid, 23, 24-dihydrocucurbitacin-F-25-acetate, alpha-spinasterol-3-O-beta-D-glucopyranoside; gypsogenin and hederagenin by spectroscopic (MS, IR, ¹HNMR, ¹³CNMR and DEPT) data. All of them were found to possess anti-cancer activities against L₁₂₁₀, CCER-CEM, Ls174T cell lines.

CONCLUSION

Momordica dioica is a medicinal plant and is also used as a vegetable; Due to rare growth habits it has high economical values and demand in the market. *In vitro* micropropagation and regeneration of the plant will be possible through plant tissue culture studies. The cell cultures have made great development in building advances in plant science. The increased level of natural products for medicinal purposes can be obtained via large-scale plant tissue culture technology. *In vitro* plant cell cultures can be used for commercial production of any secondary metabolites. Plant tissue culture technology works for biodiversity conservation and the economic development of any rare plant species. This comprehensive review analyses that the *in-vitro* culture and anti-cancerous activity of *Momordica dioica* had very less inputs. Thus, this is the thrust area to explore, the micropropagation of *Momordica dioica* with detailed experimental workout for its potential as an anti-cancerous agent.

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