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CALLUS INDUCTION AND EXTRACTING MEDICAL MATERIALS

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ABSTRACT: In recent year, the importance of plants has been Known as a source of pharmaceuticals, due to many factors, the most important of which is the rapid progress in bio molecular technologies where methods have been developed for the transfer of genes and the use of the plant as an alternative source in the production of pharmaceutical proteins, including vaccines, antibodies, enzymes, bioactive compounds and free of viral and pathogens. Therefore, the technology of tissue culture and the generation of cells and plant organs outside the living body is an indispensable tool in the production of medicinal materials derived from plants, and this method has significant productivity benefits in terms of quantity, quality and production controlled without restricting natural factors such as geographical location, seasonal changes, or environmental stresses.

Key words: Callus, extraction, natural product, medical materials

INTRODUCTION

Medicinal and aromatic plants are an important source of medicines they are play a significant role in world health care system. Today medicinal plants are important to the economical, It is source of income for rural people in developing countries. About 70% - 80% of the people worldwide rely on herbal medicines derived from plants for their primary health care needs. **(Pei, 2001; Muley *et al.*, 2009 and Maruska, 2011)**. This renaissance has led to a sudden rise in demand for herbal medicines. Generally, herbal preparation are produced from field-grown plants and are susceptible to infestation by bacteria, fungi, and insects that can alter the medicinal content of the accommodation **(Mello *et al*. 2001 & Bissa and Bohra, 2011)**. But it is difficult to ensure the quality control as the medicinal preparations are multi-herb preparations and also difficult to identify and quantify the active constituents **(Murch, 2000; Wen, 2000 and Ford, 2002)**.

Callus tissue is an irregular collection of non-specialized parenchymal cells at different stages of telogenization **(Hartmann *et at.*, 2002; Hopkin and Hiiner, 2004; Zhoo and Wu, 2006 and George *et at.*, 2008)**. The size and growth of this callus increases according to the type and amount of growth regulators present in the MS medium **(Salih and Al-Obaedi 2011 and Zhoo *et al.*, 2017)**. The tissue culture through differentiation into members of a process called organogenesis, by growing this non-specialized tissue on medium containing auxin and cytokinin, the formation of branches or roots, or both **(Al-Kanani, 1987; Muhammad and Omar, 1990; Purohit, 1999; Al-Hadidi, 2002 and Gupta, 2006). Mello (2001)** suggested the possibility of introducing callus cultures

from growing bean embryos of *phaseolus vulgaris* on MS medium prepared with 5.0 mg/L 2,4-D. **Li *et al*. (2002)** reported that growing the leaves of the Grand gala rose plant on MS medium prepared with 2.5 mg/L 2,4-D obtained callus at a rate of 93.3% and **Soomro *et al.* (2003)** reported that cultivation of the rose plant *Rosa indica*, obtained from tissue culture on MS medium provided with IBA and NAA. Led to the best growth and quality of callus from cultivation at concentration 0.6 or 0.8 mg/L respectively. **Alnazal (2005)** mentioned that callus was introduced to different plant parts of the clove plant *Dianthus caryophyllus*. (Stems, leaves, cotyledon leaves, roots, stems sub-epiphysis) where the rate of creation of callus was 100% from cultivation on MS medium prepared with 0.5 mg/L 2,4-D and 0.1 mg/L BA of cut stems and the leaves and stems of legionary plant. **Shen *et al.* (2007)** indicated that cultivated parts of *Dieffenbachia cv*. Leaf on MS medium supplemented with1.0 mg/L TDZ and 0.2 mg/L2,4-D callus gave.

NATURAL PRODUCT

Some wild and medicinal plants and ornamental plants contain chemical compounds of great benefit and importance that are by products of the metabolism processes inside the plant, used for the purposes of sustaining their life or protection and defense against other living organisms, They can be called natural secondary, or accidental products, ingredients active, and since ancient times, these compounds (in the form of raw extracts) have been used as drugs, but the purification and diagnosis of many of these active substances with a biological effect is still a concern of pharmacologists, chemists, and life sciences **(Huany and Liu, 2002; Lee, 2007; Amoian *et al*., 2010 and Bernatoniene *et al*., 2011)**. Attention has been focused on the effect of raw plant extracts on a number of pathogenic bacterial and fungal strains. And also, to find a specific extraction method or system that is adopted to extract the active substances, as these methods vary between alcoholic extracts (methanol or ethanol) or aqueous and even the use of vegetable juice sometimes for the portion for treatment purposes. The extraction methods also vary according to the plant fraction adopted for the extraction **(Harborn, 1973; Burton, 1989; Ramawat, 2004 and Sarin, 2005)**.

EXTRACTION

It is the process of dissolving, with drawing or isolating some of the compounds (active substances) contained in the plant part using a solvent, or it is the transfer of substances across the boundaries between two phases, one of which is a solid (the solute) and the other phase is a liquid (the solvent) not mixed as a result of the difference in the solubility between them **(Saeed and Al-ghubsha, 1985)**.

Methods for extracting plant samples:

1- (SFE) supercritical fluid extraction **(Al-dawidi, 2012)**.

2- (ASE 300) Accelerated Solvent Extractor **(Al-dawidi, 2012)**.

3- Roise extraction.

4- Shoxhlet extraction.

5- Grand method: It is a method used by placing an amount of the material to be extracted from it at a ratio of 10: 1 volume: ethanol is used as a solvent. The shaking continues by an electric motor for at least 72 hours, then the filtrate is taken and concentrated by a rotary evaporator to obtain crude.

6- Extraction by ultrasonic frequency ultrasonic extraction: The material to be extracted is placed in sealed bottles, and placed in the water basin of the device for the purpose of extraction.

HPLC high-performance liquid chromatography technology is one of the most important techniques in separation, diagnosis and quantification **(Hiraoka,**

1976; Ripath and Tripath, 2003; Budhiraja, 2004; Liu, 2005 and Medina, 2006). Studies have been continuous to separate, diagnose and quantify compounds from a variety of sources. Or proportions between these materials.

Wen *et al*. (2005) found different concentrations of thirteen phenolic compounds in alcoholic extracts of dry parts of the chrysanthemum plant, including vanillic acid, caffeic acid, syringic acid, sinapic acid, p-coumaric acid, ferulic acid, anisic acid, rosmarinic acid, salicylic acid, cinnamic. acid, chlorogenic acid gallic acid and acid gentsic, as they were detected by HPLC technology and the maximum concentration was for Gallic acid, while the lowest concentration was for Cinnamic acid.

Khalil *et al*. (2007) mentioned the presence of a large number of phenolic compounds in the different stages of the life cycle of the chrysanthemum plant, including Resorcinol and hydroxyl benzoic acid, Chlorogonic acid, Salicylic acid, Coumrin, P-coumric acid, and Cinnamic acid, the researchers showed the presence of high levels of salicylic acid in the alcohol extracts of the plant. **Perez-Alonso *et al.* (2009)** indicated that extracting Digoxin and Digitoxin from dried foliage and the product from tissue cultures of *Digitalis purpurea* L. plus 70% ethanol using an ultrasound device at 70 °C led to results. Excellent in extracting the two materials through the results obtained from the HPLC device, and **Pellati *et al.* (2009)** explained that he used an ultrasound device to extract Digoxin from the plant samples of the dried *Digitalis lanata* leaves using methanol dissolved at 70% at room temperature. **Gurel *et al*. (2011)** used Ultrasound machine to extract Digoxin from the dried foliage of the *Digitalis davisiana* at 65 - 70 °C, using methanol, a 70% solvent. In a comparison between the alcohol extracts of the vegetative parts of the field growing plants and the vegetative parts of tissue culture, **Kaskoniene *et al*. (2004)** confirmed the presence of varying concentrations of phenolic compounds and that their concentrations in the parts obtained from tissue culture were 2.0 mg / 1 g dry weight while their concentration was in field parts, 05,0 mg / 1 g dry weight.

Al-Abbasi (2012) showed that the *Digitalis purpurea* L. callus growing on MS medium supplied with different concentrations of 2,4-D at the age of 12 weeks gave the highest amount of digoxin, and that the increase in age after that led to a decrease in the amount of digoxin in the callus. The largest amount of digoxin compared with the rest of the other treatments and that from the growth of callus on MS medium supplied with 0.5 mg / L CaCl2 with 0.1 mg / L 2,4-D and BA.

Aljibouri *et al*. (2012) indicated that adding 200 mg / L sodium chloride to the MS food medium of *Hyoscyamus niger* L. resulted in a significant increase in the amount of Scopolamine and a decrease in Hyoscyamine compared with the comparison treatment. And between **Abdel Rahman *et al.* (2013)** for the production of Atropine and Scopolamine in *Datura metel* and *Datura stramonium.* The addition of 2 mg / L sodium chloride to MS dietary medium led to an increase of Atropine and Scopolamine in the novel callus compared to the comparison treatment without NaCl after one month. Farming.

AlMemary (2014) reported that cultivating portions of *Catharanthus roseus* L. leaf on MS medium supplied with 0.5 mg / L Vinb. With 0.75 mg / L 2,4-D it generated the highest wet weight of callus and the highest protein content of 3.230 g and 16.34%, respectively, after 60 days of cultivation. **Al-Akidi (2017)** found that the leaves of *Atropa belladonna* L. grown on MS medium prepared with 0.25 mg / L of glutamine with 1.0 mg / L 2,4-D plus 0.5 mg / L BA contained the highest amount of alkaloid Hyoscyamin sulfate 0.29 mg / g wet weight after 4 weeks of planting, and the callus obtained from leaf planting on MS medium prepared 0,5 mg / L NaCl with 1.0

mg / L 2,4-D plus 0,5 Mg / L BA gave the highest amounts of Scopolamin and Tropine 1.01 and 1.20 mg / g wet weight, respectively, after 4 weeks of cultivation. Some phenolic compounds were diagnosed from **Al-Dulaimi and Jasim (2019)** in flaxseed seeds using HPLC these are (Qurcetine, Kaemferol, Gallic acid, Catechine, Epigene).

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