PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 3 (2&3), 2019: 305 – 313
www.jpsr.in

AN OVERVIEW ON *IN VITRO* REGENERATION OF MEDICINALLY VALUED ENDANGERED HIMALAYAN FLORAL SPECIES OF UTTARAKHAND

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Received: 12 July, 2019 – Accepted: 25 July, 2019

Abstract

Indian Himalayan Region (IHR) spans most of the northern states of the nation. This incredible mountain is a hub for highly rich diversity in terms of flora and fauna. Some species are even endemic to this region. From the past few decades, this glorious habitat is facing many threats due to climatic change and increased human activities. Because of these factors, many species have become endangered and striving with their existence in nature. The floral species of this region are of great therapeutic values, some species have not yet been inspected for their medicinal value and for other industrially important products. In-situ and ex-situ methods of protection have been implemented to save these threatened species. Plant tissue culture in ex-situ terms, has proved to be an effective means for conservation of these threatened plant species as it provides a large-scale multiplication of plants under sterile lab conditions. Present review deals with results and conclusions of mass multiplication of endangered species with the help of *in vitro* regeneration technique, their therapeutic values and the future perspectives related to protection of these endangered plant species.

Key words: Endangered, Endemic, Ex-situ, Indian Himalayan Region (IHR), In-situ

Introduction

Indian Territory (6˚45' to 37˚6' N and 69˚ 7' to 97˚ 25' E) has a land perimeter of 15,200 km and a shoreline of 5400 km (R.R. Rao, 1997). Indian terrain can be classified into three distinct regions namely Himalayan province in northern part, the Indo- Gigantic plain and the plateau expanse in southern and central portions of the nation. The central Himalayan region is rich in medicinal and aromatic flora. India inhabits barely 2.4% of the earth's land area but its contribution to world's biological diversity is round about 8% of the total number of species (Khoshoo, 1996). In terms of floral diversity, India holds tenth rank in the world while fourth in Asia (Rodgers and Pawar, 1988).

India is a habitat to more than 48,000 species of plants, near about 3,000 species possesses therapeutic values and has been used in conventional medicament. Although a diversified species of plants has been reported in India, but wistfully this scenario has been reduced. According to investigators and botanists, at least 20 percent of numerous species of plants found in India are classified as either threatened or endangered. This species declination is due to threats such as habitat destruction, altered climate, or pressure from intruding species, increased urbanization. Pollution of soil, water and air, in addition to unrestricted usages of chemicals, fertilizers, plastics and expansion of factories and industries are devastating the natural necessity for flourishing these plant varieties.

Habitat alteration not only accelerate depletion of plant resources, but also affect conventional community life, cultural divergence, and accompanying proficiency of medicinal value of various endemic species (UNESCO, 1994b). On an average, 28 percent of plants species reported

in India are endemic to the nation and hence, have to preserved and secured from getting extinct. If these medicinally important plants species get extinct in the near future, the world will be devoid of therapeutic products derived from these plants.

Uttarakhand is one the most important region of Indian Himalayan Region (IHR) as the state is specifically enriched with variegated collection of natural ecosystems hence, is considered as home to numerous variety and exclusive range of plant diversity of India. It is apparent that endemism is quite high in species of Uttarakhand in which some of the species has a high threat status. As claimed by scientific studies, diversity under 1503 genera and 213 families of flowering plants, including 93 endemic species is harbored in various vegetation types, extended from sub-tropical forests in upper Gangetic plain and Shivalik zone in the south to arctic-alpine vegetation of trans-Himalayan cold desert in the north of Uttarakhand. Besides 487 species of ferns of which 15 species are endemic, 18 species of gymnosperms have also been reported from the state. These plant species have economic, medicinal value as these contains, anti- oxidants, anti- inflammatory, and anti-cancerous activities that helps in curing various diseases.

These endangered plant species are at the verge of extinction, so if not provided with firm policies and strategies for their conservation, then there is every likelihood of their extinction. In-situ and Ex-situ, both the measures have been taken for the protection of such threatened species so far. Under In-situ conservation Forest Department had established different National Parks, Sanctuaries, Reserved Forest and Biosphere Reserve in order to ensure good survival rate of threatened floral and faunal species.

Along with these in-situ programs, ex-situ program such as Plant Tissue Culture was also employed to safeguard these species. Plant tissue culture, also called micro-propagation, is a practice used to propagate plants under sterile conditions or in a controlled environment, often to produce clones of plant. This process involves maintenance of tissues or cells, as suspensions or as solids under conditions, which encourages their growth and multiplication.

Plant tissue culture is based on the characteristic feature of plant cell i.e. totipotency which allows plant cells to regenerate into a whole plant, when placed on suitable nutrient medium in sterile conditions. Leaves pieces, nodes, internodes, single cells, less commonly roots can generally be used to regenerate a new plant on culture media when provided required nutrients and Phyto- hormones (Vidyasagar, 2006).

Tissue culture protocols for near about 70 angiosperms and 30 gymnosperms has been established thus far (Thorpe *et al*., 1991). *In vitro* propagation is a promising technique to proliferate and preserve critically challenged plants genotypes as the method is independent of environmental factors, geographical and seasonal variations, and offers an explicated production system, which corroborates the steady supply of plantlets, consistent in superior quality and yield (Kala, 2005).

Uttarakhand: Devbhumi ('Land of Gods')

Uttarakhand earlier known as Uttaranchal is a state situated in the northern region of India. It is often referred as Devbhumi ('Land of Gods') due to many Hindu temples and pilgrimage centers found throughout the state. Uttarakhand is known for its natural environment of the Himalayas, the Bhabhar and the Terai.

Uttarakhand is located at the foothills of the snow-covered Himalayas with flourishing green vegetation. The State has rich and diverse floral, faunal and microbial abundance inclusive of unique and threatened species of plants and animals.

Conventionally, the mountains present in the lower areas of Uttarakhand are roofed with damp deciduous forest. Large-scale dominant natural vegetation entails of Pine, Oak, Rhododendron, Walnut and Larch are present between the elevations of 1,500-3,000m.Vegetations like forests of Spruce, Fir, Cypress, Juniper and Birch are present below snow line, while going in the higher altitude of the State *i.e.* above the snow line, is Alpine vegetation, which consists of Mosses, Lichen and a variety of wildflowers like Blue Poppies and Edelweiss. Loads of pioneer flora and fauna of the Himalayas are confined to protected areas and sanctuaries because of deforestation.

Species Threatening

Miscellaneous developmental procedures and approaches such as industrialization, urbanization, hydroelectricity, global warming, oppression of lucrative plants, deforestation, alteration in climate have emerged as responsible factors for massive forfeiture of biological assets (Gaur 2005, 2007a, 2007b). As specified by an estimate, approximately 75 percent diversity dissipation is because of habitat variation while the remaining 25 percent losses are due to over exploitation (Gaur, 2008). Himalayan flora

has been in use for miscellaneous purposes, which includes some scientific medicinal uses from pre-historic times. Plants of Himalayan range have many uses that are well described in ancient Indian Literature such as Rigveda, Charak Samhita, Sushruta Samhita, Atherveda, Upanishada.

Applications and usages of indigenous plants and natural assets and resources are increasing very abruptly for the past few decades. This put together massive hazards to the survival of numerous wild species and ecosystems, which are of enormous lucrative value to the society. There is a huge region in Shivalik Himalayas, where the woods have demeaned and transformed into small packets (Sharma *et* al., 2011). It is proposed that if no genuine attention and awareness will be provided in the direction to these rich economic diversities of this region, then it will hardly last for future.

Medicinal value of some endangered species of Uttarakhand

S. N.	Plant species	Medicinal values
1.	*Aconitum balfourii*	An alkaloid pseudacnitine is present in roots, which is biologically 1.5 times as active as aconitine and is highly toxic. Root Paste can be applied for rheumatism, against neuralgia, fever and bone complaints .it is used in gastric disorders, leprosy, swelling and sciatica and wound.
2.	*Aconitum heterophyllum*	Roots are used to cure dysentery, diarrhoea, fever, malarial fever, cough, cold colic, headache, piles, hysteria, throat infection, cure for dyspepsia, especially when appetite is lost after illness and also in vomiting, abdominal pain and diabetes. It also monitors sun controlled menstrual flow. Fresh leaves are used to cure toothache.
3.	*Aconitum violaceum*	An alkaloid called indoconitine is present in roots, which serves as tonic and is used in cough, cold, stomach pain, fever, bronchitis, epilepsy, headache, inflammations, snakebite, renal pain and rheumatism.
4.	*Eremostachys superba*	Gujjars of some states give tubers of this plant to buffaloes to increase the milk production.
5.	*Gentiana kurroo*	It is known to have beneficial effects on liver so acts as tonic, anthelmintic, emmenagogue, blood-purifier, and carminative, digestive agent and also used for the medicament of diabetes, digestive disorder, hepatic disorder, bronchial asthma, and urinary infection. Along with roots, whole plant can be used as therapeutic agent.
6.	*Nardostachys grandiflora*	The rhizome and the oil derived from the rhizome are regarded as tonic, stimulant, diuretic, emmenagogue, stomachic and laxative and is used in hysteria, insomnia, dysmenorrhea, skin diseases, throat trouble, lumbago, ulcer, rheumatism, paralysis etc. it is known to provide poise and calmness of mind and used conventionally as a hair tonic to cure hair loss as well.
7.	*Phaius tankervilleae*	it is used as tonic (personal communication). Paste of wild ginger along with plant acts as medicine in dysentery and healing bone fractures (Roy *et* al., 2007). Grounded pastes of pseudobulbs, roots and leaves are used as remedies for boils, infected wounds and abscesses (Chowdhary, 1998). Smoked flower of this plant along with food is taken by the women of Papua New Guinea as an aid to conception (Powell, 1976).
8.	*Cyathea spinulosa*	The whole plant parts are useful as remedies. It is used as general hair tonic, fronds powder is used as sudorific and aphrodisiac (Singh and Upadhyay, 2010). In case of indigestion and hair loss due to various reasons, mixtures of stem powders of *Cyathea spinulosa* and *Angiopteris helferiana*

		is administered orally with water to the cattle such as cows, buffalos and goats. For rapid growth of hair, trace amounts of potion are also applied on the skin (Upadhyay *et* al., 2011).
9.	*Schrebera swietenioides*	The leaves are thought to contain stomachache-healing properties and are used in the medication of urinary discharges. Boils and burns can be treated by bark while roots can be beneficial to cure for leprosy. Local grazers also use crushed roots as an application for killing worms in infested wounds.
10.	*Meizotropis pellita*	------
11.	*Turpinia nepalensis*	------
12.	*Indopiptadenia oudhensis*	------
13.	*Trachycarpus takil*	------
14.	*Pinguicula alpine*	------
15.	*Pecteilis gigantean*	------
16.	*Diplomeris hirsuta*	------

Plant Tissue Culture

In vitro propagation is one of the modern techniques for propagation of plants. Apart from mass multiplication, it holds promise in multiplying genetically engineered, high yielding and disease resistant plant material. It saves time, requires less space and allows freedom from seasonal variations. In the nineteenth century the idea of experimenting with tissue and organs of plants under controlled laboratory conditions was born. For the first time in 1902, a German physiologist, Gottlieb Haberlandt, developed the concept of *in vitro* cell culture.

Micro-propagation has a high level of commercial potential and this potential is due to many reasons *viz*; high speed of propagation, germplasm conservation, genetic transformation, clonal propagation and it has ability to produce disease- free plants. It offers a rapid means of afforestation, multiplying woody biomass, conservation of elite and rare germplasm (Bajaj, 1986; Karp, 1994; Roja and Rao, 1998), regeneration of plantlets from both callus cultures and organ cultures (Chalupa, 1987), development from single cells into callus (Muir *et* al., 1958). *In vitro* plant regeneration is also used for conservation of those species that are at risk. Rare, endangered or of special cultural, economic or ecological value. Micro propagation of mature trees with vegetative explants has been a difficult task due to various factors i.e. presence of phenolic compounds, exogenous and endogenous infection, maturity, juvenility, slow growing habit, long genetic variations (Durzan and Gupta, 1986; Zimmerman, 1985; Bajaj, 1991).

Plant tissue culture of different endangered species of Uttarakhand

1) *Aconitum balfourii*

Pandey *et* al., 2004, had established a protocol of *in vitro* regeneration of *Aconitum balfourii*. Leaves were used for either callus induction using 4.5mM 6-benzyladenine (BA) and 26.9mM alpha-naphthaleneacetic acid (NAA) or multiple shoot induction using 1.1 mM BA only. Root was induced by supplementing 12.3mM indole-3-butyric acid (IBA). *In vitro* regeneration of *Aconitum balfourii* was also established by taking roots as explants by Sharma and Gaur, 2012, in which for the induction of callus 13.4 µM NAA with 5.55 µM BA was used. Shoots were regenerated in medium supplemented with 0.54 µM NAA and 8.88 BA. For complete regeneration of plant, rooting media was supplemented with 1.43 µM IAA and 1.23 µM IBA.

2) *Aconitum heterophyllum*

In vitro regeneration protocol was established and published in a book by Nandi *et* al., 2016 by taking seeds of *Aconitum heterophyllum*. When the seedlings (without radicle) were cultured on the medium containing BAP (0.01, 0.1 and 1.0 µM), best response (80 %) was obtained on a medium containing 1.0 µM BAP with maximum shoot formation (six shoots/explants) after 8 weeks.

Giri *et* al., 1993 and Jabeen *et* al., 2006-used BAP for shoot induction while micro- propagating *Aconitum heterophyllum.* The establishment of hairy root culture in *Aconitum heterophyllum* and the production of active ingredients was successfully demonstrated (Giri *et* al., 1997). Total aconitine content in transformed roots was found out to be 3.75-fold higher than the non-transformed roots.

3) *Aconitum violaceum*

An *in vitro* regeneration protocol was established by Mishra-Rawat *et* al., 2013 in which leaves shoot tips and nodal segments of the plants were used as explants. Basal MS medium with different plant growth regulators (PGRs) at various concentrations (0.0–2.5 l M BAP and 0.0–1.0 l M NAA) was tested for multiple shoot formation. Proliferating shoots (about 3 cm in length) were transferred to half-strength MS medium supplemented with IAA (0.0–0.5 l M) and NAA (0.0–1.0 l M) either individually or in combination for rooting.

4) *Eremostachys superba*

G. S. Panwar, S. K. Srivastava and P. L. Uniyal in 2015 established *in vitro* regeneration protocol for *Eremostachys superba* by taking seeds. Shooting was done with MS medium supplemented with most suitable concentration of BAP (6.6 µM), TDZ (4.54 µM) and kinetin (6.9 µM) was tested in combination with different concentration of NAA (0.53-1.59 µM). To prevent the browning of culture medium and necrosis of tissues from white-milky exudates of explants, the medium was supplemented with activated charcoal (1.0 g l-1) and poly-vinyl-pyrrolidone (PVP: 1.5 g l-1). MS medium supplemented with IBA (7.36 µM) for root regeneration. A subsequent rooting experiment was performed involving activated charcoal, PVP and gelling agents such as agar (0.8 and 0.6% w/v), agar gel (0.4% w/v), gelrite (0.2% w/v).

5) *Gentiana kurroo*

For the first-time *in vitro* regeneration of *Gentiana kurroo* was established by Sharma *et* al., 1993. Shoot multiplication of *Gentiana kurroo*, a threatened medicinal plant species, was achieved *in vitro* using shoot tips and nodal segments as explants. Fifteen-fold shoot multiplication occurred every 6 weeks on Murashige and Skoog's medium (MS) containing 8.9µM Benzyladenine and 1.1 µM 1-naphthaleneacetic acid. Rooting was accomplished successfully in excised shoots grown on MS basal medium containing 6% sucrose. Sharma and Kaur, 2014, developed efficient protocol for *in vitro* regeneration of *Gentiana kurroo* using different explants (leaves, petioles, and roots) and those explants responded differently for regeneration according to different combinations of growth regulators. They found out that the petiole explants were responding best for callus induction and consequently for indirect and direct regeneration. The callus induction was achieved on MS basal + 1.0 mg/l benzyladenine (BA) and 3.00 mg/l naphthalene acetic acid (NAA). MS medium supplemented with 0.10 mg/l NAA and 1.0 mg/l thidiazuron (TDZ) was recorded as the best medium for indirect regeneration. However, for direct regeneration the maximum number of shoot emergence was observed on MS basal fortified with 0.10 mg/l NAA + 0.75 mg/l TDZ. Half strength MS basal supplemented with indole-3-butyric acid (IBA) 1.00 mg/l gave best response for root induction. Actively growing shoots 3–4 cm in length derived directly from petioles (direct) and multiple shoots derived from calli induced from petiole (indirect) were transferred to MS basal solid and liquid medium of different strengths supplemented with various growth regulators like NAA (0.50 mg/l), IBA (0.50 mg/l–1.0 mg/l) (Kaur *et* al., 2007). Kaushal *et* al., 2014 developed *in vitro* regeneration protocol for *Gentiana kurroo* by using apical meristem as explants. The apical meristem was excised to a length of 0.2-2 mm from the shoot tips. Among different treatments of growth regulators either alone or in combination, the growth of meristem was best observed on Murashige and Skoog (MS) medium supplemented with 6- Benzyl amino purine (BAP) (1.0 mg/l) and Indole acetic acid (IAA) (0.5 mg/l). The maximum response for meristem proliferation was 83.3% with an average mean number of 8.1 ± 0.2 leaves /explant. Three weeks old sprouted meristems were transferred to the MS medium supplemented with 0.5 mg/l each of Kinetin (KN) and BAP for shoot elongation and proliferation resulting in 5- 6 shoots/ explant. When transferred to half strength MS medium supplemented with 0.5 mg/l Indole butyric acid (IBA), *in vitro* regenerated shoots developed roots in six weeks with a survival rate of 86%.

6) *Nardostachys grandiflora*

Callus cultures of *Nardostachys grandiflora* was maintained on Murashige and Skoog's medium containing 3.0 mg 1−1 of α-naphthaleneacetic acid and 0.25 mg 1−1 of kinetin when shifted to medium containing 0.25–1.0 mg 1−1 of indole-3-acetic acid or indole-3-butync acid showed profuse rhizogenesis. The callus-regenerated roots when transferred to medium containing 2.0–6.0 mg 1−1 of kinetin produced shoot buds. The *de novo* shoot bud regeneration took place either directly from cortical cells or from the inner stellar region. In addition, concomitant root-shoot development was also observed (Mathur, 1992). Callus cultures of *Nardostachys grandiflora*, were established using petiole explants on MS medium supplemented with 16.1 µM ∝-naphthaleneacetic acid and 1.16 µM kinetin. Embryogenesis in these callus cultures took place only upon sequential subculture of the callus on media having gradually decreasing auxin (16.1 to 1.34 µM NAA) and simultaneously increasing cytokinin (1.16 to 9.30 µM kinetin)

concentrations over a period of 7 months. Somatic embryo to plantlet conversion took place on a medium containing 9.30 µM kinetin and 1.34 µM NAA (Mathur, 1993).

7) *Phaius tankervilleae*

In vitro seed germination and seedling development of *Phaius tankervilleae* technique was successfully established for rapid multiplication using 0.8% (w/v) agar solidified MS medium supplemented with different concentrations and combinations of Kinetin (Kin) and NAA. MS medium supplemented with 1.0 mg L-1 Kin + 1.0 mg L-1 NAA was the most ideal condition for early seed germination (2.87 weeks) (Thokchom *et* al., 2017).

8) *Cyathea spinulosa*

In vitro regeneration protocol for *Cyathea spinulosa* was developed by Shukla *et* al., 2004 by - employing leaf primordium explants excised from *in vitro*-raised sporophytes through spore culture. Calli were induced from the explants on Parker's and Thompson (P&T) media using 8.87 µM 6-benzylaminopurine (BAP) and 2.21µM 2, 4-dichlorophenoxyacetic acid (2,4- D). Maximal multiple shoots (12.5±0.45) were differentiated from callus and elongated on P&T media with 4.52 µM BAP and 5.36µM α -naphthalene acetic acid (NAA). *In vitro*-raised shoots rooted on P&T with 2.24µM indole- 3-butyric acid (IBA).

9) *Meizotropis pellita*

An efficient protocol for high frequency *in vitro* regeneration of *Meizotropis pellita* an endangered and endemic plant was developed by taking seeds. Leaf was taken as explants for callus induction and proliferation. Callus initiation stated within 15 - 20 days of incubation in MS medium containing 2 - 4, D (9.06 µM) alone or in combination with 2 - 4, D (9.06 µM) + 2-iP (7.38 µM). Shoot regeneration was attained callus as explant in MS medium boosted with BA (17.6 µM) + GA_3 (1.0 µM). Proliferation of shoots was also achieved from cotyledonary node of *Meizotropis pellita* in MS medium using Kinetin + GA_3 (4.6 µM + 1.0 µM) or BA (13.2, 17.6 µM) + GA_3 (1.0 µM) after 30 - 45 days of incubation. IBA (4.9 µM) was found out to have more effect for root regeneration from micro shoots (Singh *et* al., 2013).

Future Perspectives

The Indian Himalayan Region (IHR) is a mega hot spot of biological diversity (Myers 2000). It comprises about 18% of India, is more than 2,800 km long and 220 to 300 km wide, with altitudes from 200–8000 m. The flora includes about 8,000 species of angiosperm (40% endemic), 44 species of gymnosperm (16% endemic), 600 species of pteridophyte (25% endemic), 1737 species of bryophyte (33% endemic), 1,159 species of lichen (11% endemic) and 6,900 species of fungi (27% endemic) (Singh and Hajra 1996; Samant *et* al., 1998).

Conservation of bio-diversity is an important task for well sustenance of life. Plants are crucial component of our daily life. They maintain a proper environment by exchange of gases in atmosphere, checks soil erosion, reduces levels of certain pollutants, such as benzene and nitrogen dioxide, reduces airborne dust levels, keeps air temperatures down, conserve water and energy. Along with that, plants are the great source of energy and nutrient rich foods like fruits, cereals etc. They are abundant source of essential oils, have therapeutic value as these contain anti-inflammatory, anti-oxidants, anti-cancerous activity-based compounds, which helps in curing many diseases. Plants are also beneficial to animal and birds as it is habitat, provides food and fodder to them. Plants also offer great economical and aesthetical value

Due to large-scale human activity and interference, increasing urbanization, extreme deforestation, intruding wild species reduces the habitat of native species. In addition to physical encroachment, human development of animals' habitats pollutes the natural landscape with petroleum products, pesticides, and other chemicals, which destroys food sources and viable shelters for the creatures and plants of that area. Environmental pollution, illegal smuggling of exotic plant species, natural calamities like forest fires, earthquakes etc, along with extreme research and collection of plant species for institutes, nurseries etc also affect the existence of plant species in nature. Improper handling, negligence of people towards floral habitat, diseases in them is some other important causes because of which floral diversity is at the verge of extinction. Due to extreme human actions and other environmental factors, some plant species have become threatened but needs to be conserved for future as they are of great medicinal and economical value.

Plant tissue culture offers *in vitro* regeneration of plants under sterile conditions in laboratory. For the conservation of threatened species, tissue culture is one of the best medium, which is used ex-situ. This technique has many advantages over conventional method of propagating plants. It provides mass multiplication, off-season and disease-free plant variety.

There are many endangered plant species found in Uttarakhand in which some are endemic

to the state. *In vitro* regeneration has been used for conservation of these endangered species, but still no proper evidence was found for some species *viz*; *Schrebera swietenioides*, *Turpinia nepalensis*, *Indopipta deniaoudhensis, Trachycarpus takil, Pinguicula alpina, Pecteilis gigantean* and *Diplomeris hirsuta.* Plant tissue culture can be done for future references and investigations in order to find out the requirements of phytohormones for these endangered plant species. With the combination of recombinant DNA technology plant with desired characteristics can be developed in order to have proper balance and mass production of these threatened plants in nature.

Some endangered floral species such as *Aconitum balfourii, Aconitum heterophyllum, Aconitum violaceum, Eremostachys superba, Gentiana kurroo, Nardostachys grandiflora*, *Phaius tankervilleae***,** *Cyathea spinulosa* are rich in disease curing properties for various disease like cancer, rheumatism, gastric disorders, fever, headache, bronchitis, leprosy, dysentery etc. if one plant species gets extinct, the potential benefits, such as a source of medicine, will be forfeited. Pharmacological investigations can be carried out in future in order for the betterment of medicine preparations by using active compounds of these medicinally value plants.

There is no proper evidence, which suggests the medicinal and therapeutic value of certain endangered species like *Turpinia nepalensis, Indopiptadenia oudhensis, Pinguicula alpine, Diplomeris hirsuta, Trachycarpus takil.* Further analysis can be done on this area so that a potential drug and medicines can be developed by finding out the active ingredients present in these plant species in future. Other research and studies may include the anti- microbial activity, anti-inflammatory activity, anti-cancerous activity that can be researched in future for these plant species. Plants are also a great source of active industrial products such as flavonoids, pigments, alkaloids etc.so, a phytochemical analysis can be done in these species in order to find out the type and number of active compounds.

According to the U.S. Fish and Wildlife Service, one lost plant species can lead to the loss of 30 other insects, plant, and other animal species found in the higher levels of the food chain. These individual species of plant or animal are sometimes called the keystone species. If that species is removed, the whole ecosystem will be changed drastically.

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