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Review Paper

Initiation of Pharmaceutical Factories depending on more Application of Biotechnology on some Medicinal Plants Review Article
(In Vitro Production of some Antioxidant, Analgesic, Antibacterial, Antidiabetic agents)

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Abstract

Higher plants are an important source of all type of substances, especially medicines (glycosides, ethereal oils, steroids, flavonoids, anthraquinones, alkaloids, tannins and saponins, etc.,). Traditionally the medicinal plants have been grown and then the active components extracted and this is likely to remain the normal procedure. However the production of medicinal plants can present problems, which have load to the search for other ways to produce naturally accruing substances: i. Production in the field is strongly dependent on season, weather, climate, diseases and pests. ii. Naturally occurring sources, especially in the tropics and subtropical zones, are becoming limited and some medicinal plants are extremely scarce. iii- There may be technical and economic problems in production. iv. Production is labor intensive and therefore costs are high. v. There may be political instability in the country where the plants are available resulting in an interrupted supply. For the above mentioned reasons, attempts have been made to obtain substances from cell suspension cultures of higher plants, either through accumulation in the callus (biomass) or sometimes by the release into the nutrient medium. In this review we will discuss in vitro production of some antioxidant, analgesic, antibacterial, antidiabetic agents.

Keywords: Glycosides, ethereal oils, steroids, flavonoids, weather, climate, production antibacterial, antidiabetic agents.

Introduction

In vitro production of secondary metabolites advantages, the effective factors etc[1,2] : Organ, tissue and cell culture and other biotechnological techniques are useful ways to obtain biologically active constituents those play an important roles in our life (They have pharmaceutical, medicinal and economical importance in our life). These ways (in vitro) of obtaining secondary metabolites are better than the classical methods since:

They are natural in origin, so they are of little toxic side effects compared with synthetic drugs. They are safe sources and do not cause any environmental pollution as like as those occurred by applications of pesticides and insecticides to farm lands. They are performed under controlled conditions (since the yield can be increased, with increasing replicates number and by using elicitors, fomenters and bioreactors in a large application scale), they are performed under aseptic conditions (This means that, they are system free of contaminating microbes " fungi and bacteria", consequently they are clean sources of drugs).

Using these techniques we can direct the culture for producing the organ that contains the highest amounts of the product we need (for example, we can produce root culture using Agrobacterium rizogenesis to obtain substances that produced in root cells only).

Production cycle is smaller than that of normal culture in land (it takes little time), since callus cultures with short life cycle may be a good source for production of phytochemicals needed. Using these methods we can conserve our natural resources (wild plants) instead of over-collection by herbalists. Finally, the cost can be decreased if done on a large scale (instead of Fedens language "large areas" we use jars in small place = higher productivity of secondary metabolites).

Two main approaches have been followed in connection with the production of secondary metabolites in vitro : The rapid growth of suspension cultures in large volumes which are subsequently manipulated to produce secondary metabolites. The growth and subsequent immobilization of cells which are used for the production of compounds over a prolonged period.

The production of secondary metabolites is strongly dependent on the rate of cell division, so the yield of metabolites is dependent on a large number of factors: The starting material. The pre-treatment before the in vitro culture. Physical growth factors: light, temperature, aeration. The composition of the medium (carbohydrate sources,

growth regulator etc.,). The two stage culture system is necessary sometimes. The first stage involves growing the cells on maintenance medium and the second stage involves transferring the cells to a production (of secondary metabolites) medium. Inducing morphological structure in culture can have effects.

i- To enable the accumulation of a group of compounds such as the appearance of leaf bearing shoots in the culture, these leaves contain oil glands. ii -To alter the qualitative composition of the products accumulated, such as the furanocoumarin, psoralen, is present at much higher levels in the shooty culture. Also, the different compounds are elicited to various levels depending on the concentration of elicitor applied. This different culture types can be used to study the responses of different chemical components to exogenous factors. Chrosomal stability of the culture.

The development of biosynthetic production in vitro has been extremely rapid because: There has been a gradual introduction of growth and production from plant cells in fermentors and bioreactors. The growth of plant cells can be optimized by changes in the nutrient medium and the physical growth factors (aeration, stirring); also it has been attempted to increase biosynthesis by the addition of precursors.

Immobilized plant cells can be used (packed in a jelly-like mass) to increase production of the products and also to accumulate the metabolites in the medium. This accumulation means that, substances excreted by the cells can be obtained by simply exchanging the medium. The interest in the in vitro production of other compounds (e.g., biodegradable nematicides and insecticides) increases. It is hoped that, it will be possible to produce substances in vitro which are impossible to normally biosynthesize with plants in the field.

With this in mind it is hoped that, genetic manipulation of cells can result in them gaining the characteristic required of being able to produce a particular substance. Selection and screening techniques have been developed for the growth of plant cells which is hoped will result in a higher production of secondary metabolites. Biotransformation becoming more important. This is a technique which utilized enzymes located in the plant cells to alter the functional group chemistry of externally supplied chemical compounds. There are two types of biotransformation.

Via whole cells (immobilized or non-immobilized). Via the use of immobilized compound preparations. A good example of biotransformation is the conversion of digitoxin to diogioxin by cells of Digitalis lanata. Interest has grown in the increase in accumulation of secondary metabolites by the use of elicitors. Elicitors are strictly speaking compounds of biological origin involved in plant-microbe interaction. Elicitors such as phytoalexins (biotic elicitors) which are mediator compounds of microbial stress or stress agents such as osmotic pressure or heavy metal ions, UV light, polymers like chitosan, or dilution into fresh medium (abiotic elicitors) are used to increase accumulation of products in plant cell cultures. Multiple shoot cultures are becoming a viable alternative in in vitro systems for the production of plant constituents.

Some examples of secondary metabolites obtained in vitro from some medicinal plants will be discussed in the review: Antioxidant agents, Analgesic agents,Antibacterial agents, Antidiabetic agents.

Material and Methods

In vitro production of some antioxidant agents: Many tests are used to determine the antioxidant activity in different in vitro culture systems obtained from different plants (such as; flower cell, cell suspension, callus, shoot in bioreactor, hairy roots, UV irradiated callus and regenerated plantlets cultures etc.,) such as β-carotene bleaching, lipid peroxidation, oxidative DNA damage in cell culture, DPPH, cell membrane peroxidation, XOD/NBT, singlet oxygen quenching, alleviation of oxidative stress in cultured mammalian cells, TBARS, lipid peroxidation, overexpressing CHI, H_2O_2 induced cell damage, carotene-linoleic acid oxidation, brain lipid peroxidation and hydroxyl radical scavenging and FRAP activity[3].

In vitro production of some analgesic agents: Different cultures of Hyoscyamus niger and H. albus such as; callus, cell suspension culture and root culture can be used as a source of analgesic agents such as; tropan alkaloids[2].

In vitro production of antibacterial and antidiabetic agents: Callus cultures of different species of Fagonia are studied as an antibacterial agents[4-6], while callus cultures of Zygophyllum coccinum are used as an antidiabetic agents[7]

Results and Discussions

Examples of *in vitro* production of some antioxidant agents: Example of in vitro production of some analgesic agents[2]: Production of tropan alkaloids from callus, cell suspension culture and root culture of Hyoscyamus niger and H. albus. Root culture was found to be the most containing culture of this compound.

Examples of in vitro production of antibacterial and antidiabetic agents " organ culture and Zygophyllaceae": In our works[4-7], we focused and will focus in the future on Zygophyllaceae regarding using organ culture technique as a tool for producing large amounts of active constituents.

In vitro production of antibacterial agents " organ culture and Fagonia[4-6] Callus of F. arabica: Callus of F. arabica leaf explants (photo-1) is a good source of

antibacterial agents such as phenolics, saponins, alkaloids and flavonoids. These substances gave the callus its importance regarding antibacterial activity against many infectious human pathogenic bacteria that cause many dangerous diseases such as vomiting, diarrhrea, urinary infections, gastroenteritis "Escherichia coli", infections around nose and spreading over the face, piles, carbuncles may be also caused by bacteria "Streptococci", bacteria also considered to be the major cause of impetigo "Staphylococcus", fever "Salmonella typhi", urinary tract infections may be also caused by bacteria "Klebsiella".

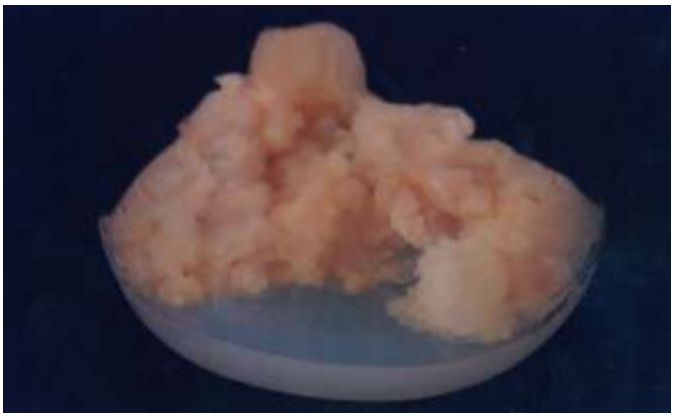
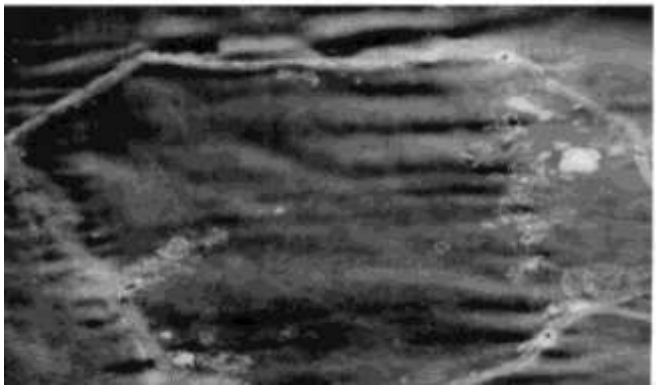


Photo-1
Callus of F. arabica leaf explants Ultrastructural study of callus cells of F. arabica leaf explants using Transmission Electron Microscope (TEM)

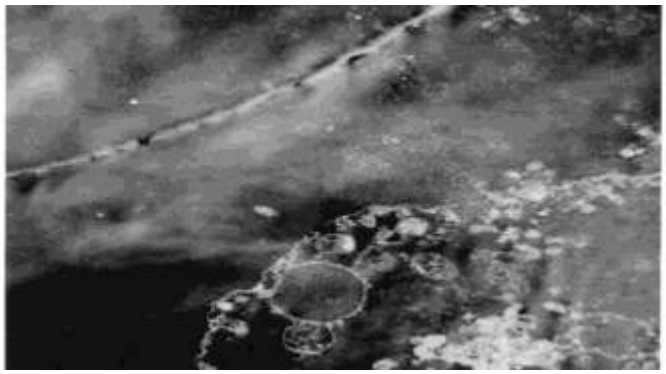
Ultrastructural study (Photo-2) of these callus cells using Transmission Electron Microscope (TEM) shows the study of the internal cellular structure of callus. Ultra structural study on the callus showed large cells with normal structure. Cell organelles such as vacuoles, dense cytoplasm, nuclei, endoplasmic reticuli, mitochondrion, golgi apparatus enveloped by cell wall appeared after using Transmission Electron Microscope.

Our work regarding phytochemical screening on both calli of F. indica and F. bruguieri (table-5) revealed the presence of many important bioactive constituents such as saponins, flavonoids, alkaloids, tannins, cardiac glycosides, coumarins, fatty acids and other constituents. In the future, we will try to investigate calli of F. indica and F. bruguieri (those calli are rich sources of many bioactive constituents) regarding their medicinal importance as antioxidant agents, especially that our results revealed that, these plants are good antioxidant agents.

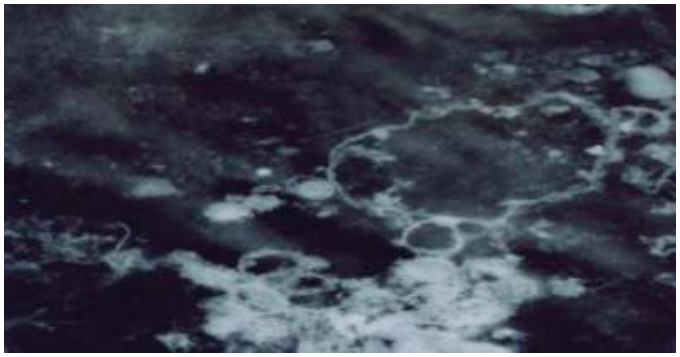
Callus of Zygophyllum coccineum[7]: Callus of Zygophyllum coccineum (photo-5) was obtained in our work without any contamination, however it is difficult to obtain callus from this succulent plant without any contamination in the culture. This plant is an important plant in antidiabetic worlds, in our next studies, we will investigate this callus regarding its chemical composition and medicinal importance, especially as antidiabetic agent.



(a)



(b)



(c)

(Photo-2 a,b,c)
Ultrastructural study of callus cells of F. arabica leaf explants using Transmission Electron Microscope (TEM)

(Where:1=Klebsiella pneumoniae, 2=Proteus mirabilis, 3=Salmonella typhi, 4=Providencia alcalifaciens, 5=Serratia marcescens, 6= Escherichia coli, 7=Acetobacter aceti subsp. Liquefaciens, 8=Staphyl ococcus aureus, 9=Streptococcus salivarius and 10=Streptococcus faecalis).

Table -1 (A)
Examples of in vitro production of some antioxidant agents (3)

Species	Compound	Culture system	Antioxidant testing
Ajuga reptans	Anthocyanins	Flower cell culture	β-carotene bleaching and lipid peroxidation
Anchusa officinalis	Rosmarinic acid	Cell suspension	Many in vitro chemical assays
Anthoceros agrestis	Rosmarinic acid and its glucosides	Cell suspension	Many in vitro chemical assays
Arachis arabica	Piceatannol (a stilbene)	Callus	Oxidative DNA damage in cell culture
Artemisia judaica	Flavonoids	Shoot cultures in bioreactor	DPPH
Carthamustinctorius	Kinobeon A	Cell suspension	Cell membrane peroxidation, XOD/NBT and singlet oxygen quenching
Cistanche eserticola	Phenylethanoid glycosides	Cell suspension	DPPH
Crocus sativus	Crocin	Callus	Alleviation of oxidative stress in cultured mammalian cells
Cynara cardunculus	Cynarin and chlorogenic acid	Callus	TBARS
Daucus carota	Anthocyanins	Callus and cell suspension	Lipid peroxidation
Fagopyrum esculentum	Rutin	Hairy roots	Many in vitro chemical assays
Glehnia littoralis	Anthocyanins	Callus and cell suspension	Many in vitro chemical assays
Hemidesmus indicus	Rutin	Callus, shoot culture	Many in vitro chemical assays
Hyssopus officinalis	Rosmarinic acid and lithospermic acid B	Hairy roots	Many in vitro chemical assays
Ipomoea batatas	Anthocyanins	Callus and cell suspension	DPPH
Lavandula officinalis	Rosmarinic acid	Callus, cell suspension and bioreactor	Superoxide radical scavenging
Ocimum basilicum	Rosmarinic acid	Bioreactor culture of nodal explants and cell suspension	Many chemical in vitro assays
Passiflora quadrangularis	Flavone-C-glycosides	UV irradiated callus	DPPH
Petroselinum sativum	Flavonols and flavones	Cell suspension	In vivo (rats)
Rosmarinus officinalis	Carnosic acid	Callus and shoot culture	Oxidative stress reduction in living cells and many chemical in vitro assays
Salvia officinalis	Rosmarinic acid, abietane and diterpenoids	Callus, shoot culture, hairy roots and cell suspension	DPPH and P-Mo, lipid peroxidation
Salvia miltiorrhiza	Lithospermic acid B and rosmarinic acid	Callus, regenerated plantlets and hairy roots	DPPH

Table -1 (B)
Examples of in vitro production of some antioxidant agents 3

Saussurea arabica	Apigenin	Hairy roots	Overexpressing CHI , H_2O_2 induced cell damage
Scutellaria baicalensis	Baicalin, wogonoside	Hairy roots and cell suspension	Many in vitro chemical assays
Stevia rebaudiana	Flavonoids	Callus	FRAP and DPPH
Torreya nucifera	Abietane and diterpenoids	Cell suspension	LDL oxidation and nitric oxide inhibition
Vaccinium pahalae	Anthocyanins	Cell and aggregate suspension	Many in vitro chemical assays
Vitis vinifera	Stilbenes, procyanidins	Cell suspension	DPPH and lipid peroxidation
Withania somnifera	Withanoloids	Hairy roots	DPPH, carotene-linoleic acid oxidation, brain lipid peroxidation and hydroxyl radical scavenging

Table -2
Preliminary phytochemical screening on intact leaf and callus of F. arabica leaf explants

Callus	**Callus of F. Arabica leaf explants**	**Experiment**
Carbohydrates and / or Glycosides	+	+
Saponins	+++	++++
Tannins	++	-
Sterols and / or Triterpenoids	+	+
Alkaloids	++	++++
Cardiac glycosides	+++	+
Cyanogenic glycosides	+	+
Flavonoids	+	+
Anthraquinones	+	-
Coumarins	+	++
Irodoids	+	+
A-Chlorides	+	++
-B-Sulphates	+	+

Table - 3
Determination of total phenol contents, total alkaloids, total flavonoids and total saponins in callus of F. arabica leaf explants

Concentration of different active ingredients (mg/g fresh weight)			
Total phenols	Total alkaloids	Total flavonoids	Total saponins
1.95	113.40	0.78	10.00

Study of antibacterial activity of both callus of *F. arabica* leaf explants and intact leaf:

Conclusion

To conclude, Plant biotechnology is an alternative and effective source for production of valuable phytochemicals. It is also useful for propagation and germplasm conservation, and thus represent a way to protect plant biodiversity. As well as being an alternative source of bioactive compounds[8-12].

Table - 4
Study of antibacterial activity of both callus of F. arabica leaf explants and intact leaf

Tested Organisms	**Clear inhibition zones (mm), (volume of extract = 0.1 ml/disc)**	
	Leaf extract	Callus extract
Gram -ve		
1-Klebsiella pneumoniae	**1.833****	**12.417****
2-Proteus mirabilis	**16.083****	**21.000****
3-Salmonella typhi	**10.417****	**22.667****
4-Providencia alcalifaciens	**13.167****	**27.083****
5-Serratia marcescens	**21.417****	**32.667****
6- Escherichia coli	**6.083***	**33.917****
7-Acetobacter aceti subsp. Liquefaciens	**16.167****	**34.833****
Gram + ve	**Leaf extract**	**Callus extract**
1-Staphylococcus aureus	**.000**	**2.000**
2-Streptococcus salivarius	**9.877****	**26.420****
3 –Streptococcus faecalis	**21.880****	**26.920****
L.S.D. (0.05)	**5.434**	**4.311**
L.S.D.(0.01)	**7.443**	**5.906**

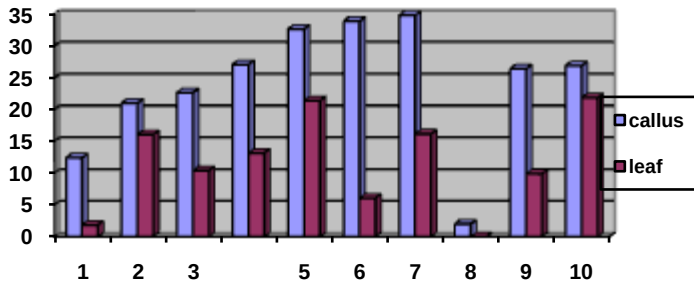


Figure -1
Antibacterial activity study on intact leaf and callus of F. arabica leaf Callus of F. indica:

Photo-4
Callus growth of terminal bud explants of F.bruguieri

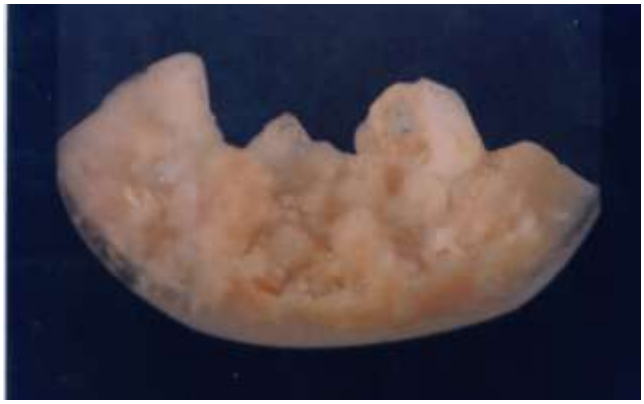


Photo - 3
Callus growth of stem segments explants of F. indica Callus of F. bruguieri

Photo-5
Callus of stem segments explants of Zygophyllum Coccineum

Table- 5
Preliminary phytochemical screening on both calli of F. indica (stem segments explants) and Fagonia bruguieri (leaf and terminal bud explants) on different media

Experiment	Calli of *F. indica* stem explants				Calli of *Fagonia bruguieri* (leaf and terminal bud explants)			
	1a	1d	1e	2b	3a	3c	3d	3f
Carbohydrates and / or Glycosides	+	+	+	+	+	+	+	+
Saponins	-	-	+	-	+	++	-	-
Tannins	+	+	+	+	++	+	+	+
Unsaturated sterols and / or Triterpenoids	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+
Cyanogenic glycosides	+	+	+	+	-	+	+	+
A- Chlorides	-	-	+	-	+	-	-	-
B- Sulphates	+	+	+	+	-	+	+	+
Irodoids	-	-	-	-	-	-	+	+
Flavonoids	-	-	+	-	+	-	-	-
Coumarins	+	+	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-

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