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Merging tradition and innovation: A review on the botany, chemistry and biotechnology of *Ephedra foliata* ex C. A. Mey

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Abstract
The genus *Ephedra* is distributed across arid and semi-arid regions globally, except in Australia. It has attracted substantial attention as a result of its traditional uses in various cultures, as well as its diverse physiological, morphological, phytochemical, and pharmacological properties. Phytochemically, *Ephedra* species are renowned for their alkaloid content, particularly ephedrine and pseudoephedrine, which possess bronchodilator properties and possess stimulant effects on the nervous system. *Ephedra foliata* is a common gymnosperm found in hot desert regions, specifically in some African countries, Afghanistan, Pakistan and India. *E. foliata*, like a myriad of other therapeutic plants, is confronted with a variety of challenges that jeopardize its survival. Due to the destruction of its natural habitat, it is seriously threatened. This review offers a comprehensive examination of the physiology, phytochemistry, pharmacology, and traditional use of *E. foliata*.

Keywords: Desert, *Ephedra foliata*, Endangered, Ephedrine, Gymnosperm

Introduction

Ephedra, known as joint firs, is an interesting genus of the s eed bearing non-flowering family *Ephedraceae*. The Genus *Ephedra* is classified under the advanced order Gnetales of Gymnosperm, with around 55-65 species, with 14 species and a variety are found to be distributed and noticed in different biogeographical zones of India *viz*., *E. foliata, E. gerardiana, E. intermedia, E. nebrodensis, E. saxatilis, E. regeliana, E. pachycla E. przewalskii. E. pangiensis, E. kardangensis, E. khurickensis, E. sumlingensis, E. yangthangensis, E. karumenchiana* (Patel *et al*. 2021) [35]. The genus *Ephedra* is found in dry and semi-dry areas of Asia, Europe, Northern Africa, Southwestern North America, and South America (Hollander *et al*. 2010) [21]. It is also present in the northern temperate zone, spanning from the Canary Islands through the Mediterranean region and Central Asia to Shandong in China, and extending to the arid regions of the USA and Mexico (Caveney *et al*. 2001) [9]. The Indian species of *Ephedra* are found in a range that stretches from Sikkim in the Eastern Himalayas to the north-west in Uttarakhand, Himachal Pradesh, Kashmir, Rajasthan, and Gujarat. *Ephedra* species inhabit arid and frigid environments and primarily consist of shrubs, vines, or occasionally small trees. Some species can climb up to 4 meters in height. These plants are commonly found in dry and open environments such as deserts, rocky slopes, grasslands, and coastal areas with a Mediterranean climate (Ickert-Bond, Renner 2016) [23]. Various countries' traditional medicine often employs certain species of the *Ephedra* to alleviate symptoms of asthma, colds, flu, chills, fever, headaches, nasal congestion, and coughs.

Ephedra foliata Boiss. ex C. A. Mey. is widely distributed in Afghanistan, Syria, Pakistan and India. It is the only gymnosperm species found in the dry and semi-arid regions of the Thar Desert in India (Bhandari 1990) [6]. The Thar Desert, located in northwestern India, is renowned for being one of the densely inhabited deserts globally. The Thar Desert is situated within the geographical coordinates of 24° to 28°N latitude and 68° to 71°E longitude, covering an approximate area of 200,000 km^2. *E. foliata* is an evergreen Shrub growing 0.15m to 1m. The plant prefers acid, neutral and basic (alkaline) soils (Bissa 2015) [8]. This plant is xerophytic, adapted to survive in dry settings and commonly found in arid and semi-arid regions of northwestern India. It can grow in challenging environments with poor soil quality and extreme weather conditions, including intense sunlight and high temperatures. In India, it is indigenous to Rajasthan and can also be found in Gujarat and the Districts of Haryana state that border Rajasthan (Gavali, Sharma 2004) [16]. Rajasthan is situated within the geographical coordinates of 23°.3' to 30°.12' North latitude and 69°.30' to 78°.17' East longitude. It covers a surface area of 342,239 km^2, accounting for 10.41% of India's total land area. The distribution of this species in Rajasthan includes the Districts of Ajmer, Bikaner, Churu, Barmer, Jaipur, Jodhpur, Jaisalmer, Jhunjhunu, Pilani, Jalore and Sikar (Kotiya *et al*. 2020) [29]. It is considered a threatened species in India, while it is listed as least concern in the IUCN Red List of vulnerable species. It was included in the Critically Endangered (CR) category by (Gowthami *et al*. 2021) [20]. However, the excessive exploitation, extensive destruction of its habitat, slow growth rate, and inadequate regeneration, as well as grazing and other human-induced pressures, have led to a significant decline in its natural population. Therefore, the natural habitats of this species have transitioned into a state of rarity or endangerment, moving from a previously vulnerable classification (Joshi *et al*. 2013) [25]. It is a perennial shrub indigenous to several global locations, has attracted the interest of botanists and horticulturists due to its compelling nature and remarkable resilience. It exemplifies the remarkable diversity and adaptability of the natural world. The plant is a dioecious climber, with the female plant producing semi-transparent, nutritious edible seed cones that resemble berries (Bissa 2015) [8]. These berries have a sweet taste because of the fleshy bracts surrounding them (Meena *et al*. 2019) [33]. The fleshy bracts have a crucial role in times of food scarcity in dry locations (Kotia 2008) [28]. It has gained attention for its biotechnological potential due to its production of secondary metabolites, particularly alkaloid content, which are widely used in medicine.

Fig 1: Habitat of *E. foliata*

Morphological characteristics

A dioecious, much branched, climbing shrub. Branches slender, smooth, striate, the older 7-8 cm in diam., more or less knotted. Leaves 2-3 at each node, shortly connate at base, 25-30 x 1-1.5 mm, setaceous. Male flowers: spikeletes ovate, tetragonous, 1-3 together, on 1-2 cm long peduncle; flowers 6-24; bracts 1.5-2mm long, as much broad, round, obtuse, connate upto their length from the apex, ciliate. Petals 2-2.5mm long, obovate, ciliate. Staminal column shortly exserted. Female flowers in sessile, 2-3 flowered cymes or fascicles or on 2-10mm long peduncles; bracts 3 pairs; outer1x1 mm long, margins scarious, ciliate; the inner most 5 mm long, connate. Fruit Ovoid-globose, 7-8 x 6-7 mm, milky-white, semi-transparent, fleshy. Seeds 2-6.7 x 2-2.5 mm, plano-convex, brown, acute at apex, rounded at base, glabrous, dorsally keeled **(**Bhandari 1990) [6].
Fl. & Fr.: January-April.

Common names

Arabic: Alanda warakia, fedr waraki, Al-Kuood al-waraki (Al-Snafi 2017) [2]
English: Shrubby horsetail. (Al-Snafi 2017) [2]
Indian: Unth phog, Suo-phogaro and Andho-khimp (Kotiya *et al*. 2020) [29].

Synonyms

Ephedra aitchisonii (Stapf) V. A. Nikitin, *Ephedra alte* Brandis, *Ephedra alte* C. A. Mey., *Ephedra asparagoides* Griff., *Ephedra ciliata* Aitch., *Ephedra ciliata* Fisch. & C. A. Mey., *Ephedra ciliata* var. *polylepis* (Boiss. & Hausskn.*)* Riedl, *Ephedra foliata* var. *aitchisonii* Stapf, *Ephedra foliata* var. *ciliata* (Fisch. & C. A. Mey.) Stapf, *Ephedra foliata* var. *polylepis* (Boiss. & Hausskn.) Stapf, *Ephedra kokanica* Regel, *Ephedra peduncularis* Boiss., *Ephedra polylepis* Boiss. & Hausskn., *Ephedra rollandii* Maire

Botany and Physiology

The remarkable range of morphological variations indicates that *Ephedra* was a survival of an old group, and its ancient origins are further corroborated by fossil evidence. *Ephedra* is prevalent in cold and arid regions of both the ancient and modern worlds (Elhadef *et al*. 2020) [13]. The genus exhibits a great tolerance to extreme aridity. This results in a decrease in the vegetative growth of plants (Faried *et al*. 2018) [14]. *E. foliata* can either climb or grow as woody shrubs. According to a study (Dhiman *et al*. 2010) [10], it thrives in infertile soil, intense sunlight, hot temperatures, and limited water supply. The cultivation and harvesting of this plant are conducted on a small commercial scale in Gujarat and Rajasthan (Gavali, Sharma 2004) [16]. The plant population has a high proportion of male plants compared to female plants, resulting in a major lack of successful seed production. Additionally, the seeds are frequently consumed by rats and birds, which greatly hinders natural proliferation through seeds (Singh 2004) [44].

Traditional Uses

Ephedra species have played a crucial role in traditional remedies of various civilizations for thousands of years. The ancient Indian Ayurvedic tradition identifies *Ephedra* as a soma plant. In Traditional Chinese medicine (TCM), the dried stems of *Ephedra* species, known as Ma Huang, have been used for thousands of years to relieve symptoms associated with the common cold, influenza, asthma, bronchitis, nasal congestion, and hay fever (Dhiman *et al*. 2010) [10]. For decades, the plant has been historically employed in Russia to treat respiratory ailments and rheumatism. The indigenous American peoples and Spanish settlers in the southwestern region of the United States utilized *Ephedra* for several medical applications, particularly in the treatment of venereal illnesses (Al-Snafi 2017) [2]. *Ephedra* plants are highly sought after globally for their dual purposes: traditional medical applications and as valuable sources of raw materials for ephedrine production all over the world. In addition to its traditional usage in Asia, it has also gained popularity as a nutritional supplement in the United States and Europe in recent years. Nevertheless, the improper or excessive use of dietary supplements containing ephedrine, with the intention of achieving weight loss or experiencing euphoric stimulation, has led to a range of negative consequences, including headaches, sleeplessness, heart palpitations, and in some cases, fatalities. As a result, the United States of America and several Western nations have implemented rules or prohibited the distribution of dietary supplements containing ephedrine since 1994 (Kitani *et al*. 2009) [26]. The seed cones of *E. foliata* possess a delightful sweet flavor because of the fleshy bract they contain. The succulent bracts of the fruits are consumed as a last resort for sustenance in dry locations during periods of shortage (Bhandari 1990; Kotia 2008) [6, 28]. The fruits of *E. foliata* have the potential to be utilized as a commercially viable nutraceutical food ingredient. These fruits may be collected and stored for extended periods of time at a temperature of 10±2˚C. Ethnobotanical research highlights the cultural importance of *Ephedra* species in indigenous traditions. In addition to their medical use, these plants also serve significant functions in ceremonial rites and spiritual contexts, which are indicative of long-standing cultural beliefs and traditional ecological knowledge. The *Ephedra* plant species have been used for thousands of years across different cultures, demonstrating its lasting importance in healthcare and cultural traditions.

Phytochemistry

Ephedra species possess bioactive natural compounds that have the potential for application in pharmaceuticals, cosmetics, nutrition, and agro-industries. Flavonoids and phenolic compounds are the most diversified classes of specialized metabolites produced from species within the *Ephedra* genus. The chemical components of *Ephedra*

species have been the subject of investigation for many years due to their presence of ephedrine-type alkaloids and their associated pharmacological characteristics. *Ephedra* species also contain other chemical components, including phenolic and amino acid derivatives. (Gonzalez-Juarez *et al*. 2020). The soluble extracts obtained from both the aerial parts and roots of *Ephedra* contain secondary metabolites. These metabolites can be classified into three groups: 26 alkaloids, 75 phenolic compounds and 7 amino acid derivatives. The alkaloids are represented by compounds which are mainly of the ephedrine-type framework. The phenolic compounds are represented by compounds include aromatic compounds, flavonoids, lignans, and proanthocyanidins. Lastly, there are seven amino acid derivative compounds. In addition, the essential oils of *Ephedra* species yielded 98 volatile organic compounds (VOCs) during hydro-distillation and approximately 70 compounds through extraction with supercritical CO_2 (Elhadef *et al*. 2020; Ibragic, Sofic 2015) [13, 22]. The physiological and pharmacological actions of *Ephedra* have been ascribed to six ephedrine alkaloids showing in figure 2. (Caveney *et al*. 2001) [9].

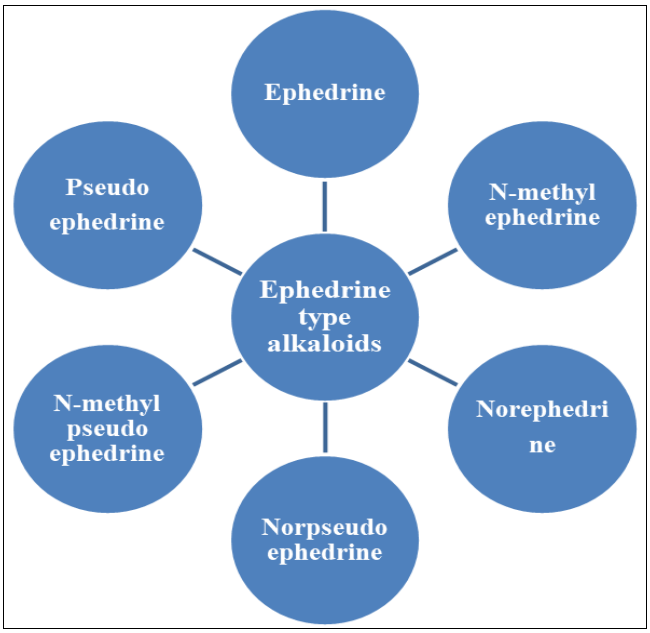

Fig 2: Types of ephedrine alkaloids.

The composition and proportions of ephedrine alkaloids differ across different species of *Ephedra*. Ephedrine acts as a stimulant by activating both alpha- and beta-adrenergic receptors. Ephedrine undergoes metabolism to form nor-ephedrine, which is perhaps responsible for the central nervous system stimulant effects (Dollery 1991) [11]. Total alkaloids content (TAC), total phenolics content (TPC), and total flavonoids content (TFC) are determined using spectrophotometric methods. The separation and quantification of E-type alkaloids from different *Ephedra* species was achieved using ultra-performance liquid chromatography (UPLC-UV) with UV detection, marking the first instance of its application. The variable content and ratio between secondary metabolites determined in different *Ephedra* species reflects their metabolic activities. *E. foliata* exhibits a greater concentration of secondary metabolites in contrast to other species within the *Ephedra* genus (Ibragic, Sofic 2015) [22]. *E. foliata* possesses a non-protein amino acid with a cyclopropyl ring structure, as well as other nitrogenous secondary compounds (Kynurenates) which has been observed to exert potent pharmacological effects on the sympathetic nervous system of mammals (Caveney *et al*. 2001) [9]. 6-hydroxykynurenic acid occurs as the major quinoline alkaloid in *E. foeminea* and *E. foliata* (Gonzalez-Juarez *et al*. 2020). In a study**,** the stem extract of *E. foliata* was analyzed and shown to contain all the evaluated phytochemicals, with a higher concentration of alkaloids (Bissa 2015) [8]. When *E. foliata* extracts (100 g dry wt) were nitrosated under physiological circumstances, they yielded 0.77 mg of N-Nitrosoephedrine (NEP) and 8.3 mg of N-nitroso pseudoephedrine (NPEP) (Alwan *et al*. 1986) [3]. A recent study examined the overall phenolic and flavonoid content in *E. foliata*. The total phenolic content was measured to be 52.6±0.1 mg/g of dry weight, whereas the total flavonoid content was found to be 25.0±0.0 mg/g of dry weight (Ibragic, Sofić 2015) [22]. The combined amount of ephedrine and pseudoephedrine in *E. foliata* ranged from 0.04% to 0.2% (Bahernik *et al*. 2000) [4].

Pharmacology

The medicinal significance of *Ephedra* is based on the sympathomimetic properties of ephedrine alkaloids. Nowadays, medicinal use of *Ephedra* herb is limited, but the abuse of its psychostimulants is rising (Ibragic, Sofic 2015) [22]. Prior pharmacological investigations have demonstrated that *Ephedra* species have antibacterial, antimicrobial, antioxidant, antidiabetic, hepatoprotective and cardiovascular properties. The therapeutic effects of *Ephedra* are notable due to the presence of diverse secondary metabolites (Bissa 2015) [8]. Ephedrine functions as a bronchodilator by inducing relaxation in the smooth muscles of the airways, so enhancing the passage of air. Recent investigations have confirmed once again the effectiveness of this treatment in addressing respiratory problems such as asthma exacerbations.

Ephedrine and pseudoephedrine are the compounds act as adrenergic receptor agonists, principally causing constriction of blood vessels and bronchial spasms waning cough and asthma symptoms (Garbis 2007) [15]. Multiple *in silico* studies have been conducted to forecast the possible antidiabetic activities of various chemicals found in *Ephedra*. The findings of these research indicate that ephedrine and five derivatives of ephedrine has promising antidiabetic characteristics through the inhibition of dipeptidyl peptidase IV (DPP-IV) (Wang *et al*. 2018) [49]. Ephedrine activates both α and β receptors. This impact was partially achieved through the direct stimulation of receptors and partially through the indirect mechanism of releasing noradrenaline from its tissue reservoirs and it acts as a gentle central nervous system stimulant (Al-Snafi 2017) [2]. Scientific research corroborates the conventional utilization of *Ephedra* for facilitating weight loss and augmenting metabolic rate. The stimulant characteristics of ephedrine enhance thermogenesis and calorie expenditure, which is why it is included in weight reduction pills. Recently, there has been an expansion in research to investigate the diversity of endophytic fungi that are linked with *Ephedra* species. Researchers have been studying the chemical components produced by these fungi and their potential for use in pharmaceutical exploration. The entire plant is traditionally utilized for its medicinal properties in treating fever, stomach ailments, worms, purifying the blood, asthma, dropsy, snake bites, and as a cardiac tonic (Quattrocchi 2012) [36]. *E. foliata* gave the best results in

hepatoprotective study using the liver enzyme levels as an indication for hepatoprotection and revealed a tremendous progress with disappearance of fatty deposition and necrosis. It's crude extract of (whole plant) at a dosage of 500 mg/kg proved a significant hepatoprotective effect in all parameters examined with 42.6, 39.5, 21.2 and 46.2% reduction in SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase), ALP (alkaline phosphatase) and bilirubin respectively (Alqasoumi 2008) [1]. Bissa (2015) [8] conducted an evaluation of the antimicrobial properties of aqueous, alcoholic, chloroform and petroleum ether extract from different parts of *E. foliata* to determine the effectiveness of these extracts against both human pathogenic bacteria (*E. coli, Salmonella typhi, Klebsiella pneumoniae,* and *Enterobacter aerogenes*) and plant pathogenic bacteria (*Agrobacterium tumefaciens*). The petroleum ether extract of dried stem of *E. foliata* exhibited the highest antibacterial activity against *Klebsiella pneumoniae*, while the dried leaves showed the highest antibacterial activity against *Enterobacter aerogenes*. Ghiasvand *et al*. (2019) [18] investigated the contribution of endophytic bacteria derived from *E. foliata* to the synthesis of bioactive chemicals. Bacterial strains were obtained from the surface-sterilized stem of healthy plant. The bacterial metabolites were isolated using chloroform and analyzed for their antibacterial, antioxidant and anticancer activities. The study demonstrated that Strain F5 (*Paenibacillus*) had a moderate level of antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Escherichia coli.* In addition, the extracts were chemically identified using tandem mass spectrometry which indicated the potential presence of alkaloid, aminoglycoside, flavonoid and terpenoid chemicals similar to those previously taken from other medicinal plants. This study demonstrated the significant potential of endophytes in synthesizing bioactive chemicals that are beneficial for plants.

Molecular Work

In order to effectively conserve, maintain and utilise plant genetic resources (PGR), it is important to understand the molecular basis of the fundamental biological processes in plants. A thorough understanding of the genetic variety that exists, where it is located in plant populations and how to effectively utilise it, is particularly important for both basic science and practical fields such as the effective management of plant genetic resources. Research on the genetic diversity of *Ephedra* has provided critical insights into its evolutionary history, adaptation and conservation. This type of research work conducted on *E. foliata* is summarized in table no. 1. In previous studies, researchers have used nuclear and plastid markers to investigate the phylogeny of *Ephedra* (Ickert- Bond, Renner 2016) [23]. However, only a few studies have employed more easily accessible markers for studying the relationship between species or populations of *Ephedra* such as RAPD (Ghafoor *et al*. 2007; Ehtesham- Gharaee *et al*. 2017) [12, 17], ISSR (Zhu *et al*. 2013) [50] or a combination of RAPD and ISSR (Saeed *et al*. 2015) [40]. More recently, researchers have also used DAMD and ISSR in their investigations (Meena *et al*., 2019) [33]. The karyotype of *E. foliata* has been studied from root tips, stem apices, female gametophyte and male gametophyte. It has also been reported as having heteromorphic sex chromosomes (Mehra, Khitha 1981) [34].

Table 1: Molecular work on *E. foliata* using different markers for different purposes

S. no.	Species	Work done	Markers used	Result	Reference
1.	*E. major, E. strobilacea, E. sarcocarpa, E. foliata, E. intermedia and E. distachya*	To investigate genetic relationships	RAPD	highest genetic diversity (25%) between *E. foliata* with other species	(Ehtesham-Gharaee *et al*. 2017) [12]
2.	*E. alata, E. aphylla, E. foliata, E. foeminea* and *E. pachyclada subsp. sinaica*	Taxonomic, DNA Barcoding and Phylogenetic Reassessment	Intergenic spacer *trn*-H/*psb*-A, ITS	*E. ciliata* was illegitimate and became a synonym to *E. foliata*	(Faried *et al*. 2018) [14]
3.	*E. foliata*	estimate genetic diversity and population structure	ISSR, DAMD	high genetic diversity was found in *E. foliata* at the species level (HT = 0.23 and 0.25) than at population level	(Meena *et al*. 2019) [33]
4.	*E. major* subsp. *procera, E. foliata* and *E. intermedia*	Measure genetic diversity	RAPD, ISSR	The closest relation was found between *E. procera* and *E. foliata*	(Basit *et al*. 2021) [5]
5.	50 specimens of *Ephedra* including *E. foliata*	Phylogeny of Ephedra	Plastid genome, Nuclear ribosomal cistron	Display incongruent phylogenetic positions	(Rydin *et al*. 2021) [39]

Application of Biotechnology in the development of plant

Ephedra, known for its medicinal properties, has attracted significant interest in biotechnological research due to its production of bioactive compounds, particularly ephedrine and pseudoephedrine. The majority of the *in vitro* research on this plant is concerned with the alkaloid concentration of callus (Dhiman *et al*. 2010) [10]. The table no. 2 provides a quick summary of this work that has been done on *E. foliata*. *E. foliata* stem tissues grown *in vitro* or *in vivo* under 16 hours of light or darkness are reported for their alkaloid content (Ramawat, Arya 1979) [37]. Under white light *E. foliata* callus generated ephedrine and pseudoephedrine at a concentration of 0.1%. When *E. foliata* callus was exposed to blue or red light, there was a threefold increase in the synthesis of ephedrine and pseudoephedrine (Shukla 1980) [43]. Process of sterilising seed explants of *E. foliata* by varying the concentration and duration of four sterilising agents-sodium hypochlorite (NaOCl), mercuric chloride ($HgCl_2$), hydrogen peroxide (H_2O_2) and Bavistin has been studied. NaOCl and $HgCl_2$ were the most effective sterilising agents at lower quantities and hydrogen peroxide in some way prevented the infection

at greater concentrations (Singh *et al*. 2018) [45]. The consequences of potassium nitrate, potassium dihydrogen orthophosphate and magnesium sulphate on *E. fotiata* germination was investigated, the findings showed that magnesium sulphate and potassium nitrate were more successful in fostering germination (Kshetrapal, Goel 1989) [30]. The biotechnological applications could be used for rapid, large-scale multiplication that minimises the male-to-female plant ratio, provides appropriate planting material for commercial cultivation, establishment of plants in natural habitat and conserves the germplasm of this endemic and threatened arid gymnosperm species in situ.

Table 2: Biotechnological work done by different Scientists

S. No.	Source of Explant	Culture Medium	Plant Growth Hormone	Result	Reference
01.	Female gametophytic tissue	White's modified medium (WBM)	2,4-D (5 ppm) + KN (1 ppm) +CM (20%) +inositol (100 ppm).	*In vitro* Proliferation	(Sankhla *et al*. 1967a) [41]
02.	Callus derived from root-tip of excised embryos	WBM	2,4-D (1ppm) + CM (20%) + KN (1 ppm).	Production of Plantlets	(Sankhla *et al*. 1967 b) [42]
03.	Female gametophyte	MS medium	Shooting and rooting- 2 ppm KN (auxin free medium)	Production of Plantlets	(Konar, Singh. 1979) [27]
04.	Female gametophytes	MS medium	Shoot buds- 2,4-D + KN Rooting- BAP (0.05 mg/l) + NAA	Haploid plantlets formation	(Singh *et al*. 1981) [48]
05.	Female gametophyte	B5, modified White's and MS medium	Shoot bud production- KN at 4.0 parts 10^{-6} with 0.5 parts 10^{-6} 2,4 D, Root formation- 2,4-D at 2.0 parts 10^{-6} with 0.5 parts 10^{-6} KN	Organogenesis	(Bhatnagar, Singh 1984) [7]
06.	Embryos	MS medium	Somatic embryos- 2 and 5 µM 2,4-D with 2 to 15 µM KN	Somatic Embryogenesis and Plant Regeneration	(Dhiman *et al*. 2010) [10]
07.	Nodal explant of mature female plant	MS medium	Budbreak-1.5 mg l^{-1} BA +additives Shooting- BA (0.25 mg l^{-1}), KN (0.25 mg l^{-1}), IAA (0.1 mg l^{-1}), additives Ex *vitro* rooting-500 mg l^{-1} of IBA	*In vitro* propagation	(Lodha *et al*. 2014 a) [32]
08.	Axillary buds	MS medium	Shooting- 0.25 mg l^{-1} each of KN, BA and 0.1 mg l^{-1} of NAA. Ex *vitro* rooting- 500 mg l^{-1} of IBA	*In vitro* plantlet regeneration	(Lodha *et al*. 2014 b) [31]
09.	Nodal sector explants of male plant	MS medium	Shooting-KN (2.5 mg/l) with IAA (2.5 mg/l) Rooting- NAA	*In vitro* Multiple Shoot Induction and Plant Regeneration	(Joshi, Deokule 2018) [24]
10.	Seeds	MS medium (half strength)	2mgl^{-1} GA3 and combination of 2mgl^{-1} BAP + 0.2mgl^{-1} KN	*in vitro* seed germination	(Singh, Singh 2019) [46]
11.	Intermodal explants	MS medium	Shooting- 6.0 mg L^{-1} KN Rooting- 3.0 mg L^{-1} NAA	Indirect organogenesis	(Singh *et al*. 2022) [47]
12.	Nodal and internodal explants	MS medium	Callus formation- 2, 4-D (2.0 mg/l) +KN (0.05 mgL^{-1}) and 2, 4-D (4.0 mg/l) + KN (1.0 mgL^{-1})	*In vitro* Callus Induction	(Rastogi, Srivas 2024) [38]

Challenges to the Continued Existence of Species and Measures for Their Protection and Preservation

E. foliata like many other medicinal plants, faces various challenges that put its survival at risk. It is facing a major threat of extinction as a result of the destruction of its native environment. The exponential urbanization, agricultural expansion and infrastructural development in arid and semi-arid regions have resulted in significant fragmentation and degradation of ecosystems. The introduction of invasive species into the natural habitats of *E. foliata* may lead to resource competition which directly endangers its survival. Invasive species such as *Prosopis juliflora* compete with *E. foliata* for water and nutrients, leading to negative effects on the growth of *E. foliata*. To mitigate the dangers faced by *E. foliata*, various conservation methods can be devised and implemented. The programs should give priority to the conservation of habitats, implementation of sustainable harvesting practices, development of climate resilience and control of invasive species. Conserving and restoring the natural habitats of *E. foliata* is crucial for its preservation. This can be achieved by establishing and managing protected areas and enacting land-use policies that minimize harm to habitats. The creation of protected areas in significant habitats of *E. foliata* in India, coupled with efforts to rehabilitate deteriorated areas. Implementing sustainable harvesting practices is essential to prevent the excessive exploitation of *E. foliata*. This involves developing protocols for the collection and cultivation of the plant to ensure its continued availability in the future. A management strategy focused on community involvement, which includes educating local communities about sustainable harvesting techniques and establishing certain zones where harvesting is limited. In order to improve the ability of *E. foliata* to withstand and adapt to changing climate conditions, it is imperative to conserve its genetic diversity and implement adaptive management measures. The implementation of ex-situ conservation measures such as seed banks and botanical gardens, to protect genetic diversity and facilitate the reintroduction of species into suitable habitats, efficient management and regulation of invasive species are essential to preserve the populations of *E. foliata*. Implementation of invasive species management programs that include mechanical eradication, chemical regulation and public awareness initiatives to reduce the spread of exotic plants.

Future Prospectives and Conclusion

The present investigation gives a deep insight of distribution, botany, physiology, morphological description, traditional uses, phytochemistry, pharmacology, molecular work and *in vitro* regeneration protocols for *E. foliata.* This species is the storehouse of numerous bioactive compounds such as alkaloids, phenolic compounds, amino acid derivatives and some special alkaloids which are mainly of the ephedrine-type framework with wide range of therapeutically active properties *viz*., antibacterial, antioxidant, antidiabetic, hepatoprotective, bronchodilators, decongestants and cardiovascular properties. The *in vitro*

regeneration protocol briefed from the present study can be employed for the large-scale propagation of *E. foliata.* Instead of the extraction of secondary metabolites from whole plants or plant parts, it could be convenient to produce these compounds from callus and cell suspension cultures which may not only help in addressing the urgent local needs for effective conservation but also meet the high pharmacological requirements of the country. Despite the great importance of *E. foliata* as economical, ethanobotanical and medicinally important plant, attempts for its conservation, sustainable utilization and genetic improvement are by and large lacking. To maintain a sustainable supply of high-quality plant material, it is important to prioritize extensive phytochemical profiling, pharmacological validation, and the optimization of cultivation and extraction procedures. Incorporating both traditional knowledge and modern scientific methods can help establish consistent standards and ensure the quality of products derived from plants. The plant's physiological adaptations such as its ability to efficiently utilize water in dry settings, highlight its resilience and evolutionary tactics. *E. foliata* has potential in pharmaceutical research, particularly in the areas of innovative medication discovery and ecological restoration. As our knowledge of science expands, *Ephedra* continues to be an intriguing topic that connects traditional wisdom with modern achievements. It has the potential to greatly influence future medical and ecological developments.

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