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REVIEW

Compounds (Secondary Metabolite) Identified from Cultured Hairy Roots of *Catharanthus roseus*

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In this paper, the isolated or extracted present in hairy culture roots of *Catharanthus roseus* (L.) compounds identified by spectroscopic studies and HPLC methods are reviewed. Several classes of compounds (sesquiterpene glucosides, flavonoid glucosides, steroidal glucosides, major ones belong to alkaloids and especially indole alkaloids and other classes of compounds) have been reported from the culture hairy roots of this genus and their culture conditions. Lot of work available on hairy culture roots of *Catharanthus roseus*, relevant compounds are reviewed. The *Catharanthus rosesus*, the most important medicinal plant extensively studied because of their several activities like anticancer and others were reported. The relevant literature is reviewed and 49 references are cited.

Key Words: *C. rosesus*, Compounds (secondary metabolites) from cultured hairy roots, Identified by spectroscopic or HPLC.

INTRODUCTION

The periwinkle, *Catharanthus roseus* (Apocynaceae), is widely used ornamental and medicinal plant. *C. roseus* is a herbaceous shrub[1] that has been extensively studied because of its production of two valuable alkaloids, vincristine and vinblastine, which are used in the treatment of human neoplasms[2]. Ajmalicine, also produced by *C. roseus*, is used in the treatment of circulatory disorders and hypertension[2]. Biologically indole alkaloids produced by plants are believed to play a role as antimicrobial and antifeeding compounds[3,4]. Phenolic compounds have been reported in this genus[5,6]. Recently, two flavonol trisaccharides of kaempferol and quercetin have been reported[7]. Several indole alkaloids have been reported from *C. roseus* cell suspension cultures[8,9]. However, the production of the most valuable compounds reported from this plant, vincristine and vinblastine, has not yet been achieved in these cultures[2]. The presence of anthocyanidins[10] phenolics[9,11] and terpenoid compounds[8,9] in cultures of *C. roseus* have also been reported.

Previously all compounds were reported from leaves, stems and cell cultures major ones belong to class of alkaloids[2-11].

The review covers several groups of compounds (sesquiterpene glucosides, flavonoid glucosides, steroidal glucosides, major ones belong to alkaloids and especially indole alkaloids, other classes of compounds) have been reported from the hairy culture roots of *C. roseus* either isolated or extracted and identified by IR, mass and NMR or mostly identified compounds especially alkaloids by HPLC methods. The culture conditions are also reported in this review. All the data reported in this review were collected from the literature. All relavant compounds from hairy root cultures are reported in Table-1 provides evidence of continuous progress in this area.

High stability of the production of secondary metabolites is an interesting characteristic of hairy root cultures. For the last several years, hairy roots have been investigated as a biological system for the production of valuable compounds from medicinal plants. A better understanding of the molecular mechanism of hairy root development, which is based on the transfer of *Agrobacterium rhizogenes* into the plant roots, has facilitated its increasing of secondary metabolites. Hairy roots can also produce recombinant proteins from transgenic roots and there by hold immense potential for the pharmaceutical industry. In addition, hairy roots offer promise for phytoremediation because of their abundant neoplastic root proliferation. Recent progress in the scaling-up of hairy root cultures is making this system an attractive tool for research and industrial processes.

A number of references available on compounds of hairy culture roots of *C. roseus*, but only relevant compounds regarding the isolated or extracted and identified from culture hairy roots on the basis of identification method IR, NMR and Mass or HPLC are reported here.

TABLE-1
COMPOUNDS FROM *Catharanthus roseus* CULTURED HARIY ROOTS

Compounds name	Identification method	Reference No.
Some general work regarding compounds isolated from other parts and cell suspension cultures and biological activities	As given in references	1 -11
Ajmalicine, serpentine, vindolinine, catharanthine. Vinblastine	HPLC and radio immunoassay	12
Five indole alkaloids, belonging to a new series of yohimbine type bases (anthraserpine, dimethoxyanthraserpine).	Spectral analysis	13
Catharanthine	HPLC	14
Ajmalicine, serpentine and catharanthine, vindoline	HPLC and GC-MS	15
Production of secondary metabolites through hairy culture roots	HPLC	16
Ajmalicine and catharanthine	HPLC	17
Tabersonine, ajmalicine and serpentine	HPLC	18
Ajmalicine, serpentine, catharanthine, tabersonine and tryptamine	HPLC	19
Sterols and some indol-alkaloids	HPLC	20
Tabersonine and löchnericine (indole alkaloid)	HPLC	21
Tryptophan and tryptamine, lochnericine,	HPLC	22
Serpentine, ajmalicine, yohimbine, akuammicine analogs fluorinated and methylated	LC-MS and NMR	23
2,3-Dihydroxybenzoic acid; 2,3-dihydroxybenzoic acid glucoside; salicylic acid; salicylic acid glucoside; benzoic acid; gallic acid; glucovanillin; vanillic acid; glucovanillic acid; vanillyl alcohol; vanillyl alcohol phenyl-glucoside; *trans*-cinnamic acid; hydroxytyrosol; ferulic acid; chlorogenic acid; kaempferol; kaempferol trisaccharides; quercetin; quercetin trisaccharides; syringetin glycosides; malvidin; malvidin 3-Oglucosides; malvidin 3-O-(6-O-pcoumaroyl); petunidin; petunidin 3-oglucosides; petunidin 3-O-(6-Op-coumaroyl; hirsutidin; hirsutidin 3-oglucosides; hirsutidin 3-O-(6-O-*p*-coumaroyl)	Reported from review	24
Caffeic acid; chlorogenic acid; *trans*-cinnamic acid; *p*-coumaric acid; ferulic acid; *p*-hydroxybenzoic acid; salicylic acid; syringic acid; (+)-catechin; hesperidin; myricetin; naringenin; naringin.	HPLC	25
3,7,11,19,23,27-Hexamethyl-15-hydroxymethylene-*n*-octacos-5,8,20-triene-10β,18α-diol-10β-D-glucopyranoside; 3-epibetulinic acid, *n*-pentadecanyl octa-dec-19-en-oate and β-sitosterol	Column chromatography, IR, mass and NMR	26
Lanast-5,8-dien-3β-ol-27-oic acid-3β-D-glucopyranosyl (4'-1")-10",11"-dimethoxy anthracene; 2-methoxy-6-(*n*-nonacontan-5",6"-dionyl)-11-hydroxy-13-methyl-11β-D-rhamnopyranoside anthracene	Column chromatography, IR, mass and NMR	27
Guaia-2,8-dien-4α, 15-diol-15β-D-glucopyranoside; cadin-3-en-2β-ol-2-β-D-glucopyranoside	Column chromatography, IR, mass and NMR	28
Heptacosan-13α-ol-13β-D-glucopyranoside; *n*-henetetracont-36-3*n*-5β-ol	IR, mass and NMR	29
3-Methoxy-6,8-dimethyl-β-naphthyl-β-D-glucopuranosyl-6'-pimaran-17''-oic acid ester; 1-methoxy-7,8-dimethyl-β-naphthyl-β-D-glucopuranosyl-4'-pimaran-17''-oic acid ester	Column chromatography, IR, mass and NMR	30
3,5,7-Trihydroxy-3',4'-dimethoxyflavone-7β-D-glucopyranosyl (4"-13"')2"',6"',10"',14"'-tetramethyl hexadec-14-ene; 3,5,7-trihydroxy-4'-methoxyflavone-3β-D-glucopyranosyl (4"-13"')2"',6"',10"',14"'-tetramethyl hexadecane; 6"-(3"',11"'-dimethyl dodec-3"',7"'(14"'),10"'-trienyl glucopyranosyl-7-hydroxy-3',4'-dimethoxyflavonone; 6"-(3"',11"'-dimethyl-7"'-hydroxymethylene dodecanyl)glucopyranosyl-7, 3'-dihydroxy-4'-methoxyflavonone	Column chromatography, IR, mass and NMR	31
Indole alkaloids and terpenoid indole alkaloid	HPLC	32, 33, 35, 38, 39, 40, 46
Hairy roots model in plant metabolic engineering.	HPLC	34
Secondary metabolites	HPLC	36
Production of chemicals through root cultures	HPLC	37
Review on plant secondary metabolites of large-scale culture of plant cells	HPLC	41
Catharanthine	HPLC	42
Ajmalicine, serpentine, ajmaline and catharanthine	HPLC	43
Alkaloid production	HPLC	44
Genetic engineering and expression of vindoline biosynthesis	HPLC	45
Serpentine	HPLC	47
Patent: Process for production of antidiabetic compound (serpentine) in root culture of *C. roseus*	Spectroscopic methods	48
Patent: Natural products from *Vinca* (compounds reported same as reference in 26 -31)	IR, Mass and NMR	49

Normally, adventitious root cultures need an exogenous phytohormone supply and grow very slowly. Hairy roots can be produced by transformation with the soil bacterium *Agrobacterium rhizogenes*, in addition they produce similar secondary metabolites to the normal roots and much higher levels than do cell cultures.

Transformed roots of *C. roseus* were obtained following infection of detached leaves with *Agrobacterium rhizogenes*. Roots would not grow in full strength Gamborg's B5 medium but would grow satisfactorily if the medium was diluted to one half strength. Little alkaloid appeared in the growth medium but root tissue contained a high level and wide variety

of alkaloids. Ajmalicine, serpentine, vindolinine and catharanthine were prominent components. Vinblastine could also be detected by a combination of HPLC and radioimmunoassay, though at a level of only 0.05 μg/g dry weight[12].

From the *in vitro* hairy-root cultures of *Catharanthus* have been reported five indole alkaloids, belonging to a new series of yohimbine type bases. Anthraserpine and dimethoxyanthraserpine have been found to contain the 11-methoxyepiallo-yohimbine skeleton, esterified at C-18 with 2-acetamidobenzoic acid and 2-acetamido-4,5-dimethoxybenzoic acid, respectively. Their structures have been established by detailed spectral analysis. Isolated in trace amounts, three other congeners of the same series have been identified on the basis of their mass spectral data[13].

Improvement of the catharanthine productivity in hairy culture roots of *C. roseus* by using monosaccharides as a carbon source[14]. Sucrose, glucose and fructose as carbon sources in culture medium were assessed in hairy root cultures of *C. roseus*. The cultures preferentially consumed sucrose, resulting in about 40 % (dry wt.) higher growth rate. However, fructose enhanced the cathranthine yield about two-fold. The elevated yield was not seemingly ascribed to the higher osmolarity per unit weight of fructose than sucrose. A two stage culture using sucrose (1st) and fructose (2nd) improved volumetric yields of catharanthine about two-fold, *i.e.*, 41 mg/L[14].

Hairy culture roots of *C. roseus* were established by infection of seedlings with *Agrobacterium rhizogenes* 15834[15]. About 150 transformants from four different. *C. roseus* cultivars were screened for desirable traits in growth and indole alkaloid production. Five hairy root clones grew well in liquid culture with doubling times similar to those reported for cell suspensions. Fast growing clones had similar morphologies, characterized by thin, straight and regular branches with thin tips. The levels of key alkaloids, ajmalicine, serpentine and catharanthine, in these five clones, also compared well with literature data from cell suspensions, yet HPLC and GC-MS data indicate the presence of vindoline in two clones at levels over three orders of magnitude greater than the minute amounts reported in cell culture. These results suggest that further optimization may result in hairy roots as a potential source of vindoline and catharanthine, the two monomers necessary to synthesize that antineoplastic drug, vinblastine[15].

In this review, the establishment and cultivation of hairy root cultures as well as their properties and application for production of secondary metabolites was discussed[16].

Two year old, transformed root cultures of *C. roseus* accumulate ajmalicine and catharanthine (0.57 and 0.36 mg g^{-1} DW or 7.0 and 3.0 mg L^{-1}, respectively). Changes in the concentration of the medium components, as well as the addition of hydrolytic enzymes and biotic elicitors, were used as strategies to increase these alkaloid yields. Regarding the components of the medium, the results obtained, when sucrose was raised from 3.0-4.5 %, are noteworthy[17].

The kinetics of growth, the uptake of macronutrients and the accumulation of indole alkaloids were investigated in long-term, heterotrophically cultured transgenic hairy roots of *C. roseus*. Tabersonine, ajmalicine and serpentine were monitored over a 70-day period[18].

Two direct HPLC analytical methods for the screening of the major indole alkaloids of *C. roseus* hairy roots and their iridoid precursors have been developed. Photodiode array and fluorescence detection were performed. The separation was achieved on a reversed-phase C_{18} column. The first method allowed the separation of catharanthine, serpentine, tabersonine, vindoline, vinblastine and vincristine in 20 min. Ajmalicine, tryptophan, tryptamine and secologanine were separated using the second method in 13 min. The identification of the compounds was based on the retention time and the comparison of UV spectra with those of authentic standards. A simplified alkaloid extraction method was developed in order to accelerate sample preparation. The assays were successfully used to quantify major compounds of the secondary metabolism of hairy root cultures of *C. roseus*, thus providing a reliable tool for rapid screening of *C. roseus* secondary metabolite samples. In these cultures, ajmalicine, serpentine, catharanthine, tabersonine and tryptamine were detected, but tryptophan, vindoline, vinblastine and vincristine were not identified[19].

Catharanthus roseus (L.) G. Don hairy roots harboring hamster 3-hydroxy-3-methylglutaryl-CoA reductase without membrane-binding domain were evaluated by quantifying the levels of sterols and some indole-alkaloids[20].

Continuous selective extraction of secondary metabolites (indole alkaloids tabersonine and lochnericine) from *C. roseus* hairy roots were reported [21].

Different plant species produce a variety of terpenoid indole alkaloids, which are of interest as plant defensive secondary metabolites and as valuable pharmaceuticals[22]. Although significant progress has been made, the mechanisms regulating the levels of this important class of compounds require continued elucidation. Previous precursor feeding studies have indicated that alkaloid accumulation can be improved during the exponential growth phase of hairy root cultures through enhanced tryptophan availability. To test this relationship, transgenic hairy root cultures of *C. roseus* were established[22] with a glucocorticoid-inducible promoter controlling the expression of an arabidopsis feedback-resistant anthranilate synthase α-subunit. Despite the large increases in tryptophan and tryptamine, the levels of most terpenoid indole alkaloids were not significantly altered, with the exception of lochnericine, which increased 81 % after a 3-day induction period. These results suggest that terpenoid indole alkaloid levels are tightly controlled[22].

Terpene indole alkaloids (TIA) are plant natural products with diverse structures and biological activities. A highly branched biosynthetic pathway is responsible for the production of approximately 130 different alkaloids in Madagascar periwinkle (*C. roseus*) from a common biosynthetic intermediate. The well known of these alkaloids in the anticancer agent vinblastine. High resolution (HR) LC-MS is used in conjunction with the extensive knowledge of the *C. roseus* metabolome to predict the structures of the analogs. Most abundant alkaloid analogs are isolated and subjected to NMR spectroscopy. This work demonstrates that the terpene indole alkaloids biosynthetic machinery can be used to produce many novel alkaloid structures and also highlights the potential of

this pathway for future metabolic engineering efforts. Serpentine, ajmalicine, yohimbine, akuammicine analogs fluorinated and methylated were reported[23].

Thirty three phenolic compounds have been reported from *C. roseus* of cell suspension culture, plant, leaves, flower, stem, flower callus culture, flower and cell suspension culture in a review[24].

Thirteen phenolic compounds in response to ectopic over expression of tryptophan feedback-resistant anthranilate synthase holoenzyme in *C. roseus* hairy roots have been reported through HPLC identification methods[25].

Four compounds 3,7,11,19,23,27-hexamethyl-15-hydroxymethylene-*n*-octacos-5,8,20-triene-10β,18α-diol-10β-D-glucopyranoside; 3-epibetulinic acid; *n*-pentadecanyl octa-dec-19-en-oate and β-sitosterol were reported from the methanolic extract of the hairy culture roots of *C. roseus*. The structures all compounds were elucidated using one- and two-dimensional NMR in combination with IR, EI/MS, FAB/MS[26].

Two compounds lanast-5,8-dien-3β-ol-27-oic acid-3β-D-glucopyranosyl (4'-1'')-10'',11''-dimethoxy anthracene; 2-methoxy-6-(*n*-nonacontan-5'',6''-dionyl)-11-hydroxy-13-methyl-11β-D-rhamnopyranoside anthracene have been reported from hairy culture roots of *C. roseus*. Their structures have been elucidated with the help of NMR using one- and two-dimensional NMR in combination with IR, EI/MS, FAB/MS and HRFABMS spectroscopy[27].

Two compounds cadin-2-en-1β-ol-1β-D-glucuronopyranoside; guaia-1,7-dien-3β,13-diol-13α-D-glucofuranoside have been reported from the culture hairy roots of *C. roseus*. Their structures have been elucidated with the help of NMR using 1D and 2D spectral methods of NMR aided by EIMS, FAB-MS, HR-FABMS and IR spectroscopy[28].

Three compounds *n*-heptacosan-13α-ol-13β-D-glucopyrasnoside, *n*-hentetracont-36-en-5β-ol and β-sitosterol were reported from the methanolic extract of the hairy culture roots of *C. roseus*. The structures of these compounds were elucidated by a combination of spectral methods *viz.*: IR, EIMS, FABMS, ^{1}H and ^{13}C NMR[29].

Two compounds catharanthusopimaranoside A and catharanthusopimaranoside B have been reported from the hairy culture roots of *C. roseus*. Their structures have been elucidated with the help of NMR using 1D and 2D spectral methods *viz.*: NMR, EIMS, FABMS and IR spectroscopy[30].

Four flavonoid glucosides, 3',4'-di-O-methylquercetin-7-O-[(4''·13''')-2''',6''',10''',14'''-tetramethylhexadec-13'''-ol-14'''-enyl]-D-glucopyranoside; 4'-O-methylkaempferol-3-O-[(4''·13''')- 2''',6''',10''',14'''-tetramethylhexadecan-13'''-olyl]-D-glucopyranoside; 3',4'-di-O-methylbutin-7-O-[(6''·1''')-3''',11'''-dimethyl-7'''-methylenedodeca-3''',10'''-dienyl]-D-glucopyranoside and 4'-O-methylbutin-7-O-[(6''·1''')-3''',11'''-dimethyl-7'''-hydroxymethylenedodecanyl]-D-glucopyranoside were reported from the methanol extract of *C. roseus* culture hairy roots. Their structures were elucidated spectroscopically. The new flavonoid glucosides inhibited both MMP-9 activity and TNF-R production in THP-1 cells treated with lipopolysaccharide[31].

Culture conditions from reference 26-31 were reported same: The hairy root line used in this study was previously generated by infection of *C. roseus* cv. Little bright eye seedlings with *Agrobacterium rhizogenes* 15834. The culture media consisted of a filter-sterilized solution of 3 % sucrose, half-strength Gamborg's B5 salts and full-strength Gamborg's vitamins with the pH adjusted to 5.7. The 50 mL cultures were grown in 250 mL Erlenmeyer flasks to late exponential phase in the dark at 26 ºC and 100 rpm.

Cultures of *C. roseus* transgenic hairy root clones LBE-6-1 and LBE-4-2 were adapted with periodic daily illumination to investigate the effect of light on growth and nutrient utilization and the accumulation of the indole alkaloids. Light-adapted roots appeared green and had radially thickened morphology compared with dark-grown controls. Their growth rates were higher than dark-grown controls, with 45 % lower doubling times: LBE-6-1, 3.6 days; LBE-4-2, 2.8 days[32].

Late exponential phase hairy culture roots of *C. roseus* were elicited with pectinase and jasmonic acid. The effects of elicitor concentration and exposure time on growth and levels of several compounds in the indole alkaloid biosynthetic pathway were monitored[33].

Due to their fast growth rates and biochemical stability, 'hairy root' cultures remain unsurpassed as the choice for model root systems and have promise as a bioprocessing system. Applications are wide-ranging, from the production of natural products and foreign proteins to a model for phytoremediation of organic and metal contaminants. Hairy roots will have a continuing role as an experimental model in plant metabolic engineering[34].

Precursors from the terpenoid and tryptophan branches were fed to *C. roseus* to determine which of the two branches limits metabolic flux to indole alkaloids. The feeding of tryptophan at 17 days of the culture cycle produced auxin-like effects. Addition of low levels of auxin or tryptophan resulted in significant increases in flux to the indole alkaloids. Conversely, feeding higher levels of auxin or tryptophan resulted in increased branching and thickening of the hairy root cultures[35].

Agrobacterium rhizogenes conn. causes hairy root disease in plants. Hairy root-infected *A. rhizogenes* is characterized by a high growth rate and genetic stability. Hairy root cultures have been proven to be an efficient means of producing secondary metabolites that are normally biosynthesized in roots of differentiated plants. Furthermore, a transgenic root system offers tremendous potential for introducing additional genes along with the Ri plasmid, especially with modified genes, into medicinal plant cells with *A. rhizogenes* vector systems. The cultures have turned out to be a valuable tool with which to study the biochemical properties and the gene expression profile of metabolic pathways. Moreover, the cultures can be used to elucidate the intermediates and key enzymes involved in the biosynthesis of secondary metabolites. The present article discusses various applications of hairy root cultures in plant genetic engineering and potential problems associated with them[36].

This paper reviews recent studies of secondary metabolism in higher plants, with emphasis on the use of organized systems such as the 'hairy roots' obtained from transformation of plant cells with *A. rhizogenes*. The potential contribution of these

novel systems to the production of speciality chemicals through scaled-up cultures is discussed[37].

In vitro cultures of hairy root derived from *C. roseus* accumulate higher levels of indole alkaloids than cell suspension cultures[38]. Growth and terpenoid indole alkaloid production in *C. roseus* hairy root clones[39]. Increase in the indole alkaloid production and its excretion into the culture medium by calcium antagonists in *C. roseus* hairy roots[40]. Review on plant secondary metabolites by means of large-scale culture of plant cells in bioreactors is technically feasible[41].

Hairy root cultures offer promise for production of valuable secondary metabolites in many plants. The effects of the concentrations of inorganic salts in Schenk and Hildebrandt (SH) medium on catharanthine production in hairy root cultures of *C. roseus* were investigated[42].

After the elicitation of *C. roseus* hairy roots with different concentrations of methyl jasmonate (MeJA), changes in the accumulation of alkaloids such as ajmalicine, serpentine, ajmaline and catharanthine were observed. In addition to the increased accumulation of alkaloids in the tissues, the root exudation of phytochemicals increased compared to that of the non-treated control hairy roots. Moreover, methyl jasmonate induced differential secretion of several *C. roseus* hairy root metabolites[43]. Effect of precursors and organic compounds on alkaloid production in transformed root cultures of *C. roseus*. var. nirmal[44].

Madagascar periwinkle [*Catharanthus roseus* (L.) G Don] is a pantropical plant of horticultural value that produces the powerful anticancer drugs vinblastine and vincristine that are derived from the dimerization of the monoterpenoid indole alkaloids (MIAs), vindoline and catharanthine. The present study describes the genetic engineering and expression of the terminal step of vindoline biosynthesis, deacetylvindoline-4-O-acetyltransferase (DAT) in *C. roseus* hairy root cultures[45]. Studies on the optimization of growth and indole alkaloid production by hairy roots cultures of *C. roseus*[46]. Chemical induced production and release of alkaloids serpentine from hairy roots cultures of *C. roseus* var. Nirmal[47].

Process for production of anti-diabetic compound in root culture of *C. roseus* have been patented[48]. The present invention provides treatment of diabetes using serpentine. The present invention particularly provides a process of production of serpentine in *C. roseus* hairy root culture, effect of hairy root extract in reducing blood glucose level in a subject. The present invention further provides identification and isolation of biologically active compound from the extract[48].

Natural products from Vinca have been reported in US patent[49], it should be assessed whether there are flavonoids in hairy root cultures of *C. roseus* present. Hairy roots of this medicinal plant *C. roseus* are rich of phenolic compounds. For the basic study in the production of flavonoids in hairy roots of *C. roseus*. The following compounds were reported in this patent[49]. A pharmaceutical composition comprising an isolated compound selected from the group consisting of: 3,5,7-trihydroxy-3',4'-dimethoxyflavone-7β-D-glucopyranosyl (4"-13'") 2'",6'",10'",14'"-tetramethyl hexadec-14-ene (**1**); 3,5,7-trihydroxy-4'-methoxyflavone-3β-D-glucopyranosyl (4"-13'")-2'",6'",10'",14'"-tetramethyl hexadecane (**2**); 6"-(3'",11'"-dimethyl dodec-3'",7'"(14'"), 10'"-trienyl glucopyranosyl-7-hydroxy-3',4'-dimethoxyflavanone (**3**); 6"-(3'",11'"-dimethyl-7'"-hydroxymethylene dodecanyl) glucopyranosyl-7,3'-dihydroxy-4'-methoxyflavanone (**4**); *n*-heptacosan-13α-ol-13β-D-glucopyranoside (**5**); 3,7,11,19,23,27-hexamethyl-15-hydroxymethylene-*n*-octacos-5,8,20-triene-10β,18α-diol-10β-D-glucopyranoside (**6**); lanast-5,8-dien-3β-ol-27-oic acid-3β-D-glucopyranosyl (4'-1")-10",11"-dimethoxy anthracene (**7**); 2-methoxy-6-(*n*-nonacontan-5",6"-dionyl)-11-hydroxy-13-methyl-11β-D-rhamnopyranoside anthracene (**8**); 1-methoxy-7,8-dimethyl-β-naphthyl-β-D-glucopuranosyl-4'-pimaran-17"-oic acid ester (**9**); 3-methoxy-6,8-dimethyl-β-naphthyl-β-D-glucopuranosyl-6'-pimaran-17"-oic acid ester (**10**); guaia-1,7-dien-3β,13-diol-13α-D-glucofuranoside (**11**); cadin-2-en-1β-ol-1β-D-glucuronopyranoside (**12**) and combinations there of, together with a pharmaceutically acceptable carrier[49].

Concluding remarks: At last culturing hairy roots, a promising system to produce valuable metabolites through different conditions. Many plant secondary metabolites of interest are accumulated in roots. Harvesting roots is destructive for the plants and hence there has been increasing interest in developing hairy root cultures from several medicinal plant species. A survey of literature on hairy root cultures compounds are reported in Table-1 which provides evidence of continuous progress in this area. Recent developments indicate that the hairy root culture technology for the production of chemicals as pharmaceuticals useful techniques. Therefore, hairy roots can offer a valuable source of root derived secondary metabolites that are useful as pharmaceuticals, cosmetics and food additives. Transformed roots of many plants species have been widely studied for the *in vitro* production of secondary metabolites.

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Prof. (Dr.) Ram K. Agarwal, Editor-in-Chief, Asian Journal of Chemistry and Prof. Sultan T. Abu-Orabi, President of Yarmook University Irbid, Jordan with her Royal highness Princess Sumaya Bint El-Hassan, President of El-Hassan Science city and Royal Scientific Society, Jordan during inaugural session of 6th Jordanian International Conference of Chemistry at Yarmook University, Irbid, Jordan on 19th April 2011.