Improvement of secondary metabolites for *Cucurbita moschata* through tissue culture techniques: An overview

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Abstract: *Cucurbita moschata* is a plant which belongs to Cucurbitaceae family and it is widely used in traditional medicine. As there is lack of information available on the tissue culture and secondary metabolites of *Cucurbita moschata*, plant tissue culture appears to be a viable option for the improvement of the Cucurbita species by means of plant tissue. The establishment of an efficient *in vitro* plant regeneration system suitable for genetic transformation is the key step in this approach. Plant growth regulators hold great potential for their effect and impact on callus development and regeneration in *Cucurbita moschata*. Callus formation and regeneration in plants is not only effected by PGRs but also dependent upon the moisture content, light intensity, temperature and type of explant. Present study highlighted the medicinal importance of *Cucurbita moschata* secondary metabolites production. The tissues having higher rates of cell division produce growth regulatory substances including cytokinins, auxins and many other secondary metabolites. Secondary metabolites regeneration and effectiveness in tissue culture is generally a quantifiable trait that repeatedly varies between plant species and within a plant species among, varieties, subspecies cultivars and ecotypes. So tissue culture regeneration of secondary metabolites can become variable particularly when many metabolic actions have to be established within the similar species and different types of secondary metabolites identified by the plant callus.
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Introduction

Cucurbita moschata, contains numerous cultivars of pumpkin and winter squash. It belongs to Cucurbitaceae family and is cultivated in warm areas of all over the world. It is grown not only as a food crop but also for animal food. Butternut, cushaw crookneck and winter, are most popular cultivars of this family. *Cucurbita moschata*was initiated in Central America and Mexico, and is extensively cultivated in US (v). The plants are frost-intolerant annuals with bald stem and climbing nature growing up to 3 meters. The fruit of *Cucurbita moschata*is big with extensive range of shapes and seed are 16–20 mm long. The name *Cucurbita* derives from the latin word which means gourd or pumpkin, and moschata means “musk-scented”, derived from Italian word and literary it means plants bearing squash having elongated recurved necks and hard rinds.

Plants have been used in folk medicine widely and maximum number of traditional medications were derived as of nearby growing wild plants which are locally available. The usages of the plants was hoarded by trial and error and approved to generations verbally. Global markets are rotating to plants as a basis of elements in healthy foods. *C. moschata* has wide range of pharmaceutical uses meanwhile centuries. Seeds are heated and eaten to kill caterpillars and other parasites and used as a diuretic, a research from the flowers used to treat smallpox and measles and *C. moschata* seeds are from time to time used as a natural worming agent for goats and sheep by farmers.

The pharmacological and chemical properties of *C. moschata* extracts from its stalks, fruits and seeds have been examined. These researches confirmed that *C. moschata* has wide-ranging bioactivities, such as anti-diabetes, hepatoprotection, anti-cancer, and anti-obesity properties (Magdeleine *et al*., 2011). It is used as a medicinal plant for bladder problems and prostate, and as an anthelmintic, anti-emetic and galactogogue. It have wonderful medicinal properties such as anti-HIV, anti-diarrhoeal, anti-diabetic, antibacterial, laxative, carminative, anxiolytic, anti-pyretic, antioxidant, anthelmintic, anti-tuberculosis, and purgative (Khattak *et al*., 2014; Wajid *et al*., 2014; Yaqoob *et al*., 2015; Zhang *et al*., 2012). The

consumers are preferred to be chosen to use plants as makers of secondary metabolites. Plant secondary metabolites were found to be foundations of a variety of phytochemicals which are used directly or indirectly for the making of medicines, cosmetics, food or drink enhancements (Ramya and Perumandla, 2010). Secondary metabolite is not involved directly in growth processes, but secondary metabolites generally have not only tremendous biological function but also used in practical daily approaches. Examples are pigments and anti-biotics. Secondary metabolites are organic mixtures which not only mobilize the growth process and power to generate young ones. Secondary metabolites deficiencies directly do not cause death as compared to primary metabolites, but cause in enduring deficiency of organism's fecundity, aesthetics and survivability. Secondary metabolites always plays a vital part in defense from herbivore and other interspecies defenses in plant species. Human being use secondary metabolites as recreational drugs, flavors, dyes and medicines.

Phytochemicals of *Cucubita moschata*:

Cucurbita moschata as a source of α and β-carotene, vitamin C, minerals, dietary fiber and phenolic mixtures highlighted in the research studies. β-carotene decreases skin harmness by sun and deal as an anti-inflammatory agent. α-carotene is understood to slow down the aging procedure, decrease the danger of mounting cataracts, and saves from tumor growth. Vitamin E (tocopherols) defends the cell from oxidative damage by saving the oxidation of unsaturated full of fat acids in cell membrane (Valenuela *et al*., 2011a). These nutrimental and bioactive components are found very significant in providing benefits of human beings health. *Cucurbita moschata* seeds, usually thrown away but the seeds are a great source of oils and nutrients (Dhiman *et al*., 2009).

Cucurbita moschata seeds subjected to industrial processing and commercially used as a savory appetizer. These seeds are reported as a good alternative for nutritional enrichment of food products (Gorgonio *et al*., 2011). *Cucurbita moschata* seeds have a high nutritious value, delivers good quality oil, and outstanding source of protein. *Cucurbita moschata* fruits are usually sweet when ripe with yellow and orange flesh, rich in β-carotene which is a precursor of vitamin A and reported to have antioxidant components including vitamin A, vitamin E, C, K, B2, carotenes, xanthophylls and phenolic compounds which shows potential role in protection against oxidative tissue damage (Chanwitheesuk *et al.,* 2005). *Cucurbita moschata* flesh and seeds are opulent in proteins, antioxidant vitamins, such as tocopherols and carotenoids as it has a low energetic content and a great quantity of fiber. The cucurbitacins are a clutch of bitter tasting, mainly high oxygenated, tetracyclic, triterpenic plant materials resulting from the cucurbitane skeleton (Miro, 1995). *Cucurbita moschata* seeds are a worthy source of polyunsaturated fatty acids, phytosterols, and zinc, which can prevent chronic diseases (Abrie and Staden, 2001).

Antiviral and antimicrobial activities:

The seed of pumpkin has pharmacological activities such as anti-diabetic, antifungal, antibacterial, anti-inflammation activities and antioxidant effects (Abd El-Aziz and Abd El- Kalek 2011).

Anti-cancer activity:

Cucurbita moschata seeds are deliberated as another treatment for stage I and II benign prostatic hyperplasia and for ill-tempered bladder. The seeds considered mildly diuretic and vermifuge (Oswell *et al*., 2007). The pharmacological activities of cucurbitacin are purgative activity and their cytotoxic and hepatoprotective, antitumoral, antifertility in feminine mice and stomachic between other properties. They also can perform other biological parts like as plant growth regulators and insect feedant.

Research studies have described that *Cucurbita moschata* can value the dealing of benign prostate hyperplasia, since of its high β-sitosterol contented. β-Sitosterol has been specified to decrease blood cholesterol and to shrinkage risks of firm sorts of cancers. Muscatine is a unique ribosome–inactivating protein that has been just filtered from *Cucurbita moschata* seeds, and exposed to have a strong inhibitory activity to protein amalgamation and selectively kill refined human melanoma cells (Konoshima and Takasaki, 2002). A novel immunotoxin Muscatine (Ng76) was structured effectively form *Cucurbita moschata* extract and capably inhibit growth of directed (M21) melanoma cells. The anticancer and anti-inflammatory events of *Cucurbita moschata* are conveyed by a study conceded out on lung, breast, and central nervous system cancer cell lines (Konoshima and Takasaki, 2003).

Curative properties and Folk medicine of *Cucurbita moschata*:

Tetrasaccharide and Glyceroglycolipids were acquired from *Cucurbita moschata* and revealed significant glucose-dropping effects in streptozotocin- and high-fat-diet- persuaded diabetes in mice (Jiang and Du, 2011). Water soluble extract PG105, arranged from stalk parts of *C. moschata*, and contains compelling anti-obesity actions in a high fat diet-induced obesity mouse model (Choi *et al.,* 2007). The plant is also used as purge, and in the dealing of heart

disease, headaches, high blood pressureand high blood cholesterol treatment (AL-Syed, 2001). *C. moschata* seeds have been using for very long time in traditional and folk medicine in Mexico and North America as an antihelmintic agent and as a sympathetic treatment of the bladder illnesses and urination complications (Adolfo, 2005). The childhood enuresis nocturnal and ill-tempered bladder has also been treated effectively with its seeds. Seeds have been used to eliminate tapeworm (Pinho *et al*., 2011). *Cucurbita moschata* faces disease attacks include bacterial wilt, blossom end rot, fusarium wilt, downy mildew and powdered mildew and insect pests comprise squash bugs, aphids, squash vine borers, cucumber beetles, pickleworm, cutworms and stink bugs (Popovic, 1971). Because of its ethno-medicinal properties, its demand has been increasing incredibly. Biotechnological application of plant cell and tissue culture techniques shows the most efficient research evaluations on current methods. Plant cell culture technology was presented at the last of 1960s as a potential source for both study and production of plants secondary metabolites. Undistinguishable cell cultures like as callus had been primarily considered, but a great curiosity has been shown for production of plants secondary metabolites. The evolving importance of secondary metabolites commercially leads to a great attention in the current years, to modify detectection of bioactive plant secondary metabolites due to callus culture techniques.

In future, more drugs will be synthesized from plants, thus to study improved and enhanced understanding of plant metabolism. Secondary metabolites which do not generally occur in nature can surely be produced by plant cell cultures. The best identified feature of tissue culture is the testing of plant elements in research laboratory circumstances. Plant pharmaceutical use involved consideration for money-making exploitation of the plant to fulfil the necessities of the developing medicinal industry. Plant cell culture system signifies a potential re-newable cause of valuable pharmaceutical mixtures, smells flavors and colorants that can't be formed by microbial cells (Bourgaud *et al.,* 2001).

Plant cell and tissue culture methods could be another method as justifiable source of plant material for making bioactive secondary mixtures regularly under exaggeratedly well-ordered aseptic conditions (Everaldo and Anthony, 2001). *In vitro* callusing has been confirmed as a vital tool in rapid plant propagation. *In vitro* propagated plants are generally better than of clones *in vivo*. Cell cultures have been demonstrated appropriate for constant and pure availability of biochemical. Cells are also used for molecular and biochemical analysis of metabolites (Pavlov *et al*., 2007).

Tissue Culture of Cucurbitaceae:

Plant tissue culture seems to be a possible option for the enhancement of the *Cucurbita moschata* by means of improvement in secondary metabolites. *In vitro* plant regeneration practice appropriate for secondary metabolite production is the main step in this approach. Plant tissue culture practice among vegetative proliferation practices may successfully be useful among many gourd species of the Cucurbitaceae family. Callus culture in plant tissue culture might give certain benefits over outdated approaches of propagation of secondary metabolites of *Cucurbita moschata*.

In Cucurbitaceae, regeneration of plants *in vitro* had reported in *Cucurbita pepo* L. (Ananthakrishnan *et al.,* 2007), *Cucurbita maxima* L., *Cucumis melo* L. and *Cucumis sativus* L. (Abrie and Staden 2001) *Cucurbita moschata* (Chee 1991, Gonsalves *et al.,* 1995, Kintzios *et al.,* 2002, Leljak-Levani *et al.,* 2004) in cabbag (Qamar *et al*., 2014; Butt *et al*., 2015), in sugarcane (Jahangir *et al*., 2014).

GC/ MS Technique:

Different chemicals and metabolites in the plants possible to identify and examined through GC/MS. Gas chromatography/Mass Spectrometry instrument splits chemicals and secondary metabolites and detects the components at molecular level. Since plants had complicated profile of secondary metabolites, an approach for their identification is of vital significance. Therefore different methodologies used to distinct polyphenols and vitamins found in plants or any other sample (Helmja *et al*., 2007). GC/MS analysis of metabolites of plants and biological samples is speedily becoming bases of systems biology and functional genomics. Collections of mass spectra of metabolites using in most of laboratories in world proves it most efficient tool for identification (Schauer *et al.,* 2005).

The volatile compound mixtures can be investigated by GC (Silverstein, 1981). GC splits the different mechanisms depending upon their collaboration with SP and mol. mass of the mechanisms. These mechanisms can be spotted by a detector qualitatively & quantitatively (Rose and Johnstone). GC/MS is applied to diverse fields of science like biochemistry. The GC/MS showed results in a line spectrum. In MS the formed ions are recognized because of their changed mass to charge ratios (Younas, 2008). Plants are very significant according to pharmaceutical point of view and are supportive for providing the bioactive mixtures. Research have been accompanied to study the secondary metabolites of *Cucurbita moschata* Duch. ex. Poir. and to check its medicinal properties. There has been some considerations like as callus culture and secondary metabolites of *Cucurbita moschata*

which are in chronological order in literature review. Rakha *et al.,* 2012 reported *Cucurbita pepo* L. regeneration of plants *in vitro* having double haploid character by anthers and ovules causing hybridization of *Cucurbita ficifolia*, *Cucurbita moschata* and *Cucurbita martinezii* and suggested 2,4-D (1mg/L) and sucrose (90g/L) combination was found suitable among six combination for calli production by anthers and ovules. Usman *et al.,* (2011) established cucumber cultivars regeneration by embryogenesis under different plant growth regulators (PGRs) by seed explants. Highest response of callus was reported in leaves on medium of 2mg/L 2,4-D, NAA+BAP (1.5mg/L) respectively. Cotyledon showed maximum response (77%) on 4mg/L BAP+0.75mg/L NAA. Highest level of 2,4-D in medium which was 5 mg/L induced embryogenic response and formed embryos. The concentration of 5mg/L BAP+1mg/L NAA produced 12% and 14% shoots.

Kurtar *et al.,* 2010b firmed response of hydrophilic polymers (hydrogels) as well as growth mediums to observe growth and survival of *in vitro* plantlets of *Cucurbita moschata* and pumpkin during acclimatization. Eight different growth mediums with AAm EDTA JEL4, PCrA 0.18JEL3 and VPITA JEL4 were used. The plantlets were propagated by in vitro embryo culture in solid E20A medium via irradiated pollen method. Plantlets were transplanted to soil and then to soil with polymers after that in sand and on fourth phase sand with polymers used. The perlite, perlite with polymers, peat moss, peat moss with polymers were used correspondingly acclimitization. Growth mediums with polymers bigger rate of persistence and growth of *in vitro* plantlets of winter squash and pumpkin during acclimitization. Marta *et al.,* 2009 produced *Cucurbita moschata* cultivars which are profitable in the regeneration of plants and fruits. In this report reproducible technique for somatic embryogenesis used and tested genotype and source of explants using different PGRs (2,4-D, BAP and 2,4,5-T) concentrations. Embryogenic calli with friable texture was produced by zygotic embryo (56%) and cotyledon (70%) of *Cucurbita moschata* culture on 0.5mg/L and 2.5mg/L 2,4-D. *Cucurbita moschata* pure cultivars produced by embryogenic calli (75%) having calli percentage frequency ranged from 5-34%.

Mahzabin *et al.,* (2008) accomplished micro propagation of *Cucurbita maxima* was attained using shoot tip of seedings grown *in vitro*. Shoot tips were cultured on medium of BA, KIN, NAA at numerous levels of attentiveness and combination for shoot induction and proliferation, and best response was found at 3mg/L of BA. Shoots get roots most efficiently by 1⁄2 MS medium supplemented with 1mg/L IBA. The lengthiest shoots (6.1±0.85 cm) and the maximum percentage of shoot formation (90.45%) were experiential in treatment with 3mg/L BA in 30 days after culturing. The highest shoot number per shoot tip (16.5±0.95) was noted in the media comprising 2.0 mg/L BA. Further growing the attentiveness of BA did not increase the shoot size and number. Kintzios *et al.,* (2002) defined leaf explants of *Cucumis melo* and *Cucurbita pepo* were pre-treated primarily by 113.1, 226.2 or 452.4 μM 2,4-D, 46.5, 93 or 186 μM kinetin or amalgamation of both at the overhead applications, for 6, 24 or 48 hours. Explants transported to a jelled medium which had not accompanied with PGRs in pre-treatment. Preliminary pre-treatment of *Cucurbita pepo* explants by 186 μM kinetin and of *Cucumis melo* explants with 226.2 μM 2,4-D for 48 hours, considerably stimulated development embryos somatically that established more to torpedo-shaped stage or propagated.

Ravishankar and Ramachandra 2002 established the making of great value secondary metabolites containing medicinal and foodstuff essences through shoot and root culture, transgenic roots and plant cell culture gained by bio-technological techniques. Plant cell cultures are potential alternative source for manufacturing of great value secondary metabolites having industrial status. Current improvements in tissue culture have raised the probability to find biosynthetic pathways to gain more secondary metabolites. Norshazila *et al.,* 2012 purposed analytical technique to separate β-carotenoid from *Cucurbita moschata* by using open column chromatography. *Cucurbita moschata* variation had been selected because of its ease of access all year around and non-seasonal nature. This research verified purity of β carotene standard that was evaluated by HPLC ranged 92.21% to 97.95%. Peak area was plotted against different concentrations of β-carotenoid extracts having five standard curves in triplicate. Young *et al.,* 2012 studied the chemical composition to estimate some bioactive mechanisms, for example carotenoids, tocopherols, and β-sitosterol, were valued in three main species of pumpkin which are *Cucurbita pepo*, *Cucurbita moschata*, and *Cucurbita maxima* grown in Korea and also in three portions flesh, peel, and seed of each pumpkin species. *Cucurbita pepo* and *Cucurbita moschata* seeds contained expressively more γ-tocopherol than *Cucurbita maxima*, whose seeds had the premier β-carotene content. Okoye (2012) considered anti-nutritional factors of three Nigerian Cucurbits including *Cucurbita moschata*. The anti-nutritional factors found were tannin, oxalate, cyanogenic glycosides, and trypsin inhibitor. Significant differences ($p<0.05$) of anti-nutritional mechanisms of the cucurbits were detected. The each sample showed high protein digestibility but lesser amount of anti-nutrients. Valenzula *et al.,* 2011b categorized

chemical, physical and physicochemical properties of the *Cucurbita moschata* including carotenes, phenols, fiber and minerals in *Cucurbita moschata*. Their physical and physicochemical characteristics presented higher percentage of dietary fiber and carotenoids in *Cucurbita moschata*. The oil and phenols was less in comparison with some other fruit. Javid *et al.,* 2011 conceded out the study to evaluate composition of nutrients and minerals in selected species of plants i.e., *Momordica charantia*, *Abelmoschus esculentus*, *Cucurbita moschata*, *Solanum melongena*, *Allium sativum*, and *Portulaca oleracea* and taken form Mardan. Secondary metabolite study of species was examined AOAC approaches and macro-nutrients were evaluated. Fats, ash, proteins, fibers, energy value and carbohydrates of these species were found. Fe, Zn, Mn, and Cu was also found. Macronutrients gained having values of Ca (90-1850 ppm), Na (3-160 ppm), and P (220-2530 ppm), Mg (74-671 ppm), K (2771-3612 ppm). Javid *et al.,* 2010 chosed eight vegetable species comprising *Cucurbita moschata* and measured for their nutritional values using standard systems for proximate, macro and micro-nutrient analysis. In proximate examination, ash, fats, proteins, carbohydrate, fiber and moisture (both dry and wet) were evaluated while Cu,Cr, Ca, Ni, Zn,Co, Cd, Pb, Fe, and Na were assessed in micro-nutrients investigation using AOAC approaches and atomic absorption spectrometric methods. *Cucurbita moschata* have exposed higher percentage of fibers, carbohydrates, and energy values. Dhanalakshmi, 2010 valued the nutritional and chemical constituents of *Cucurbita maxima* and *Cucurbita moschata* using HPLC by the manner of Stahl Egonand antibacterial activity of both the seeds and their insufficiencies has also been measured using disk diffusion technique by measurement of diameter of growth inhibition zones. Seeds of *Cucurbita maxima* contain 8.62±1.50mg of carbohydrate, 34.93±0.42mg of protein, 20.6±0.62mg of amino acids, 4.03±0.04mg of chlorophyll and 1.15±0.96mg of carotenoids whereas the seeds of *Cucurbita moschata* appears to contain comparatively more carbohydrate (8.86±2.60mg), protein (32.03±2.62mg), amino acids (18.6±0.26mg), chlorophyll (4.65±0.03mg) and carotenoids (0.26±0.006mg). *Cucurbita maxima*and *Cucurbita moschata* contain more or less equal amounts of tannins, lignin, glycosides and serpentines.

Gohari*et al.,* (2010) evaluated chemical and physicochemical properties of *Cucurbita pepo* seeds. Seeds comprised 42.60% oils and 23.6% proteins. Gas chromatographic examination of seed showed oleic acid (38.42%), linoleic acid (36.13%), palmitic acid (11.48%) and stearic acid (9.13%) was found as vital fatty acids. Peroxide value, acid value, saponification number, iodine value, and unsaponifiable matter content (%) of the extracted oil from *Cucurbita pepo* seeds were 0.18, 0.49, 11.45, 114.36, 140.19, and 5.13 respectively. Phenols, tocopherols, sterols and waxes percentage was 66.27%, 882.65%, 1.86%, and 1.58% respectively.

Barbieri *et al.,* 2006 investigated 40 edible vegetables to evaluate protein fraction quantitatively and used the extracts of these vegetables having adenine glycosylase activity which inhibit cell-free protein biosynthesis and lose adenine from DNA. Therefore it recommended existence of ribosomes inactivating protein (RIPs) in plant extracts. This was more supported by existence of two actions after a partial chromatographic representation of three extracts of species, showed *Lycopersicon esculentum* having less action. *Cucurbita moschata* extract was utmost active one a glycoprotein of 30,665 Da was showed characteristics of RIPs.

Bojaxa *et al.,* (2012) examined phyto-constituents of *Cissus x avierensis*, *Cucurbita quadrangularis* and *Cucurbita vitiginea* and recognized by GC-MS (Gas Chromatography-Mass Spectrometry) investigation. The study was passed out with the methanol extracts of the dried wild plant and callus of the three selected plants. The results determined that the callus have more phytoconstituents than the wild plant extracts. In the GC-MS study, 23 mixtures were recognized in the wild methanol extract of *Cucurbita quadrangularis*, but in callus extract 46 mixtures were identified.

Irshad *et al.,* (2012) worked on *Plectranthus rugosus*, *Angelica glauca*and *Valeriana wallichii*, and endangered to Clevenger-type-hydro distillation equipment therefore examined by GC-MS. The main composition of *A. glauca* had α-phellandrene (12%), β-pinene (10%), β-caryophyllene (8%). *P. rugosus* yielded 0.18% oil and had 23 metabolites, 82% of essential oil. Most important metabolites were spatulenol (23%), germacene D (19%) and β-caryophyllene (11.6 %). 24 metabolites were recognized from *V. wallichii*having 81.4% oil. 3-Methylvaleric acid (24.6%), maaliol (37.1%) and β-eurjunene (5.7%) were main constituents of *V. wallichii*. The essential oil showed *in vitroMicrosporum canis*and *Fusarum solani* antifungal action.

Hemlal and Subban, (2012) implemented to estimate the chemical composition of the methanol extract of *Pseudarthria viscida*(L) and *Desmodium gangeticum* (Linn). 43 amalgams have been recognized from *P. viscida* extract and the major chemical constituents are cis-Vaccenic acid (16.47%), γ sitosterol (13.73%) and stigmasterol (6.24%).18 mixtures have been recognized from *D. gangeticum* and the major chemical constituents are 9,12-

Octadecadienoic acid (41.71%), n-Hexadecanoic acid (9.43%) and Octadecanoic acid (5.9%). Stigmasterol was enumerated from both extracts by HPTLC technique (105.15µg/ml and 20.9µg/ml correspondingly). *In vitro* antibacterial and antifungal actions of methanolic extracts of *P.viscida*and *D. gangeticum* was assessed.Ramya and Perumandla, (2010) worked on seeds of *Nigella sativa* L. (Ranunculaceae) to value secondary metabolites by qualitative and quantitative measures using the ethanolic extracts of seeds of *N.sativa* by GC-MS techniques. The seeds comprise both fixed and important oils, alkaloids, proteins and saponin. Phytochemical constituents like alkaloids, flavanoids, thymoquinone, saponins, glycosides in the ethanolic extract of *Nigella sativa*, Thymoquinone and Flavonoid were known. Oswell *et al.,* 2007 accompanied a survey in four areas of Manicaland province of Zimbabwe on *Cucurbita moschata* and acknowledged ethnobotanical understanding to classify the local landraces. These species were found grown mostly in intercrops mainly with maize, (85 % respondents) and was planted and grown in summer (67%). The leaves are consumed 3 - 4 times a week during the rainy seasons. The key problem during production of pumpkin was insect pests and infections. Farmers use mostly reserved seed (71.9%) for planting that is kept in any unfilled container in the home.

Nuchjaree *et al.,* (2007) established microsatellite symbols in *Cucurbita moschata* was accomplished using the biotin-streptavidin enrichment process. One hundred and thirty three clones were casually nominated. After arrangement analysis of 31 randomly selected positive colonies, 100% of the colonies were found to comprise microsatellite sequences, and 9 primer sets were premeditated. Five of the primers tested might amplify *Cucurbita moschata* DNA and can be used for genetic purity testing of the commercial hybrids. Ashutosh *et al.,* (2012) examined low yield and high market price of the pharmaceutically significant alkaloids of *Catharanthus roseus*such as vinblastine, vincristine, and ajmalicine for their making by using cell culture. Highest development of total alkaloid contented was found in and 1mg/L BA +0.5mg/L 2, 4-D associated with other amalgamations. In addition to plant growth controllers and strength of the MS media, numerous carbon sources and have significant response of leaf callus formation and total alkaloid content. It was testified that half strength MS basal medium supplemented by 2,4-D 0.5mg/L+1mg/L BA and 6% sucrose was best for biomass production of leaf callus and improvement of alkaloid accumulation in *C. roseus*.

Elfahmi *et al.,* (2011) examined the chemical elements of *Cucurbita pepo* and valued the phytochemicals by origination of cell and organ culture from diverse areas in Indonesia. Callus and suspension cultures of have been recognized using Murashige and Skoog medium and growth hormone NAA 2 mg/L: BAP 0.5mg/L. Leading mixtures that were noticed by GCMS are hydrocarbon such as 2-heptenal, hexsadecane, decadienal, cyclooctane pentadecane, etc, fatty acid like as octadecanoate acid, ethyl stearate, ethyl linoleate, hexadecanoate acid and steroid like as fucosterol, stigmasterol, sitosterol.

Conclusions

Cucurbita moschata is a rich source of bioactive secondary metabolites including carbohydrates, carotenoid, protein, fats, glycosides and sitosterols. It is concluded that these compounds can be produced in larger amount by using plant tissue culture. Callus culture in plant tissue culture might give certain benefits over potential approaches of production of secondary metabolites of Cucurbita moschata.

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