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Inula racemosa: An Insight into Callus Induction, Secondary Metabolites and its Therapeutic Potential

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Abstract
Inula racemosa (Hook.f), commonly referred to by the name Pushkarmula, is an important medicinal and ornamental plant of Asteraceae family and has numerous references in Ayurvedic scriptures. The plant is inhabited to the Western Himalayas and grows at an altitude ranging from 1300 to 4500 meters. The plant is a perennial, paraphyletic, stout herb with elliptical, large leaves and yellow flowers. Extracts made from different plant parts are used to treat a number of diseases in the form of oil, ointment, syrup, powder, tablet etc. either by consumption or application. Traditionally, its roots have antiseptic, anti-bacterial, anti-fungal, anti-inflammatory and analgesic properties. Because of these properties it is used in the treatment of contagious fevers, heart diseases, indigestion and other diseases related to respiratory tract. Due to over exploitation and reckless collection of the species by pharmaceutical companies, it has become critically endangered in its own habitat. To conserve the germplasm of this therapeutically important species from extinction, *in vitro* propagation strategies are required. The major constraint in micropropagation strategies is the long waiting period ranging from six to eight months. To overcome this problem, propagation of numerous calli from leaves can be obtained in just 2-3 weeks. The secondary metabolites like Isoalantolactone and Alantolactone can be obtained from callus for further investigation. This review article discusses the importance of callus induction for mass multiplication and production of secondary metabolites. Mass multiplication of *Inula racemosa* not only helps in the conservation of this medicinally important plant by preventing the uprooting of plants to extract roots for obtaining alkaloids but also in the synthesis of secondary metabolites in dried calli. Besides, the review also throws light on the therapeutic value of *Inula racemosa*.

Keywords: *Inula racemosa*, callus induction, secondary metabolites, antimicrobial properties

1. Introduction
Nature has been a source of medicinal agents for thousands of years and ever since the beginning of human civilization, medicinal plants have been used by mankind for their therapeutic values. The term "herbal drug" often used refers to the parts of a plant (leaves, flowers, seeds, roots, bark, stem) used for preparing medicines.

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30 percent of the entire plant species, at one time or the other are used for medical purposes. It is estimated that world market for plant derived drugs accounts for nearly about Rs. 3,00,000 crores in which India contributes about Rs. 3,000 crores approximately. According to an estimate, plant drugs constitute as much as 30 percent of the total drugs in developed nations, whereas, in fast developing countries like China and India, the contribution is as high as 80 percent. The economic importance of medicinal plants is therefore far more in developing countries than in the rest of the world.

In Indian system, Ayurveda has plenty of references of plants being used for medicinal purposes. For instance, 595 plants are referred for their therapeutic use in Charak Samhita (100 BC). The references of medicinal plants are also found in Rig-Veda (67 species), Atharv-veda (290 species) and Yajur-veda (81 species). India has a huge genetic pool of medicinal plants as around 20,000 medicinal plant species are found here (Dev, 1997) [1].

Among most important medicinal plants in India, *Inula* is a large genus of about 90 species of flowering plants belonging to Asteraceae family. The most common of these are *Inula acaulis*, *Inula acuminate*, *Inula candida*, *Inula cappa*, *Inula falconeri*, *Inula helvetica*, *Inula nervosa*, *Inula racemosa*, *Inula stewartii* and *Inula subfloccosa*. *Inula* is native to Europe, Asia and Africa. The genus is thought to be paraphyletic (consisting of all the descendants of the last common ancestor of the group members) based on study of the different phenol compounds they possess. Mostly they are perennial herbs varying greatly in size from small species that are few centimeters tall to enormous perennials over 0.33-2 meters tall. Some common

characteristics include pappus with bristles (feather like structures seen on magnification), flat capitulum ("false flower" or flower head with a special type of inflorescence in which several flowers are grouped together to form a flower like structure) and lack of chaff (dry, scaly plant material such as in flower).

The plant is one of the most important medicinal and ornamental plants mentioned in Ayurvedic scriptures. The plant is common to Kashmir in India because it grows in temperate and alpine western Himalayas ranging from 1300 to 4500 meter of elevation. The stem is grooved, rough and very hairy, the leaves are elliptical, large and 2-3centimeters broad, having long petioles and the flowers are yellow which are 0.5 -1 centimeters in diameter. The fresh root is brown and resembles camphor in aroma that later turns grey on drying. It starts growing in spring and at the end of the season its leaves fall off and the plant dries but can be preserved for long time as it lasts for years.

The leaves are bitter and pungent in taste, spicy in post digestive effect and has hot potency. The rhizome is sweet, bitter acrid in taste with a neutral potency. Extracts of the plant have been used to treat various diseases in the form of oil, ointment, syrup, powder, tablet etc. It can be used by consuming or applying and it shows activity against several gram-positive and gram-negative bacteria (Chourasia and Rao 1987) [2]. It is also used as an antiseptic, anti-fungal, anti-inflammatory, analgesic and mild diuretic for the treatment of contagious fevers, heart diseases, indigestion and diseases related to respiratory tract. *Inula racemosa* is also useful in boosting the appetite and is efficacious adjunct in the treatment of obesity by reducing excessive body fats. Paste of its roots is used in dressing the wounds and ulcers because this herb possesses antiseptic property by restricting the itching sensation and oozing in the skin diseases and thus facilitates wound healing which alleviates pain along with edema. It pacifies the brain and helps to strengthen mental ability. The herb also accords a stimulant action to genital system in both the sexes (used for both amenorrhea as well as dysmenorrheal).

Roots of *Inula racemosa* possess sesquiterpenes as secondary metabolites that belongs to the class of terpenes consisting of three isoprene units and have a molecular formula $C_{15}H_{24}$. Like monoterpenes, sesquiterpenes may be acyclic or contain rings, including many unique combinations. A biochemical modification such as oxidation or rearrangement produces the related sesquiterpenoids. They are found naturally in plants and insects as semiochemicals like defensive agents or pheromones. Isoalantolactone, alantolactone and their dihydro derivatives are found to be responsible for antimicrobial activity in *Inula racemosa.*

Due to over exploitation, the species has become critically endangered in its own habitat and needs to be conserved by adopting new technologies such as tissue culture. So far, this plant has not got the required attention from researchers and except for a few efforts, not much work has been done for its cultivation, conservation and its anti-bacterial activity.

2. Propagation

Among 20 species of *Inula* occurring wild in India, five have economic significance and *Inula racemosa* is one of them because of its medicinal and aromatic properties. *Inula racemosa* can be propagated by seeds as well as by divisions of rhizomes. Sanders (1926) [3] gave description of propagation in *Inula racemosa* by seeds. Seeds germinate well when sown in spring or autumn in a cold frame and germinated seedlings are picked and shifted into individual pots when they are large enough to be handled. However, propagation by seed is a time consuming technique that can be overcome by tissue culture. Propagation of *Inula racemosa* by divisions of roots was studied by Arora *et al*. (1980) [4]. The species was grown on small scale in Lahaul valley in North Western Himalaya. Its cultivation was restricted to the borders of agricultural fields of wheat through root cuttings. The collar portion of the root gave better performance in sprouting and survival percentage. In general, plant prefers porous soils for faster root growth.

Propagation of *Inula racemosa* through seeds has been reported by Thomas (1990) [5] who observed that it takes a few years for the plant to become fully established. Sharma *et al*. (2006) [6] studied germination behavior of *Inula racemosa* seeds collected from Lahaul and Spiti district of Himachal Pradesh. The efficacy of chilling, acid scarification, KNO_3 and GA_3 treatments were tried for germination and the studies revealed that the complete dormant seeds start germinating with 80 percent efficiency when treated with GA_3.

Uses and cultivation practices of *Inula racemosa* have been described by Rawat *et al.* (2011) [7] who also elaborated problems related to its cultivation in the cold desert environment of Lahaul valley of North Western Himalayas. Owing to its medicinal property in roots, the plants are economically important in the pharmaceutical industry. The survey revealed that the lengthy cultivation cycle, prevalence of small land holdings and continuously fluctuating market prices were main reasons for decline in its cultivation. The study concluded that there is a need for technical support, the establishment of value-addition centers and establishment of farmer federations as well as mechanisms to strengthen and stabilize the existing markets in order to promote the cultivation of this endangered medicinal herb.

2.1 *In vitro* propagation

Based on the success of plant cell culture techniques, many advances have been made in the area of micropropagation at mass scale, production of secondary metabolites, pathogen free plants and genetic manipulations. Stojakowaska and Malarz (2004) [8] worked on micropropagation of *Inula royleana* DC. through axillary shoot proliferation using primary explants (cotyledonary node explants) and secondary explants (node explants of *in vitro* regenerated shoots) and inoculated them on MS medium supplemented with 0.1 μgl^{-1} NAA and 5.0 μgl^{-1} Kinetin and reported that after six weeks of culturing from primary explants axillary shoots regenerated 3.4±1.2 with 100 percent frequency and from secondary explants it was 5.1±1.9 with 94 percent frequency respectively and were easily rooted and adapted for growing in soil.

Micropropagation of *Inula racemosa* has also been studied by Jabeen *et al*. (2007) [9] who reported that 2-3 centimeter long petiole pieces were best responding explants. Different levels of growth hormones (IBA, NAA and 2, 4-D in combination with BAP) were used. The results concluded that MS medium supplemented with BAP (0.25 mgl^{-1}) induced maximum number of shoots (20.7±0.8) and when shoots were rooted on half strength MS medium either alone or in combination with IBA (1.0 mgl^{-1}) the plants rooted directly. *In vitro* raised plantlets when acclimatized in greenhouse and successfully transplanted to field had a survival rate of 80 percent.

Protocol for induction of multiple shoots from nodal segments

on *Inula racemosa* has been proposed by Jabeen *et al.* (2007) [9]. Leaf and nodal segments were inoculated on MS medium containing different concentrations of BAP either alone or in combination with NAA or IBA. MS medium supplemented with BAP (0.25mgl^{-1}) induced maximum number of shoots (20.7±0.8) and the same were rooted on half strength MS medium either alone or supplemented with IBA (1.0mgl^{-1}). *In vitro* conserved *Inula racemosa* material could be retrieved and multiplied normally on MS medium fortified with 1.00mgl^{-1} BAP which has been recorded as the best performing medium for *in vitro* shoot multiplication (Kaur *et al*., 2010) [10].

2.2 Callus induction (indirect regeneration)

Callus is an amorphous mass of loosely arranged thin walled parenchymatous cells developing from proliferating cells of the parent tissue (Dodds and Roberts 1985) [11]. Kaloo and Shah (1997) [12] reported *in vitro* propagation protocol for *Inula racemosa*. They used different combinations of auxins and cytokinins for this purpose. When MS medium supplemented with 10^{-6} M Kinetin and 10^{-5}M NAA was used, callus was observed at the cut ends of the shoots when buds were used as explants. Cut ends were swollen to form green coloured calli from which arose numerous roots while MS medium supplemented with 10^{-5}M NAA and 10^{-6} M BAP produced calli at cut ends but without roots.

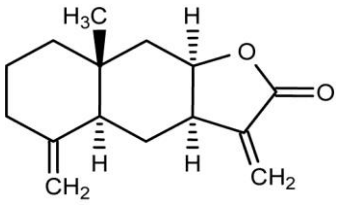
Callus induction and micropropagation of *Inula viscose* was reported by Romano *et al*. (1999) [13]. Callus cultures were initiated from leaves of *in vitro* cultured plants. The best conditions for callus induction and growth were found to be in B5 medium supplemented with 1mgl^{-1} 2, 4-D and 0.10 mgl^{-1} Kinetin under 16hrphotoperiod. Thiem *et al.* (2003) [14] studied *in vitro* culture of *Inula verbascifolia* using shoot tips of aseptically germinated seedlings. Callus was obtained which further produced adventitious shoots on MS medium containing BAP (0.88 µgl^{-1}).

MS medium with 2, 4-D 1.2 mgl^{-1} was found to be the best for callus induction using rhizomes as explants in *Inula japonica* by Bian *et al.* (2008) [15]. Hong (2010) [16] studied the effect of different concentrations of Thidiazuron (TDZ) for callus induction using leaves and roots of *Inula britannica* which was raised from sterile seedlings as explants. Their work concluded that MS medium without TDZ showed higher induction rate of callus from leaves and MS medium with TDZ 0.05 mgl^{-1} proved to be most effective for inducing callus from roots and induction rate was 100 percent for both the treatments.

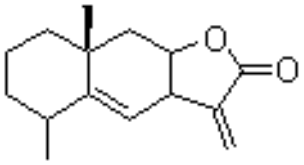
3. Secondary metabolites of *Inula racemosa*

The extracts may contain the secondary metabolites present in the plant which may show antibacterial and antifungal properties. Isoalantolactone, a major sesquiterpene lactone was found to show antifungal activity against pathogenic fungi like *Aspergillus flavus, Aspergillus niger, Geotrichum candidum, Candida tropicalis* and *Candida albicans* (Tan *et al.*, 1998) [17].

Ketai *et al*. (2000) [18] studied phytochemicals in *Inula racemosa* and demonstrated the presence of alantolactone and isoalantolactone, dihydroalantolactone, dihydroisoalantolactone, sitosterol, daucosterol, inunolide, aplotaxane, phenylacetonitrile and isoinunal. A simple micellar electrokinetic chromatography (MEKC) method was used with 20 mm borate buffer (pH 8.0) and 25 mm SDS in the presence of 10 percent (v/v) methanol to establish the identification as well as determination of two isomers, Alantolactone and Isoalantolactone. The regression equations revealed linear relationships (correlation coefficients: 0.9990 for Alantolactone, 0.9991 for Isoalantolactone) between the peak area of (AL) and (ILA) and their concentrations. Relative standard deviations of the migration time and peak area of the constituents were estimated to be 1.51, 1.62 and 2.01, 1.98 percent, respectively. The recoveries of the two constituents ranged between 95-105 percent for AL and 93-108 percent for ILA.



Isoalantolactone



Alantolactone

The pharmacological properties of large class of compounds known as the sesquiterpene lactones were reported by Neerman (2003) [19]. These constitute a large and diverse group of biologically active plant chemicals that have been identified in the oils of several plant families. They are known to possess two pharmacological properties of interest involving antibacterial and anti-fungal activity.

Extract from the dried and hard roots, leaves, stem of *in vivo* grown *Inula racemosa* was prepared by Gawai *et al*. (2007) [20] by first chopping the plant parts into small pieces and crushing in mortar pestle which was further ground in blender to make a fine powder. The powder was soaked in different solvents like n-hexane, ethyl acetate, methanol, chloroform, benzene, toluene. These organic solvents were subjected to Thin Layer Chromatography (TLC) analysis. The anti bacterial activity was then conducted by gel diffusion method on different bacterial species like *Pseudomonas aeruginosa, Bacillus cereus, Serratia marcescens, Escherichia coli, Shigella dysenteriae, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumonia.* The results show effectiveness of alantolactone against *Pseudomonas aeruginosa*, *Bacillus aureus*, *Serratia marcescens*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Isoalantolactone was found to be effective against *Pseudomonas Aeruginosa*, *Bacillus aureus*, *Serratia marcescens*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumonia*.

A quantitative RP-HPLC method for the simultaneous determination of alantolactone and isoalantolactone was developed by (Hu and Yuan 2007) [21] in herb *Inula racemosa* using column of Phenomenex Kromasil C18 (4.6 mm×250 mm, 5.0 µm). The mobile phase consisted of acetonitrile and 0.04 percent phosphate (50:50). The flow rate was 1.0 ml·min^{-1} with UV detection wavelength at 194 nm. Isomer alantolactone and isoalantolactone showed good separable degree. A good linearity was obtained with the correlation

coefficients ranged of 0.07～4.80 μgl^{-1}(r=0.999 8) and 0.07～4.85 μgl^{-1}(r=0.999 8). The average recoveries were 97.5 percent and 102.1 percent. The precision and accuracy of the assay were 1.56 percent and 1.87 percent (n=5). The method was found to be rapid, accurate and reliable and can be used to control the quality of herb *Inula racemosa* and it products. Shi Haiming *et al*. (2009) [22] developed a protocol for structural elucidation of sesquiterpene lactones in *Inula helenium* where a high performance liquid chromatography (HPLC) method was used to separate igalone, isoalantolactone and alantolactone. Column used was Agilent Zorbax XDB –C(18) (250 mm, 4.6mm,5 mm) with a mobile phase consisting of 55 percent acetonitrile and 45 percent water, at a flow rate of 1.0ml/min and detected at 210 nm. The structures of the sesquiterpenes were later elucidated on the basis of NMR analysis

A protocol for the extraction of Alantolactone and Isoalantolactone from *Inula helenium* roots was suggested by (Chanev *et al.*, 2010) [23]. The effects of ethanol concentrations, extraction time, temperature and number of extraction steps were investigated on the extraction yields of both sesquiterpene lactones. For the determination of contents in the corresponding extracts, gas chromatography method was used and compared with classical extraction methods (maceration, infusion and micro steam distillation- extraction (MSDE)). The results showed that the amounts of Alantolactone and Isoalantolactone achieved by ultrasound assisted extraction (UAE) with 70 and 96 percent ethanol for 30minutes at room temperature were higher or almost equal to those obtained by maceration for 24hrs.

Large number of secondary metabolites like alkaloids, terpenoids, polyphenols and quinines produced by plants have been reported to be utilized as natural medicines as they exhibit activity of DNA topoisomerase which are clinical targets for anticancer drugs (Baikar and Malpathak 2010) [24]. DNA topoisomerases are the cellular enzymes that change the topological state of DNA through the breaking and rejoining of DNA strands. These studies concluded that although synthetic drugs were also implicated but they have severe side effects and therefore to overcome this problem plant derived natural drugs should be designed.

A novel trinor sesquiterpene, three new and 10 known sesquiterpenes from the roots of *Inula racemosa* were isolated by (Zhang *et al.*, 2011) [25]. The structures and absolute configurations of the new sesquiterpenes were elucidated by extensive spectroscopic and computational methods when all compounds were evaluated for their inhibition of LPS-induced nitric oxide production in RAW264.7 macrophages. It was observed that compounds 9, 12 and 13 moderately inhibited the production with IC_{50} values of 7.39 ± 0.36, 6.35 ± 0.26 and 5.39 ± 0.18 µM. Sesquiterpene lactones isolated from numerous genera of Asteraceae (compositae) and some angiosperm families possess wide variety of biological and pharmacological activities such as antimicrobial, cytotoxic, anti-inflammatory activities, effects on the central nervous and cardiovascular system as well as allergenic potency due to the presence of α-methylene-γ-lactones and α, β-unsaturated cyclopentenone ring in their structure (Chaturvedi, 2011) [26].

4. Therapeutic potential of *Inula racemosa*

Glucose level reduction in albino rats in a comparative study on the ethanol root extracts of *Inula racemosa* and *Saussurea lappa* was conducted by (Chaturvedi *et al.*, 1995) [27]. The studies revealed that *Inula racemosa* reduced blood glucose earlier as compared to *Saussurea lappa* in fasting albino rats. Maximum response in case of *I. racemosa* was observed between 2-4 hrs after drug administration while for *S. lappa* it was 4-8 hrs. Bing *et al*. (2001) [28] tested Isoalantolactone, a major constituent of *Inula racemosa* for its antimicrobial action against five bacteria, six humans and six plant pathogenic fungi. Lactone exhibited absolute toxicities at 500 $ugml^{-1}$ against 3 soil borne phytopathogenic fungi (*Gaeumannomyces graminis var. tritici*, *Rhizoctonia cerealis* and *Phytophthora capsici*) with the minimal inhibitory concentrations (MICs) determined to be 100, 200 and 300 μgml^{-1}, respectively. At the minimum inhibitory concentrations, isoalantolactone exhibited its fungistatic nature of toxicity. The lowest fungicidal concentrations of lactone to *G. graminis var. tritici*, *R. cerealis* and *P. capsici* were shown to be 150, 150 and 350μgml^{-1} respectively. Moreover, isoalantolactone displayed weaker antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Sarcinalentus* and *Staphylococcus aureus* with MICs of 125, 425, 150, 150 and 100 μgml^{-1}, respectively. The investigation disclosed the strong inhibition of isoalantolactone to the phytopathogenic fungi, raising a possibility that lactone could be considered as a starting point for the project aiming at the development of new fungicides.

Studies were conducted by Liu *et al.* (2006) [29] on Isoalantolactone isolated from roots of *Inula racemosa* to examine its antifungal activity. The investigation showed that Isoalantolactone also exhibited repellent and toxic activities against rice weevil (*Sitophilus oryzae*) based on food preference apparatus and a poisoned food technique. The toxicity of Isoalantolactone was found to be dose dependent whereas the repellency was not and Isoalantolactone showed strong phytotoxic effects on seed germination and seedling growth of wheat at a concentration of 500 μgml^{-1} for 60 hrs.

Staphylococcus aureus is a versatile pathogen that can cause life-threatening infections and the growing emergence of methicillin-resistant *S. aureus* strains and a decrease in the discovery of new antibiotics warrant the search for new therapeutic targets to combat infections (Leng *et al.*, 2011) [30]. It was reported that *Staphylococcus aureus* was observed to produce many extracellular virulence factors that contribute to its pathogenicity. Therefore, targeting bacterial virulence as an alternative strategy to the development of new antimicrobials has gained great interest as α-Toxin is a 33.2-kDa, water soluble, pore-forming toxin that is secreted by most *S. aureus* strains causing pneumonia. The present studies demonstrated that isoalantolactone (IAL), a naturally occurring compound found in *Inula helenium* (Compositae), has no or very less anti-*Staphylococcus aureus* activity as per MIC evaluation *in vitro*.

A protocol for antimicrobial assay was proposed by Bhawana *et al.* (2011) [31]. The methanolic extracts of *Inula cuspidata* leaves were examined for antibacterial activity against Gram positive *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative bacteria *Escherichia coli*, *Pneumonia aeruginosa* and antifungal activity against *Candida albicans* by cup plate method. Different dilutions of the extracts with concentrations of 100 μgml^{-1}, 250 μgml^{-1}, 500 μgml^{-1} and 1000 μgml^{-1} in dimethyl sulfoxide (DMSO). 0.1 ml of each test solution and control were placed in 6mm diameter wells made at equal distance using sterile cork borer. One well was filled with 0.1ml of standard drug Amoxicillin (10µg/disc) in case of antibacterial activity whereas standard drug Fluconazole

(10µg/disc) was used in antifungal activity. It was concluded that effectiveness on ***S. aureus*** was 20 mm against 100 µgml^{-1} extract, 24 mm against 250 µgml^{-1} extract, 27 mm against 500 µgml^{-1} extract, 30 mm against 1000 µgml^{-1} extract and 13 mm against standard drug Amoxicillin. Similar results were observed in case of *B. subtilis*, *Escherichia coli*, *P. aeruginosa* and *C. albicans*.

The antimicrobial activity of Isoalantolactone against *Staphylococcus aureus* was investigated by (Wu *et al.*, 2011) [32] and its influence on the production of α-toxin Minimal inhibitory concentrations (MICs) was determined by a broth micro-dilution method and effects of isoalantolactone on α-toxin production was assessed by haemolysis, Western blot and RT-PCR assays. The studies conducted that MICs of isoalantolactone against *S. aureus* were greater than 1,024 µgml^{-1} and lower concentration of isoalantolactone could substaintially inhibit the expression of α-toxin.

Antifungal activity of *Inula* species Viz., *Inula viscose, Inula gravedens* and *Inula crithmoides* was investigated against two *Trichoderma* species (*Trichoderma harzianum* and *Tricoderma viride*) and three formae species of *Fusarium oxysporum* by (Omezzine *et al.*, 2011) [33]. Leaf organic extracts (hexane, chloroform and methanol) of *I. viscosa* showed an important inhibitory activity against all target fungal isolates with growth inhibition percentage ranging between 17 and 100 percent. Flower organic extracts reduced mycelia growth of all fungi by 32 to 70 percent. Stem with leaf organic extracts of *I. graveolens* showed more or less important inhibition depending on solvent nature, though flower organic extracts were found to be the most effective against tested fungi. Leaf organic extracts of *I. crithmoides* showed inhibition against *T. harzianum* with all three extracts with hexane and methanol fraction against *T. viride* and with chloroform fraction against FOM. *I. crithmoides* flower extracts resulted in highly significant growth inhibition between 57 and 100 percent.

Two new eudesmane-type sesquiterpene lactones, 1-one-4-*epi*-alantolactone and 4α,13-dihydroxy-5,7(11)-eudesmadien-12,8-olide isolated from the roots of *Inula racemosa* were reported by (Zhang *et al*., 2010) [34] together with six known compounds. The cytotoxic activities against five human cancer cell lines were tested and compounds exhibited moderate cytotoxic activities. Other compounds showed potent *in vitro* activities against the release of β-glucuronidase in rat polymorphonuclear leukocytes (PMNs) induced by platelet-activating factor (PAF) with the inhibitory ratios of 65.4 percent ($P < 0.01$) and 80.5 percent ($P < 0.001$), at concentration of 10 µM, respectively.

5. Conclusion

The present review has made an attempt to elaborate the medicinal properties of *Inula racemosa* and the role of indirect organogenesis in mass multiplication of this therapeutically important plant to save this plant from extinction. The role of secondary metabolites obtained from *Inula racemosa* and their comparative efficacy in antimicrobial activity for use in pharmaceutical industry has also been discussed to highlight the potential use of this important medicinal plant. It is well established that *Inula racemosa* as a medicinal plant is of multifarious use and its indiscriminate extraction from its native areas has made it a very rare plant. This review clearly indicates that if *in vitro* approaches are judiciously applied then *Inula racemosa* not only can be conserved but its supplies can also be augmented to further support Ayurvedic system of Indian medicine besides pharmaceutical industry.

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