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PLANT HORMONES; ROLE IN ADVENTITIOUS ROOTS FORMATION IN MEDICINALLY VALUABLE COMPOUNDS IN EXTINCT PLANT SPECIES: A REVIEW

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Plant hormones are small molecules resulting from various essential metabolic pathways that play a critical role in the regulation of plant growth and development. The majority of plant species are the rich source of valuable bioactive compounds, where these compounds actively participate in various aspects of biosynthesis such as drugs, fragrances, flavor, dye, pigments, and pesticides. The dynamic role of bioactive compounds on a commercial scale surrounds the interest of the researchers and pharmaceutical industry. Therefore, it is a prerequisite to explore new species that produce these compounds beyond its flora. For this, several strategies were explored keeping the objective of bioactive compounds. In recent, the induction of adventitious roots by different PGRs and culturing the production of highly valuable compounds in several endangered plant species are practicing. The adventitious roots establish from cutting not only reduce pressure on natural populations but also helps in the conservation of these species and it can further be utilized for the production of bioactive compounds to up-scale the agricultural and pharmaceutical industries. Thus overall, these extinct species playing a beneficial role in our ecosystem as well as expand the area of new drug discovery for the welfare of human beings. Based on this limelight, the current review article focused on summarizing the application of plant growth regulator, especially auxin and cytokinin, and the progress made in the recent past in the area of initiation and establishment of adventitious root cultures for the production of bioactive compounds in laboratory conditions of biologically endangered and medicinally valuable species.

Keywords: PGRs; plant growth regulators, auxin, cytokinin, secondary metabolites, adventitious roots.

INTRODUCTION

Medicinal plants are the imperative valuable source of new drug discovery and ultimately boost up the global economy. Since so far, 49% of new drugs have been developed in the form of natural products or synthetic derivatives in the last few decades, while 85% of the traditional medicine is being prepared from plants parts or directly utilize their extracts (Epstein *et al.,* 1993; Kala *et al.,* 2004; Nafees *et al*., 2019), while 25% of prescribed medicines are derived from wild plant species (Hamilton, 2004). The pharmaceutical industries largely depend on the wild plants germplasm/biodiversity and the exploration of the new raw biological materials for the production/extraction of valuable bioactive compounds, herbal drugs, and secondary metabolites. According to the conservation estimation of the International Union for Conservation of Nature and the World Wildlife Fund, the flowering species are ranging from 50,000 to 80,000, which are used for medicinal purposes, among them 15,000 plant species are highly threatened through ruinous practices of over-harvesting, habitat destruction or due to emerging global issues producing the alarming situation to the future of new drugs discovery and increase threats to the ecosystem (Ross, 2007). Based on the highly conservative estimate the losses of these species are increasing with expected 1000 times natural extinction rate after every two years. The status of extinction of medicinal plants in some countries is very critical, like China (Heywood and Iriondo, 2003; Nalawade *et al.,* 2003), India (Heywood and Iriondo, 2003; Hamilton, 2008), Kenya (Hamilton, 2008), Nepal (Hamilton, 2008), Tanzania (Zerabruk and Yirga, 2012) and Uganda (Zerabruk and Yirga, 2012).

The plant secondary metabolites have been widely derived from different plant solvents that are contributing as potent bioactivities, anti-cancer, food additives, pesticides, drugs, flavors and fragrance, pigments, ingredients, and agrochemicals (Saklani and Kutty, 2008; Ahmad *et al.,* 2015; Alamgir, 2018). Although there are some limiting consequences of the derivation of these metabolites, like the parental plants often difficult to synthesize a large quantity production in laboratory conditions and the other concern is so far only 10% of higher plant species have been chemically characterized, while the rest of diverse plant kingdom for the potential pharmacological reservoirs yet not have been explored (Yuliana *et al.,* 2011; Atanasov *et al.,* 2015). More importantly, a large number of the endangered plant species also not yet chemically characterized might they buried valuable health effects, so before they are fully extinct it is being vital to biologically conserve them and research for bioactive compounds (Saklani and Kutty, 2008; Lee *et al.,* 2010). In recent, plant cell suspension and tissue culture technology attract speedy attention for the extraction of these bioactive compounds. As the different plant parts are grown (*in vitro* system) in modified culture medium under various growth regulators in pharmaceuticals, nutraceutical and agrochemicals industries. Thus, a greater amount of these valuable compounds have been achieved *via* a synchronized cultural system and growth conditions, which promising and reliable than other methods as well as reduce efforts and increase the quantity and quality of the desired compound (Espinosa-Leal *et al.,* 2018).

In last decades we have been witnessed remarkable breakthroughs of adventitious roots (ARs) research, like the efficient induction of ARs in several plant species for the production of highly valuable bioactive compounds (agrochemicals, flavors, dyes, and fragrances, etc) (Murthy *et al.,*2008; da Costa *et al.,* 2018). It has been reported that the production of secondary plant metabolites is often differed due to species, age, variety, physiological responses, nutritional value, and stimulation by the environment and endogenous level of hormones (Aftab *et al.,* 2010)**.**In this context, several attempts have been made by researchers and entrepreneurs to explore the effect of plant growth regulator on secondary metabolite production (Weathers *et al.,* 2005; Rojbayani, 2007; Khan *et al.,* 2008; Shilpashree and Rai, 2009; Azeez and Ibrahim, 2014; Ullah *et al.,* 2019). In a comparative study of culture techniques, the adventitious root culture is the most popular method for the large scale production and derivation of valuable bioactive compounds (Jiang et al., 2015; Rahmat and Kang, 2019). The root culturing has achieved a significant impact on other cultural techniques for the exploitation of bioactive compounds as it is highly capable and identical to parental plants (Srivastava and Misra, 2017). Some studies evidenced that ARs influenced by *in vitro* method showed a high rate of proliferation and valuable active secondary metabolites (Yu *et al.,* 2000; Hahn *et al.,* 2003). Furthermore, the ARs culture system can easily up-scale to bioreactor is an additional benefit of this technique (Obom *et al.,* 2013). It is a fact that the synthesis of bioactive compounds significantly altered due to exposure to multivariate stimuli, so, logically, culturing conditions for the induction and establishment of ARs can change easily and the greater amount of bioactive compounds can be produced (Castro *et al.,* 2016). For example, the treated (KIN+IBA with thidiazuronTDZ) ARs enhanced secondary metabolite (Sandra and Anabela, 2018). The cytokinin and NAA significantly affect the production of secondary metabolites in *Aloe arborescens* (Amoo *et al.,* 2012). Likewise, 6-benzyl aminopurine (BAP) has shown an efficient role in the production of secondary metabolite in *Aconitum violaceum* (Hussein *et al.,* 2012; Rawat *et al.,* 2013). Hussein *et al.* (2012) successfully established ARs of *E. longifolia*in culturing medium using a combination of IAA and NAA, which further strengthen the potential of bioreactors for massive demand of pharmaceutical industries. Auxin influx from the site of synthesis to the site of action *via* mechanized pathways (Geisler *et al.,* 2006), that regulate various process at the cellular level in different tissues at different developmental stages, and consequently participate in various complex physiological (signal transduction/response to abiotic stresses) and morphological (embryogenesis, shoot elongation, lateral/adventitious root formation, vascular differentiation, apical dominance, and tropisms) processes in higher plants (Teale *et al.,* 2006; Mano and Nemoto, 2012; Shi *et al.,* 2014). A report summarized that different auxin levels regulate biosynthesis, transport, conjugation, and degradation processes, on the other hand, the auxin transport inhibitors significantly reduce rooting branches and showed the inhibitory effect on ARs formation (Sanchez-Bravo *et al.,* 1998; Garrido *et al.,* 2002; Ahkami *et al.,* 2013; Da Costa *et al.,* 2013). Surprisingly, the different compositions of synthetic auxin (IAA) conjugates may vary among the plant species. Likewise, the ester-linked sugars auxin conjugate is found in the majority of auxin form in maize kernels, while the *Arabidopsis* and many other dicots species mostly store IAA as amide linked amino acid conjugates (Bajguz and Piotrowska, 2009). The different forms of IAA conjugates are ester bonds with simple alcohols and sugars, e.g., inositol and myo-inositol sugars. Additionally, the IAA can also be conjugated with amino acids, peptides, or proteins through amide bond formation. Previously several studies summarized the correlation of auxin with age, plant species external stimuli, endogenous hormonal level, developmental stage, physiological and nutritional supply (Naeem *et al*.; Aftab *et al.,* 2010; Idris, 2014; Muhammad *et al.,* 2020) and the effect of various growth regulators on the production of bioactive compounds have been studied by (Weathers *et al.,* 2005; Rojbayani, 2007; Khan *et al.,* 2008; Shilpashree and Rai, 2009).

Cytokinins were initially discovered in the late 1950s, having a diver's molecular structure and specifically involved in biosynthesis, transport, perception signaling cascades and greatly impact on plant growth and development. The combination of auxin and cytokinin massively proliferate cells (callus) and induces shoot regeneration (Schaller *et al.,* 2015). The cytokinin plays role in flowering, for example, the loss of function ARR1 and ARR10 hinder normal carpel development (Andersenetal.,2018), likewise, the ETTIN/AUXINRESPONSEFACTOR3 (ARF3) is cytokinin dependent and important for flower determinacy (Liu *et al.,* 2014). Cytokinin also regulates various aspects of root development such as lateral root initiation and bilateral

Table1. Auxin and cytokinin and their available natural and synthetic types.

Key hormone categories	Natural occurred of hormones	Synthetically available hormones
Auxin	Indole-3-acetic acid (IAA) Indole-3-acetonitrile (IAN) Indole-3-acetaldehyde (IAc) Indole-3- pyruvic acid (IPyA)	Indole 3 butyric acid (IBA) Indole 3 propionic acid (IPA) Indazole 3 acetic acid (IAA) Naphthalene acetic acid (NAA) 2, 4-dichlorophenoxy acetic acid (2, 4-D) 2-napthoxyacetic acid (NOA) Thianaphthen-3-propionic acid (IPA)
Cytokinin	Ribosylzeatin Zeatin Isopentinyladenine Dihydrozeatin Ribosylzeatin	6-Benzyl amino purine 6-Phenyl amino purine Kinetin [(N-Benzyl-9- (2-etrahydropyranyl) adenine] (PBA) Diphenylurea Thidiazuron Benzimidazole Adenine 6- (2-Thenylamino) purine

symmetry and vascular tissue development (Bielach *et al.*, 2012; Lavrekha *et al.*, 2017; Andersen *et al.*, 2018). Moreover, cytokinin control radial patterning and proliferation root longitudinal sense, meristem size (Ioio *et al.*, 2008; Moubayidin *et al.*, 2010), and root nodules formation by increasing the biosynthesis of cytokinin (Chen *et al.*, 2013a; van Zeijl *et al.*, 2015; Reid *et al.*, 2017). The detail of auxin and cytokinin and their natural and synthetic available types are presented in Table 1.

The purpose of this review article is to highlight the significant aspects of the plant growth regulator auxin and cytokinin for the applied research available on medicinally and extinct plant species. Moreover, the research progress made on the induction of adventitious roots by plant growth regulator in some medicinally and endanger plant species and the appraising bioactive compounds/secondary metabolites or small molecules. This report will bring up the importance of some valuable plants, which are indispensable for biodiversity and also help to maintain our ecosystem. We are also hoping that the current information will strengthen the fundamental background of ecologist/researchers and will provide help to pharmaceutical industries.

Regulation and biosynthesis of auxin under external stimuli: The control and biosynthesis of auxin from the central fountain (flow upside-down) arecarried out by sophisticated regulatory pathways. Several logical strategies of long-distance shoot-derived auxin carriage advanced our understanding and saturated key research innovations, for example; the proteins and auxin carrier proteins enormously influenced the downward flow of auxin under adverse/stressful conditions (Fig. 1). More elaborately, the auxin efflux carrier PIN-formed 2 (PIN2) helps during halotropism on the root surface at a higher salt concentration and osmotic stress respectively (Galvan-Ampudia *et al.*, 2013; Zwiewka *et al.*, 2015). In contrast, the combined results of computational modeling and salt stress experiments revealed that PIN2 is not sufficient to elucidate the variation of auxin flow during halotropism process (van den Berg *et al.*, 2016). Although, the PIN-formed 1 (PIN1) and auxin transporter protein 1 (AUX1) have shown co-relation to facilitate auxin flow.

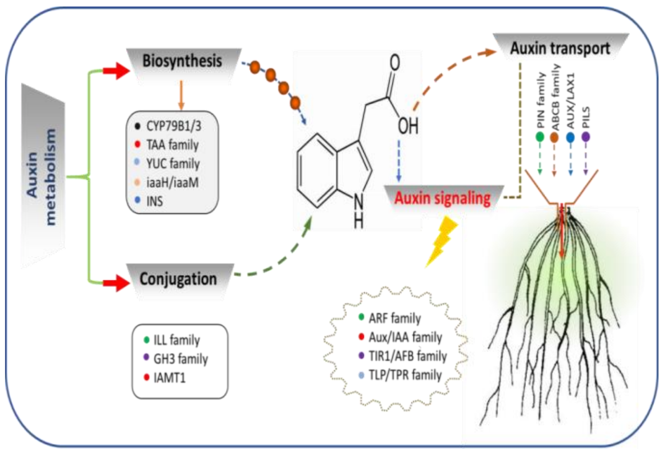

Figure 1. A schematic model of auxin metabolism, transport, and signal transduction in higher plants

Moreover, the auxin efflux carriers are thought to regulate the size of meristematic cells during root growth under salt stress (Liu *et al.*, 2015), likewise, the down-regulation effect of PIN1, PIN3, PIN7, auxin-resistant 3 (AXR3) and indole-3-acetic acid 17 (IAA17) normalize salt stress, that might influence the root meristem size by raising nitric oxide (NO) levels. Studies on the ABCB transporter family bared the influencing role of auxin flow in root under various stresses

(Geisler *et al.,* 2017), such as ABC transporters of rice (*Oryza sativa*) showed involvement during salt and drought stress (Chai and Subudhi, 2016; Han *et al.,* 2017). In contrast, the mutational studies altered the auxin flow in the root and putatively associated with abiotic stress tolerance, which may be due to the loss-of-function of the core domain in ABCB transporters (Cho *et al.,* 2014)**.** Other mutational studies of synaptotagmin 1 (ROSY1-1) and the interaction of the ROSY1-1 and synaptotagmin 1 (SYT1), enhanced salt tolerance, and reduced basipetal auxin transport (Dalal *et al.,* 2016). Furthermore, the zinc-induced facilitator-like 1 (ZIFL1) belongs to the primary facilitator of superfamily (MFS) transporter efflux auxin from roots to shoots (Remy *et al.,* 2013). When the zifl1 was mutated, it reduced the expression of PIN2 protein in the epidermal cells of the root in response to IAA and also had shown gravitropic bending defects. Therefore, to fully understand, we described diagrammatically the various roles of different transporter families involved in the regulation and biosynthesis of auxin in Fig.1.

Despite all, the transporter also determined the auxin (IAA) levels in the root by biosynthesis and conjugation, in recent decades, its cleared that both processes are substantially affected by abiotic stress (Tivendale *et al.,* 2014; Di *et al.,* 2016)**.** So far, in higher plants mainly two biosynthesis pathways are explored, such as indole-3-pyruvic acid (IPyA) and indole-3-acetaldoxime (IAOx), which are responsible for most IAA biosynthesis during stresses (Zhao *et al.,* 2002; Julkowska *et al.,* 2017; Lehmann *et al.,* 2017). However, the potential contribution of other pathways remains under debate and requires further logical research.

In vitro adventitious root culturing in medicinal plants

***Artemisia vulgaris* L.:** *Artemisia vulgaris L.* (Asteraceae) is a tall, endangered aromaticherb generally recognize as Mugwort. It is an important medicinal plant and generally serves as a traditional medicinal herb efficient in choleretic, amenorrhoeadysmenorrhoea, diabetes, liver disorders, epilepsy, psychoneurosis, insomnia, and anxiety stress (Sujatha and Kumari, 2008; Jassbi *et al.,* 2010; Nigam *et al.,* 2019). The leaves and roots of *A. Vulgaris* have numerous bioactive compounds such as coumarins, sesquiterpene lactones, volatile oils (USDA-ARS-NGRL 2004). While the extract also has the potential of repellent activities against mosquitoes (Aedesaegypti) which transmit yellow fever (Ilahi and Ullah, 2013). *In vitro,* adventitious roots were cultured of *A. Vulgaris* using leaf and root as explants on Murashige and Skoog's medium supplemented with IBA and IAA, where the highest root number (10.8) and root length (13.9 cm) delivered by 11.4 µM indole-3-acetic acid (IAA) 4.9 µM Indole -3- butyric acid (IBA). Moreover, the maximum number of the root was induced by the leaf while bulky biomass obtained from the root explants (Sujatha and Kumari, 2008).

***Eurycoma longifolia* L.:** *Eurycoma longifolia* (Simaroubaceae) is renowned due to its aphrodisiac and energy restoration properties where the high bioactive compounds are mainly stored in the taproot of this plant. Recently the importance of these compounds had proved its effect on anti-malarial, anti-ulcer, anti-microbial as well as cytotoxic behavior in the extracts of *E. longifolia's* (Ruan *et al.,* 2019). Where the most active constituents like quassinoids andcathine-6-one alkaloids are commercially extracted from its roots. Therefore, the increasing demand of *E. longifolia's* required conservation to maintain its natural habitat. It has been reported that the effects of naphthalene acetic acid (NAA), indole-3- aceticacid (IAA) and indole-3-butyric acid (IBA) enhanced adventitious roots. *E. longifolia's*where the optimum concentration NAA, 3 mg/L showed a higher number of roots than IBA and IAA that only triggered adventitious roots from the leaf of *E. longifolia's (*Hussein *et al.,* 2012). Thus, these facts provide the potential role of adventitious roots for the production of desired bioactive compounds for commercial uses.

***Morindacitrifolia* L.:** *Morindacitrifolia* (Rubiaceae) is well known as Noni and has been used for therapeutic purposes including antibacterial, antiviral, antifungal, anticancer, antitumor, anti-allergic (Abou Assi *et al.,* 2017). The *M.citrifolia* the majority of its bioactive compounds in the form of polyphenolics, organic acids, and alkaloids (Baque *et al.,* 2013; Ruhomally *et al.,* 2016). Owing to its beneficiary uses in the field of herbal medicine reduces natural/parental flora. Besides its rapid depletion, the *M.citrifolia* plant is more prone to diseases and pest attacks. Therefore, it's a prerequisite to establishing *in vitro* adventitious root culture, that combines high-density production of roots and ameliorates bioactive molecules (nthraquinones, ubiadin, phenolics and flavonoids) in the culture medium. So far a number of experimental trials were conducted such asthe adventitious roots were effectively induced from leaf explants in *M. citrifolia* by using auxins and cytokinins in various concentration on Murashige and Skoog medium (Sreeranjini and Siril, 2013), the explants were dedifferentiated on MS medium containing1 mg/L indole-3-butyric acid (IBA) and placed under florescent light (Baque *et al.,* 2010) , experiment on suspension culture for efficient adventitious root establishment not only increase the vigor of root but significantly accumulate bioactive compounds like phenols and flavonoids, were 5 mg/L IBA, 10 g/L sucrose and inoculums size (15 g/L, fresh weight) were kept for 4 weeks in a growth chamber (Baque *et al.,* 2010; Baque *et al.,* 2013; Sevik and Güney, 2013). The findings revealed that the optimized culture profoundly increased the rate of root biomass and subsequently the production of bioactive compounds that can sufficiently satisfy the thirst for industrial uses.

***Prunella vulgaris* L.:** *Prunella vulgaris* cover in the Labiatae family and recognized for self-healing (Rasool *et al.,* 2009;

Chen *et al.*, 2013b). *P. vulgaris*aqueous extracts possess inhibitory activity against human immunodeficiency virus (HIV) (Rasool *et al.*, 2009) (Table 2). Later studies on aqueous extracts of *P. vulgaris* were focused on isolation, purification, and characterization of the anti-HIV active component (Prunellin) and enhancement of its synthesis, where the biological activity such as anti-inflammatory, anti-tumor, stimulatory effect on immune system and development of T lymphocytes were explored by (Rasool *et al.*, 2009; Zdařilová *et al.*, 2009; Chen *et al.*, 2013b). The in vitro adventitious root culturing for high biomass and production of secondary metabolites were studied by (Rahmat and Kang, 2019). The adventitious roots were induced from callus on solid Murashige and Skoog (MS) medium supplemented with 6-benzyladenine (BA; 1.0 mg l^{-1}) and naphthalene acetic acid (NAA; 1.5 mg l^{-1}), where a higher amount of total phenol and total flavonoids were obtained from0.5 mg l^{-1} NAA treated cultures. Therefore, the augmented ability of culture medium for the induction of adventitious roots and extraction of a high amount of bioactive compounds can be used for commercialization.

Psoraleacoryfolia **L.:** *Psoraleacoryfolia* (Fabaceae) is another endangered plant that richin valuable bioactive compounds, mainly cultivated in the tropical and sub-tropical regions. The *Psoraleacoryfolia* produces coumarins, angelicin, psoralenanddiazein (Baskaran and Jayabalan, 2007). (Table 2) Precisely, Psoralen helps in photochemotherapy of vitiligo and skin diseases like psoriasis, mycosis, fungicides, and eczema (Yones *et al.*, 2005), *P. coryfolia* also carries some biological activates such as antitumor, antibacterial, antifungal, and antioxidant (Baskaran and Jayabalan, 2007). It is a matter of worry that the population of *P. coryfolia* has very low seedlings survival after germination and the other reason is the indiscriminate and illegal destruction for various purposes, so the only way to conserve this medicinally valuable plant for the production of bioactive compounds in the development of efficient strategies for this species. Earlier Baskaran and Jayabalan (2009) experimented on hypocotyl explants of *P. corylifolia* to establish adventitious roots by using IAA, IBA, or NAA with 1,2, 3, 4, or 5 lM concentration respectively. The results indicated that 3 µM IBA had established a maximum number of roots and significantly enhanced psoralen content in suspension culture. Therefore, the fact is quite reliable that suspension culture often produces more bioactive compounds when adventitious roots were tested (Panichayupakaranant and Tewtrakul, 2002).

Aloevera **L.:** *Aloe vera* (Liliaceae) is known for its therapeutic and commercial uses (Maan *et al.*, 2018). The plant produces an anthraquinone, aloe-emodin compounds during the synthesis of the polypeptide pathway (Diaz-Munoz *et al.*, 2018). (Table 2) The bioactive compound “aloe-emodin” have shown a positive role in anti-inflammatory and genotoxic properties (Maan *et al.*, 2018). The fluctuation in the environment and physiological alteration significantly impact on the composition of bioactive compounds (Beppu *et al.*, 2004). The *A. vera* leaf was used as explants for the induction of adventitious root culture and subsequently the root culture for the production of the aloe-emodin compound (Lee *et al.*, 2011). In this experiment, the effective root induction, adventitious roots, and root number was obtained from Aloevera leaves by culturing in media with supplementation of NAA (0.5 mg/L) and 2, 4-D supplemented in MS medium induced fragile callus, where the aloe-emodin and aloin content produced by adventitious roots were quantified by high-performance liquid chromatography (HPLC) (Lee *et al.*, 2011) (Table 2).

Table 2. The role of PGRs on adventitious roots establishment in medicinal plants.

Extinct Species	Explants	PGRs	Bioactive Compounds	Medicinal Uses	Citations
Eurycomalongifolia L.	Leaf	NAA	Quassinoids, cathine-6-one alkaloids	Antimalaria/ulcer/ microbial activity	(Hussein *et al.*, 2012)
Morindacitrifolia L.	Leaf	IBA	Polyphenolics,alkaloids	Anticancer/bacterial/viral/fungal/tumor activity	(Baque*et al.*, 2010a, b)
Prunella vulgaris L.	Leaf	BA+NAA	Prunellin, phenols, flavonoids	The inhibitory effect on immune deficiency virus (HIV)	(Rasool *et al.*, 2009).
Psoraleacoryfolia L.	Hypocotyls	IBA	Coumarins, psoralen, ispsoralen, angelicin	Antitumor/bacterial/fungal, Psoriasis/fungoides	(Baskaran and Jayabalan, 2009)
Aloe vera L.	Leaf	NAA	Anthraquinone, aloe-emodin	Anti-inflammatory genotoxic properties	(Lee *et al.*,2011)
Artemisia vulgaris L.	Leaf and root	IAA, IBA	Coumarins, Punarnavine, sesquiterpene, inulin	Diabetes/liver disorders/ epilepsy/ psychoneurosis, Depression/insomnia and anxiety stress	(Sujatha and Kumari, 2012)
Echinacea angustifolia	Root	IBA, NAA	phenols, flavonoids, total caffeic acid	lymphatic, sialagogue and support Immune system	(Jang *et al.*, 2012).

Table 3. The role of auxin on adventitious roots of extinct species.

Extinct Species	Explants	PGRs	Concentration	Techniques	Citations
G.macrocephala	Leaf node	Auxin	1:2 mg/L	callus cultures	(Vieira, 1999)
M.officinalis	leaf and stem	Auxin NAA, IBA	(10mg/L optimal for the rooting of shoots)	Tissue culture	(Meftahizade *et al.*, 2010)
***A.andrachne*L.**	basal or terminal portions of branch	IBA, NAA,	(5–48 µM (Best 24 µM)	Wounding Propagations	(Al-Salem and Karam, 2001)
T. Grandis L.	Coppice shoots	IBA and NAA	2,000 and 4,000 ppm	Clonal propagation	(Husen and Pal, 2007a)
G. biloba L.	stem cuttings	IBA and NAA	10.0 µM Maximum roots	Clonal propagation	(Gopichand and Meena, 2015)

***Echinacea angustifolia*:** *Echinacea angustifolia* is a perennial herb that belongs to the family Asteraceae, native to central Canada and the USA. It's commonly known for lymphatic, sialagogue and support Immune system. The plant produces various important secondary metabolites such as phenol, flavonoids and total caffeic acid. The induction of adventitious root culturing was assessed by various types and levels of auxin (Jang *et al.*, 2012). The *E. angustifolia* root (explant) was cultured in Murashige and Skoog medium containing with 1.0 mg /L indole -3-butyric acid (IBA produced highest (20.87 mg) weight and (3.07 mg) dry weight where the same concentration of IBA in suspension culture increased biomass production (3.07 g fresh weight and 0.38g per culture) after four weeks. Moreover, the 3.0 mg /L NAA on affected affected biomass production. The production of bio compounds like phenolic, flavonoids and total caffeic acid produced by adventitious roots were greatly enhanced by 1.0 mg /L IBA as well as the root biomass and morphology was changed significantly (Table 2)

In vitro adventitious root culturing in extinct plants

***Gomphrena macrocephala*:** *G. macrocephala* is a perennial herb having medicinal properties and abundantly found in Brazilian cerrado (Vieira, 1999). So far, numerous studies have been conducted to induce efficient propagation through cuttings, such as (Moreira *et al.*, 2000) reported that auxin-like substances (NAA and IBA) facilitate root formation of *G. macrocephala* using micro cuttings (Table 3). The three diverse combinations of auxin-induced calli to cytokinin ratios of the *G. macrocephala* (Gomphreneae, Amaranthaceae), and the callus from leaf and node tissues also have shown the generation of fructose-containing carbohydrates/starches in *G. macrocephala*. Further, the homologous arrangement of fructosepolymers (98% of the aggregate fructo-polysaccharides) identified in the culture medium of 1:2auxin to cytokinin ratio (Table 3) (Vieira *et al.*, 1995). Likewise, the nodal segments were used for adventitious shoot formation in the gelled MS medium containing 10 mg/L of indole-3-butyric acid (IBA) by (Mercier *et al.*, 1992).

***Arbutusandrachne* L.:** *Arbutus andrachne* grows naturally in thickets woody and dry rocky places of Eastern Greek. It is an evergreen shrub with smooth cinnamon-red bark and thick green foliage (Al-Salem and Karam, 2001). Several studies have been conducted so far to establish the best ratio of concentration to develop *Arbutusandrachne* from different tissues. The induction of micro shoot elongation from nodal explants under various levels of NAA and BA in the culture medium was investigated by (Bertsouklis and Papafotiou, 2007). Stem cuttings were tested from the basal or terminal portion of branches and were treated with acid or salt types of IBA or NAA at different concentrations (Al-Salem and Karam, 2001; Bertsouklis and Papafotiou, 2007). The wounding and cell proliferation significantly impact the culture medium and various concentrations/combination of growth regulators (Table 3). Additionally, the proliferation medium significantly enhanced the adventitious root and expended with the external stimulus at per liter rate in the medium up to 100% (Al-Salem and Karam, 2001).

***Tectona Grandis* L.:** *Tectona grandis* is, an essential plant species, found in tropical areas and has been used for timber production, because of its durability and delicate texture. As the demand for wood is increasing globally, on the other hand, the supply/quality of teak is significantly affected by poor genotypes and outdated propagation methods of this plant species. Therefore, it is necessitating that sufficient techniques can be screened for high quality and quantity production of *Tectona grandis* So, the clonal propagation is one of the quickest and even more efficient techniques for tree development raised from the natural population (Table 3). The rooting parameters, like root length, root size, and root percent were expelled in the rooting medium containing auxin were used for the clonal forestry program of teak (Husen and Pal, 2007a; Badilla *et al.*, 2016). The endogenous level of auxin on root activity and sensitivity of auxins to the age of the tissues were reviewed by (Olatunji *et al.*, 2017). The formation of adventitious roots from branch cuttings through auxins and the capability of *T. grandis* with relevance to tissue age/type has been studied (Husen and Pal, 2007a, b)

***Melissa officinalis* L.:** *Melissa officinalis* an aromatic perennial medicinal herb plant belongs to a mint family (Lamiaceae) is native to Europe, Central Asia, and the Mediterranean region (Tavares *et al.*, 1996; Aasim *et al.*, 2018). The plant is rich with essential oils such as neral+geranial 48%, citronellal 39.47% and β-caryophyllene

2.37% and phenolic compounds (Tavares *et al.,* 1996; Petersen and Simmonds, 2003). The other uses of *M.officinalis* are anti-tumoral, anti-virus agents and anti-oxidative agents (De Sousa *et al.,* 2004; Moradkhani *et al.,* 2010). Although, *M. officinal* is cultivated in the field the conditions, to obtain homozygous population is the major issue (Meftahizade *et al.,* 2010). So far, several attempts have been made to develop an *in vitro* micropropagation method for exploiting shoot tip or apical nodes, cotyledonary nodes, or nodal segments (Meftahizade *et al.,* 2010). Aasim *et al.* (2018) reported the combination effect of Thidiazuron (TDZ) and Indole-3-butyric acid on cotyledonary leaves to produce adventitious regeneration under in vitro conditions (Table 3). However, the higher concentration of TDZ has shown a negative impact on rooting when generated from shoots (Fratini and Ruiz, 2002). The hormonal combination of IAA, IBA, NAA, and GA3 on stem cuttings showed an influence on the morphological, while the stem tallness was only affected by GA3 application (Sevik and Guney, 2013). The auxin had shown a specific role in auxin transport, rooting beginning, lateral root inception and root gravity response (Chhun *et al.,* 2003; Štefančič *et al.,* 2005).

***Ginkgo biloba* L.:** *Ginkgo biloba* (Ginkgoaceae) is a native plant, originated in China and have been used for medicinal purposes, such as allergies, alzheimer's disease, headache, asthma, tinnitus, impotence, circulatory disorders, eye disorders, diabetes, free radical scavenger (Murray, 1996; Gopichand, 2015). The biochemistry, nomenclature, plant architecture, production of secondary metabolites, economical and pharmacological importance of *Ginkgo biloba* L. have also been extensively reviewed (Singh *et al.,* 2008). The variation of secondary metabolites and availability of terpene trilactones has been discovered by (Kaur *et al.,* 2009; Kaur *et al.,* 2012). Additionally, it has been found most effective against environmental pollution (Sharma, 1989; Neinhuis and Barthlott, 1998). Due to excessive exploitation in pharmaceutical industries and anthropogenic pressure the plant becoming extent and living Fossil under threat (Purohit *et al.,* 2009). So far, numerous scientists have conducted research on *G. biloba* related to chemistry, pharmacology, pollution, nitrogen fixation and antifungal effect by using its leaf extract. Although, there are insufficient studies on stem cutting plantlets raised under different PGRs. Gopichand and Meena (2015) investigated the stimulatory effect of auxins (indol-3-butyric acid IBA and α-naphthalene acetic acid; NAA) on adventitious root formation by using stem cuttings of *G. biloba* along with subsequent growth and survival rate of cutting raised plantlets (Table 3), where IBA (10.0 µM) at lower concentration wasmore effective, as it induced maximum rooting (88.89%) and morphologically healthy seedlings, overall the survival rate of the plantlets was improved (Kling and Meyer Jr, 1983; Nandi *et al.,* 1997). (Kling and Meyer, 1983; Nandi *et al.,* 1997) reported that species stimulated adventitious root formation in stem cuttings by auxin or/with a combination of phenolic compounds. The endogenous hormonal relationship with growth and drops in ginkgo seeds and cellular auxin transport through diffusion and carrier-mediated were reviewed by (Jian and Gang, 2001; Kramer and Bennett, 2006), respectively. The estimation of genetic diversity and chemical changes in leaves were investigated by (Gopichand, 2015; Ražná *et al.,* 2020). The performance of PGRs was propagated by semi-hard stem cuttings (Gopichand, 2015), production of active constituents, ethnopharmacology properties attempted by (Singh *et al.,* 2010; Kaur *et al.,* 2012), essential metabolites and antifungal activity studied by (Roberts, 1972; King and Roberts, 1979). In general, the propagation of gymnosperms is difficult because of low rooting efficiency. However, some success was achieved in rooting using stems cuttings of Pinus species (SMITH and THORPE, 1975), *G. biloba* (Dirr, 1987), *T. baccata* (Nandi *et al.,* 1996), and *C. deodara* (Nandi *et al.,* 2002; Tamta *et al.,* 2007). Therefore, *G. bilobaL in* necessitates effective restoration and conservation methods for sustainable use of herbal medicines, while in this regard PGRs may play an active role in developing plantlets from the stem and other plant parts.

DISCUSSION

The induction of adventitious roots through various PGRs is a relatively complicated process. Therefore, special circumstances along with growth regulators of elite clone's production and propagation for the commercial purpose required continuous attention and sufficient knowledge. As the increasing demand for medicinal plant species in agro-pharmaceutical industries for the final extraction of bioactive compounds surrounds the great interest of scientists all over the world. In this context, *in vitro* adventitious root culturing can be an effective strategy for the stable and sustainable production of secondary metabolites (Baque *et al.,* 2010; Rahmat and Kang, 2019).

The formation of ARs from different plant tissues initiates through a single or set of growth regulators, and thus pre determined cells follow a well-organized pathway. Auxin is intimately involved in the developmental process of adventitious root establishment (Pop *et al.,* 2011; Kareem *et al.*, 2013). Moreover, auxin have both effects (stimulatory and inhibitory) relies on concentration (Li *et al.,* 2006; Abts *et al.,* 2017), but as root developmental process is strongly correlated with genetic control thus the complex hormonal regulation, signal transduction and environmental imbalance certainly trigger metabolic control and root development (Nacry *et al.,* 2013; Chaiwanon *et al.,* 2016). For an instant, the natural synthesis of indole-acetic acid (IAA) performed in leaves and shoots apices and then distributed in the lower direction of the root (Aloni *et al.,* 2006; Petrášek and Friml, 2009; Mohite, 2013), and thus regulates many developmental processes such as lateral growth of root, adventitious root, vascular differentiation and apical meristem (Ponce *et al.,* 2005; Aloni *et al.,* 2006; Ling *et al.,* 2009).

Beside natural synthesis auxin is also synthetically available in the form of IBA and NAA, (Jang *et al.,* 2012) that can influence adventitious root formation in a different type of explants like adventitious roots have been efficiently induced in *A. paniculata* and *P. rosea* by the application of NAA (Sevik and Guney, 2013; Kumlay, 2014). Similarly, NAA has shown more potential than IBA in *H. perforatum,B. diffusa A. vera* and *E. longifoli* (Cui *et al.,* 2010; Lee *et al.,* 2011; Hussein *et al.,* 2012) respectively. Additionally, the IBA was also found Proficient to induction of adventitious root in several species such as *M. citrifolia* (Baque *et al.,* 2010*), O. stamineus* (Leng and Lai-Keng, 2004), *E. angustifolia* (Jang *et al.,* 2012), *O.prostrata (*Martin *et al., 2008)* and *P. corylifolia* (Baskaran and Jayabalan, 2007). Moreover, the synergistic effects of auxin in various combinations also have a positive impact on adventitious root including *T. grandis* (Husen and Pal, 2007b*), C. limon* (Wei *et al.,* 2019*), G. biloba* (Gopichand, 2015;Gopichand and Meena, 2015) and *A. vulgaris* (Rahmat and Kang, 2019).

Horticultural and medicinal plant species mostly propagate through various tissue cultural techniques, clonal propagation, and different culturing applications. Thus an accessible and widely acceptable model system is required to up-scale the multiplication process in a short period as well as reduce the intensive efforts and production cost. Therefore, the successful and broad range of culturing for the production of bioactive compounds through adventitious root development in various medicinal and endangered plant species not only help in bio-conservation but as well reduce the pressure on the natural population, moreover it also promises the production of bioactive compounds and phytochemicals (Paek *et al.,* 2005; Ponce *et al.,* 2005; Wu *et al.,* 2007a, b). Adventitious root culturing promised numerous advantages, such as controlled medium, relaxation of suitable modification, and other cultural conditions that significantly enhanced the yield of biomolecules and made available for bioreactor to produce a massive amount of desirable metabolites over a short period.

***Summary and future perspectives*:** The present review article comprehensively studied the plant species that produce bioactive compounds for beneficial uses. We highlight the model system for simple, efficient, and efficient culture techniques for mass production of these valuable compounds by employing adventitious roots. Adventitious roots have great importance especially of those species which produce secondary metabolites in the commercial sector. Besides all adventitious roots have unique characteristics like sensitivity to various PGRs and quick production/release of bioactive compounds in the culturing medium. The PGRs not only increase the biomass of ARs but also help plants in adaptation. There are two objectives of this model system, first, it provides feedback or population pressure to the over-exploitation of medicinal plants to agro-pharmaceutical industries, and it helps in bio-conservation of endangered species, thus may help to maintain the ecosystem. The future perspectives or the limitation of the study, the complex hormonal cross-talk, biosynthetic pathways, regulation of secondary metabolites, exploration of gene pool concerning the level of the product, and cellular compartmentalization are required to improve the production of these compounds. In vitro culture techniques of adventitious not only help in the rapid production of particular species but it is considered the most phenomenal approach to reduce pressure on natural plant growth. Additionally, most of the horticultural crops are grown from roots, cuttings, culms, and rhizomes, so the establishment of this model system will provide a practical solution. In last, more research work still needs to intensive/extensive efforts levels to further explore more species related to the production of bioactive compounds and biotechnological applications to meet the aforementioned challenges.

Conclusion: Numerous plant species produces various medicinally valuable bioactive compounds, such as drugs, fragrances, flavor, dye, pigments, and pesticides. These bioactive compounds also play a dynamic role in the supply of raw material to the pharmaceutical industries. Therefore, it's obligatory to search for new species or investigate the already existing species for further improvement in terms of extraction of bioactive compounds. Moreover, the highly endangered species with great medicinally valuable bioactive compounds needs to preserve and further utilization for the production of bioactive compounds to up-scale the agricultural and pharmaceutical industries. The conservation and domestication process will also help in maintaining the ecosystem and will expand the area of new drug discovery. Moreover, the establishment of adventitious roots especially in the biologically endangered species through different plant growth hormones and culture mediums will enhance the conservation process as well as provide benefits to researchers and up-scale the availability to pharmaceutical industries.

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