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Sustainable utilization of *Tylophora indica* (Burm. f) Merrill, An endangered medicinal plant

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Abstract
Tylophora indica (Burm.f) Merrill is pharmacologically important plant commonly known as Antmool or Indian ipecac, belongs to family Asclepeadiaceae. This plant is used for medicinal purposes in countries like India, Sri Lanka and Bangladesh. It is used to treat several disorders like bronchial asthma, respiratory disorders, cancer, skin disorders, cough, diarrhoea, microbial infection, inflammation. Many *in vivo* and *in vitro* studies revealed that this plant has potential for various pharmacological activities like antiasthmatic, anticancer, antimicrobial, antitumor, anti-inflammatory, antioxidant. Some alkaloids like tylophorine, tylophorinine, tylophorinidine are the important secondary metabolite which is isolated from leaves and roots of the plant. Tylophorine has been considered as a most active and explored compound. Apart from these compounds this plant is also having flavanoids, terpenoids, and sterols. This review provides the comprehensive account on the morphology, phytochemistry, medicinal value and the biotechnological aspects of *Tylophora indica*. This compiled study provide the information about the recent trends and techniques applied on *Tylophora* many of them have yet to be explored.

Keywords: *Tylophora indica*, Medicinal value, Biotechnological aspects, Pharmacological activity, Phytochemistry, *In vitro* regeneration

Introduction

Medicinal plants serve as valuable source for the treatment of various diseases. There is a continuous hike in the increasing demand for the medicinal plants. The pharmaceutical companies depend upon the secondary metabolites for the production of therapeutically important drugs. *Tylophora Indica* (Burm.f) Merillis commonly known as Antmool, Indian Ipecac, belongs to family Asclepeadiaceae. Other name of Tylophora are emetic swallow wort, vomiting swallow wort, Antamul, Dambel, Dambuti etc. *Tylophora indica* is considered as an important endangered medicinal plant which is used to treat several diseases like asthma(Mohiuddin, 2019) [32], bronchitis, and dermatitis etc and have several properties like antibacterial, antimicrobial (Reddy *et al*., 2009) [40], anxiolytic, Hepatoprotective (Kumar *et al*., 2012) [27] antiangiogenic, anti–Tumor (Saraswati *et al*., 2013) [45], anticonvulsant (Hafis *et al*., 2017) [21], and anti-neuroinflammatory etc. Phytochemically, alkaloids are the most significant fraction, which gives the plant its pharmacological properties. There are three alkaloids that are noteworthy: tylophorine, tylophorinine, and tylophorinidine. This plant is exploited extensively in the wild for its medicinal properties, and because of the uncontrolled and unmonitored harvesting from the wild, it has been listed among endangered plants (Kaur *et al*., 2011b) [24]. The main aim of this review is to describe the collective account on morphological, phytochemical, medicinal and biotechnological approach of *T.indica*. It also describes the economic and commercial value of the *T. indica*. Fig. 1 is the graphical representation of the overview.

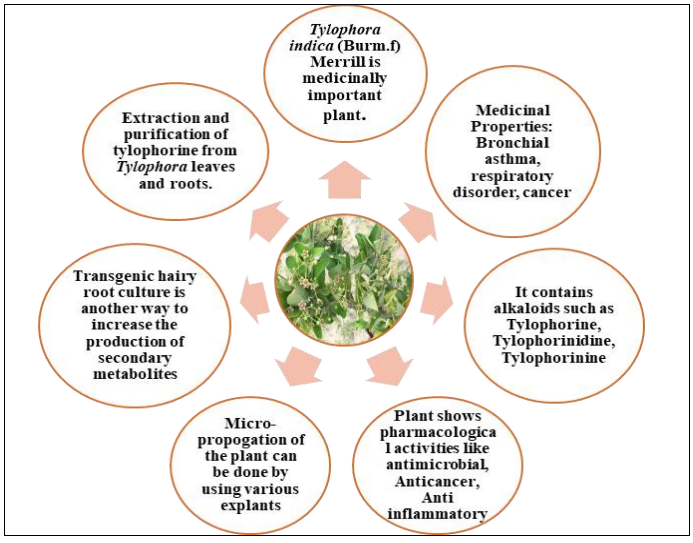


Fig 1: An overview of general characteristics of *T. indica*

1. Methodology

This study was conducted with an objective to give a comprehensive account on medicinal plant *Tylophora indica*. The literature was collected by using various online and offline resources like search engine (Google Scholar, PubMed, Science Direct), review paper, research paper and book chapters of reputed journals and publications. Several Medicinal plant databases such as, Indian Medicinal Plant Database; National Medicinal Plants Board, Ministry of AYUSH, Government of India; ENVIS centre on medicinal plants are also used of this review article. Chemical structure were drawn using Kingdraw software.

2. Distribution and Taxonomy

Tylophora indica (Burm f.) Merill (Syn: *Tylophoraasthamatica*, *Asclepias asthamatica*, *Cynanchum indicum*, *Vincetoxicum indicum*) also belongs to Apocynaceae family (Gururani *et al*., 2020) [20]. It is a perennial, climbing herb, grows in several states of the India. It is flourish well in the part of Sub Himalayan region of Uttarakhand and stretches to Meghalaya up to the height of 1260m. This plant found in the several states of India such as Bengal, Assam, Orissa, South India, North East and Central India as well as in Peninsular India. Plant can also survive in planes, hilly slopes and forests areas with lesser rainfall and area with wide ranges of soil, preferably scanty. Some other species of *Tylophora* found in the India are *Tylophora rotundifolia, Tylophora fasciculata, TylophoraEpiculata, Tylophoraanomula, Tylophora sylvatica, Tylophoraheterophyla* (Rani *et al*., 2012) [38]. In Chhattisgarh and Madhya Pradesh, this plant is categorized as Vulnerable species. In Western Ghats of Tamilnadu, South India this plant is categorized as rare species.

Table 1

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Gentianales
Family	Apocynaceae
Genus	Tylophora
Species	*Tylophora indica* (Burm. f.) Merr.

Source: Indian Biodiversity Portal
https://indiabiodiversity.org/species/show/32360#overview

3. Morphological characteristics of *T. indica*

Tylophora is a tiny, slender and climbing herb with glossy and velvety touch. The width of the roots is about 2.5-5 cm thick. Leaves are thick, ovate with pointed tip, length and breadth are approximately 6-11cm and 3.8-6 cm respectively. It is smooth at the above surface and velvety beneath. Leaves stalk is about 1.2cm. Flowers are green colored with tinge of yellow or purple color with pointed petals. Flower of *Tylophora* blooms in month of August to December. Fruitsare follicle, oval and lance shaped. Seeds are long, oval in shape and 2-2.5 cm in diameter (Gupta *et al*., 2010a) [18]. Figure2is the captured images of *T.indica*.

(2.A) **(2.B)**

Fig 2: Captured Images of *Tylophora indica*. A. Whole plant. B. Flowers of *T. indica* (DEI Herbal Garden)

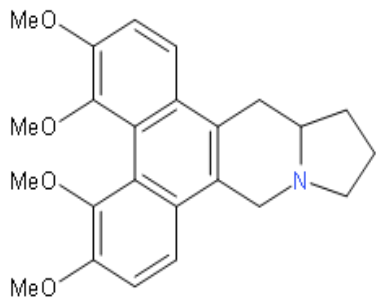
4. Phytochemistry of *Tylophora Indica*

T. indica contains an extensive phytochemical profile, such as alkaloids, saponins, tannins, and terpenoids. The plant contains several therapeutically important alkaloids such as Tylophorine ($C_{24}H_{27}O_4N$), Tylophorinidine ($C_{22}H_{22}O_4N$), and Tylophorinine ($C_{23}H_{25}O_4N$) (Jayanthi and Mandal, 2001) [22]. in roots, tylophorinidine is present in more amount and has potential to act as anti tumor drug. Tylophorine is the main alkaloid of *Tylophora indica* responsible for its strong inflammatory actions. Table 1 represents the various phytoconstituents found in the plant. Figure. 2 represents the structure of major phytochemical compound present in *T. indica* which are drawn by using kingdraw software.

Table 1: List of various phytoconstituents found in different parts of *T. indica*

Plant part used	Phytoconstituents	Reference
Whole plant	Septicine, Tylophorinidine, Tylophorine, Tylophorinine, Isotylocrebrine, Tylophoricine, resins, tannins, sterols, flavanoids, wax and resins	(Umamaheswari *et al*.,2017) [49]
	Tylophorine derivatives (PBTs) N(2,3-methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-5-aminopentanol and N-(2,3-methylenedioxy6-methoxy-phenanthr-9-ylmethyl)-l-2-peridinemethanol	(Gopalakrishnan *et al*., 1980) [15]

	Phenanthroindolizidine alkaloids known as tyloindicines A–E, 14- hydroxyisotylocrebrine,4,6-desdimethyl isotylocrebrine, Tylophorine, 6- desmethyltylophorine, 7tylophorinidine and 5-hydroxy- Omethyltylophorinidine	(Ali and Bhutani, 1989) [1]
	Anhydrous dehydrotylo-phorinine, Tylophorindine, desmethyltylophoridine, desmethyltylophorinine and desmethyltylophorine	(Gupta, 2003)
	O-Methyl tylophorinidine and simple aliphatic acid	(Reddy *et al*., 2009) [40]
	Quercetin, α- and β- amyrins, Kaempferol, quercetin, desmethyltylophorine, 4, 6- des-methylisodroxy-methyl tylophorinindine, Tyloindicines H, I and J, desmethyltylophorinine, isotylocrebrine, non-alkaloidal compounds - octaosanyloctacosanoate, tetratriacontanol, β-sitosetrol, sigmasterol, tyloindane, cetyl-alcohol, wax, resin, coutchone, pigments, tannins, glucose, calcium salts, potassium chloride	(Gupta *et al*., 2010)
	Phenanthroindolizidine alkaloid, 3-O-demethyl tylophorinidine (VI)	(Dhiman *et al*., 2013) [2]
	Alkaloids,flavonoids, phenols, saponins, steroids and terpenoids	(Ranemma *et al*., 2017) [37]
Leaf	Tylophorine, tylophorinine, tylophrinidine and septidine	(Umamaheswari *et al*., 2017) [49]
	Alkaloids, carbohydrates, steroids, saponins and triterpenes	(Gujrati *et al*., 2007) [16]
	Flavanoids, glycosides, saponins, carbohydrates, proteins and aminoacids, tannins, terpenoids and alkaloids	(Sathyabama and Kingsley, 2013) [46]
	3-O-demethyl tylophorinidine and phenanthroindolizidine	(Dhiman *et al*., 2012) [9]
	Tylophorinidine	(Manikkoth *et al*., 2016)
	Desmethyltylophorine, desmethyltylophorinine, desmethyltylophoridine, dehydrotylophorine,	(Anand *et al*., 2012; Gupta *et al*., 2010b) [19]
Root	Tylophorine, tylophorinine, tylophrinidineand septidine	(Umamaheswari *et al*., 2017) [49]
	Tylophrinidine, paramethoxysalicyldehyde and essential oil	(Ghani, 2003)
	phenanthroindolizidine alkaloid, 3-O-demethyl tylophorinidine (VI)	(Dhiman *et al*., 2013; Gujrati *et al*., 2007) [8, 16]



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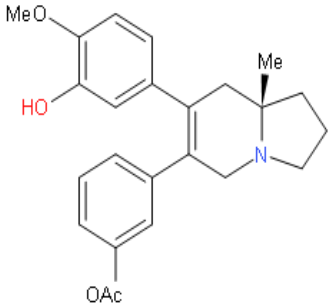

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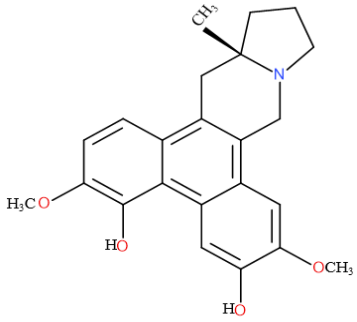

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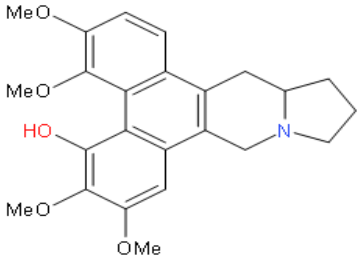

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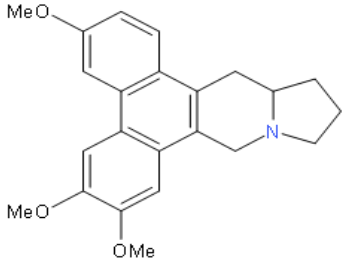

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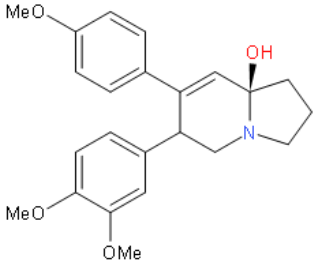

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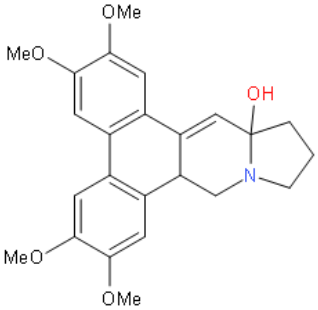

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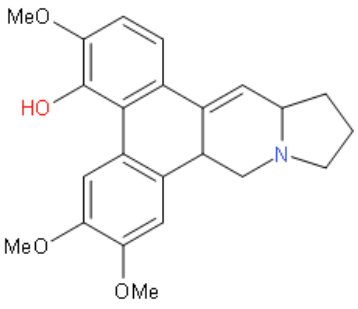

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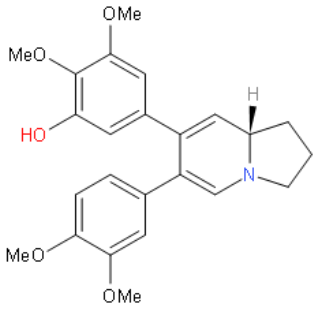

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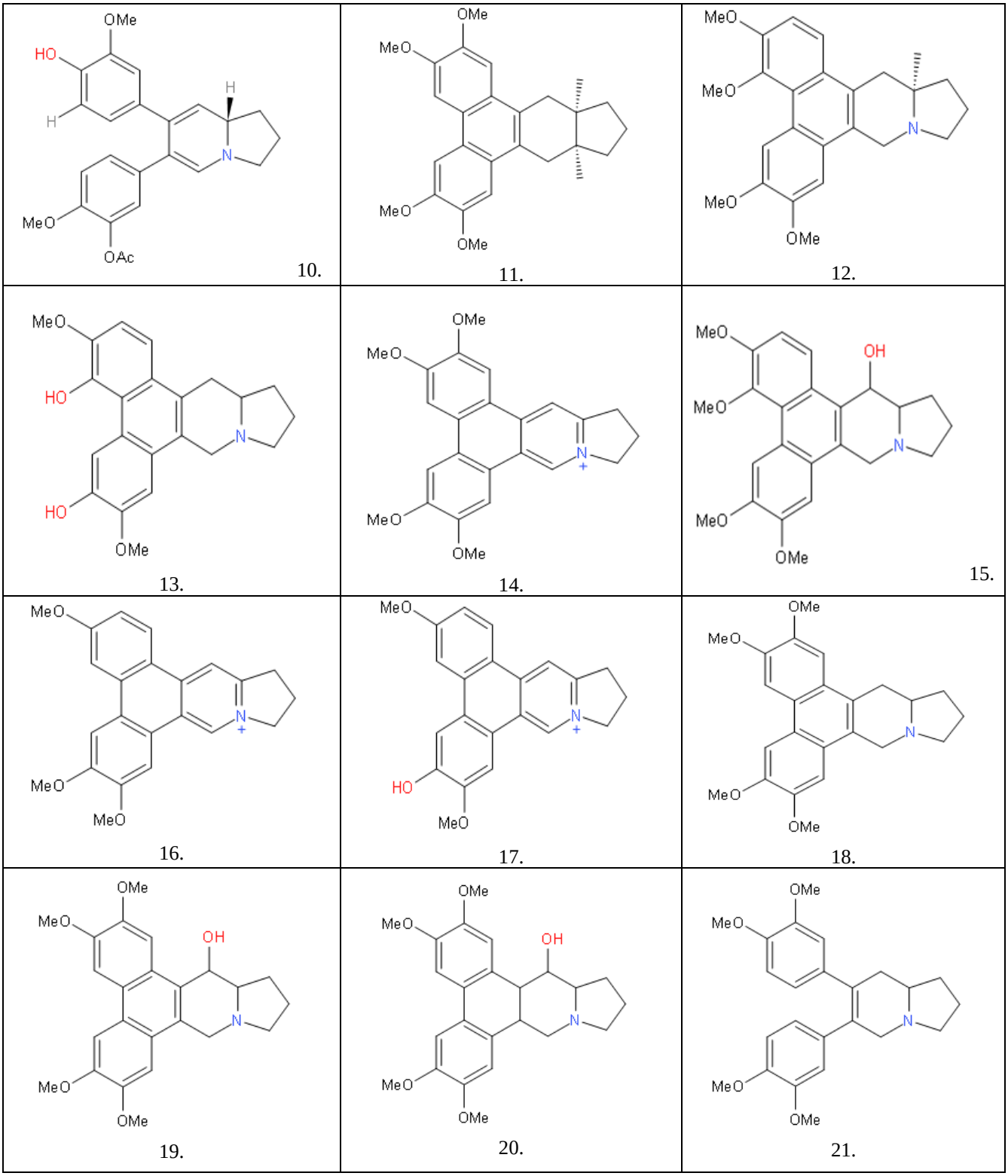

Fig 2: Structures of Major Phytochemical compounds

1.Tyloindicine – A; 2. Tyloindicine – B; 3. Tyloindicine – C; 4. Tyloindicine – D; 5. Tyloindicine – E; 6. Tyloindicine – F; 7. Tyloindicine – G; 8. Tyloindicine – H; 9. Tyloindicine – I; 10. Tyloindicine – J;11. Tyloindane; 12. Isotylocrebine; 13. 4, 6 – Desmethylisotylocrebrine; 14. Dehydrotylophorine; 15. 14 – Hydroxyisotylocrebrine; 16. Anhydrodehydrotylophorinine; 17. Anhydrodehydrotylophorinidine; 18. Tylophorine; 19. Tylophorinidine; 20. Tylophorinicine; 21. Septicine.

5. Cultivation practice

Conventionally, Tylophora is propagated through seed. Despite seeds showing good germination rate, fruit set is rare. Seeds show germination within 10 days and needs atleast 3 weeks for full growth. After growing for 3 months, plantlets are transplanted into fields (Rani *et al*., 2012) [38]. It should be done in rainy season. Proper distance should be maintained among the plantlets. The required rate of annual rainfall for growth is 1500-2000 mm. Plant also require proper humus in soil and partial shade. For the cultivation of plants loamy to clay soil enriched with manure is required.

Recent advancement of *Tylophora indica* and their economic value

T. indica has a lot of medicinal properties as well as having the commercial importance. Alkaloids of *Tylophora* are found in the leaves and roots of the plant. Many researcher are working on this plant to explore their activites. This compiled study will allow to access the advance achievements and research of *Tylophora.*

1. Medicinal Importance

Tylophora is used to treat asthma, so it was earlier called *Tylophoraasthamatica* (Gupta *et al*., 2010b) [19]. Furthermore, it is used to treat Bronchitis, rheumatism, allergies, inflammation, dysentery, dermatitis, whooping cough, diarrhoea, snake bite, muscle relaxant, laxative, expectorant, diaphoretic, purgative, stimulant, bacteriostatic, antifeedant, emetic and cathartic properties (Rani *et al*., 2012; Prasad *et al*., 2019) [38, 36]. It is reported that *Tylophora* is used as a blood purifier to treat rheumatism (Rani *et al*., 2012) [38]. Dry leaves of *Tylophora*are used as diaphoretic and expectorant. Leaves are employed in killing worms and leaf extract is used as anti-tumor. *Tylophora*is gaining worldwide interest due to its efficiency to treat asthma. It was reported that 400-500 milligrams of alkaloid in the powdered form when given daily for 6 days can cure asthma (Mandhare *et al*., 2015) [30]. It is also used as laxative, expectorant, diaphoretic, purgative, stimulant, bacteriostatic, antifeedant, emetic and cathartic properties. It also involved in some other medical properties like

1.1 Anti-asthmatic activity

T. indica plant is mainly known for the treatment of asthma, universally it is known as asthmatic herb (Gupta *et al*., 2010b) [19]. If 150 mg leaf of *Tylophora* is chewed on the daily basis than it can provide relief in asthma. If 40 mg alcoholic extract of tylophora used twice in a day than it can control asthma within 6 days.Histamine, an inflammatory mediator, decreased oxygen levels in Guinea pigs, resulting in convulsions. Convulsions are characterized by muscle contraction, hypotension, and dilation of capillaries. Histamine aerosol at a dose of 100 mg/kg induced spasms whose latent periods were prolonged by the *T. indica*. In Guinea pigs, the plant extract showed 43.15% protection against histamine-induced bronchoconstriction (Paliwal *et al*., 2011) [34].

1.2 Antitumor activity

Tylophorine is the major alkaloids of *T. indica*, showed the antitumour activity. In cell cycle tylophorine arrests the cell growth at G1 and S phase. Alkaloids, tylophorinidine and pergularine also shows the anti-cancer activity (Gantait *et al*., 2017a) [12]. There was an inhibitory effect of tylophorine analogs on transcription caused by cyclic AMP response elements, activator protein-1 sites, or nuclear factor-kappa B binding sites. A tylophorine analog is a powerful antitumor compound that differs from known antitumor drugs in its mode of action (Linyi *et al*., 2006) [28]. A series of polar phenanthrene-based tylophorine derivatives (PBTs) was synthesized, evaluated, and designed for use as antitumor agents. Against the A549 human cancer cell line, the newly synthesized PBTs were evaluated for their cytotoxic properties. Among them, *N*-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-l-2-piperidinemethanol and *N*-(2,3-methylenedioxy-6 methoxyphenanthr-9-ylmethyl)-5-aminopentanol showed the highest potency with IC50 values of 0.16 and 0.27μM, respectively which are comparable to those of drugs now being used to treat cancer (Linyi *et al*., 2006) [28]

1.3 Antimicrobial activity

Leaves of *T. indica* shows antibacterial activity against both gram positive and gram negative bacteria. Methanol extract of tylophora are more effective than the aqueous extract. It also shows the activity against viruses and parasites. There was strong antibacterial activity in all tested bacteria except *E.coli* at lower concentrations. However, all crude and pure compounds showed antifungal activity against *Aspergillus niger*, *Trichodermavirdae*, and *Aspergillus fumigates* but pure compounds had stronger antifungal activity than crude extracts (Reddy *et al*., 2009) [40]. Another study reported the bactericidal effect of solvent (benzene, isopropyl alcohol, and ethyl acetate) extracts against *S. typhi*, *E. coli*, and *S. aureus*. A maximum inhibition zone of 25–28 mm was observed for the ethyl acetate extract against *S. aureus* (Ponnanikajamideen *et al*., 2013) [35]. In both wild plants and tissue-cultured plants, methanolic extracts of *T. indica* plants exerted moderate effect with a concentration of 60μL against two fungi, *A. fumigatus* and *Verticillumlecanii*. For wild plants, the zones of inhibition were 7 and 8 mm, while for tissue-cultured plants, they were 8 and 9 mm (Vanitha *et al*., 2019) [50].

1.4 Antiinflammatory activity

The plant is used to control the rheumatism and inflammatory effects. The phenanthroindolizidine alkaloids such as ficuseptine – A and tylophorine suppress the nitric oxide production in RAW264.7 cell without having cytotoxicity (Gantait *et al*., 2017b) [15].

Commercial importance

An U.S. company Sabina Corporation developed the standardized extract of *Tylophora*which having 0.1% alkaloid content for the treatment of asthma. Ayush Herbs developed an herbal ayurvedic formulation of *Tylophora* Plus capsules to support the lung conditions. Geriforte Aqua is another drug developed at the commercial level by Himalaya Company which is used for delayed Hypersensitivity.

This was observed that, the combination of *Tylophora indica*, *Piper longum*, *Zingiber officinale* and *Emblica officinalis* extracts is used to exhibit the property of boosting immune system (Rani *et al*., 2012) [38]. Besides, the therapeutic value the plant also yields a fine, silky & strong fiber which can be used for the production of fine fabric.

Biotechnological approaches used for *Tylophora indica*

Plant genetic materials are important source to maintain diversity. With increased man made activities, biodiversity is facing a major threat which may lead to the extinction of species. Therefore, conservation is the only need of the present time. Medicinal plants have great importance to humans; their conservation is another important aspect to protect the critical elite genotypes and wild types. Micropropagation has been considered as one of the most viable biotechnological approach for conservation of germplasm.

1. Conventional growing method, propagation

Conventionally, *Tylophora* is propagated by means of seeds but seed germination is slow. It took around days to week for germination. Vegetative methods like stem cutting are also employed for propagation (Gantait *et al*., 2017a) [12]. Plants derived from seeds show unnecessary genetic variation which is detrimental to commercial propagation. Large scale demands needs fast multiplication methods which are difficult by vegetative methods which eventually led to requirement for effective micropropagation protocol so that the rising demand can be fulfilled (Kaur *et al*., 2011a) [23]. Due to these growing and propogation problem can be listed this plant among the endangered plant category (Sharma *et al*., 2014a) [47].

2. *In vitro* regeneration techniques

Generally, it can be propagated through seeds or vegetative cutting, but due to low seed viability and germination, it is quite difficult. Low yields were reported when regenerated through axillary bud culture (Prasad *et al*., 2019) [36]. Therefore, *in vitro* propagation is required for large scale regeneration and conservation. Rapid mass propagation of *T. indica* via leaf derived callus culture resulted in differentiated callogenic propagation routes that could be used for large scale multiplication of the plant. In *in vitro* regenerated plants, callus and suspension cultures have been used for mass propagation and also for the extraction of secondary metabolites (Anand *et al*., 2012) [2]. As the seeds of *T. indica* are small and do not germinate readily in the wild, large-scale propagation is difficult. *T. indica* was regenerated *in vitro* (indirectly, directly, and by somatic embryo mediated) and genome size was estimated by flow cytometry. There was the highest frequency of callus formation (87.75%) in the 6.7 µM 2,4-D with MS medium, which metamorphosed into progressive embryo stages (globular, heart, torpedo, and cotyledonary) (Mamgain *et al*., 2022) [29]. Murashige and Skoog's (MS) media supplemented with 15 and 10 mM of Benzyl Amino Purine provided an efficient and reproducible protocol for the *in vitro* cloning of *T. indica* through multiple shoot proliferation from nodal segments. Rooted plantlets of regenerates were successfully acclimatized and transferred to open field conditions, with 90% of the plantlets surviving after contamination and soil contamination from search overgrowth. Table 2 shows the *In vitro* regeneration studies on *T. indica.*

Table 2: *In vitro* regeneration studies in *T. indica*

S.No.	Explant used	Plant Growth Regulators used	Method used	Reference
1.	Leaf	29.4 µM NAA, 4.65 µM kinetin	callus and suspension cultures	(Anand *et al*., 2012) [2]
	Shoot	8.8 µM BAP	Micropropagation	
2.	Nodal	3mg/l 2, 4 D, 3 mg/ml BAP	shoot proliferation	(Kaushik *et al*., 2010) [25]
	Root	2 and 4mg/l IBA and NAA respectively		
3.	Leaf	0.5 µm TDZ, 1.5 µm 2,4-D	Somatic Embryogenesis	(Chandrasekhar *et al*., 2006) [5]
4.	Root	BAP	Organogenic nodular meristemoids	(Rani *et al*., 2012) [38]
5.	Leaf	0.5µm TDZ, 1.5µm 2,4 D	somatic embryos	(Gupta *et al*., 2010a) [18]
		7µM2,4-D and 1.5µM BAP	Organogensis callus	
		3µM IBA	Root formation	
		10µM 2,4-D and 2.5µM TDZ	Callus	
	Internodal	4 µM/L 2, 4-D	Embryogenic calli	
		5µM TDZ and 0.4 µM NAA	Optimum shoot Regeneration	
6.	Node	0.5mg/l IAA and 2mg/l Kinetin	Micropropagation	(Soni *et al*., 2015) [48]
	Leaf	0.5mg/l IAA and 1mg/l BAP		
7.	Various explants	BA	Shoot formation	(Kaur *et al*., 2011a; Kaur *et al*., 2011b; Reddy *et al*., 2010) [41]
8.	Nodular Segments	BA and NAA	Shoot formation	(Faisal and Anis, 2010) [10]
9.	Shoot	GA and BA	multiple shoots	(Rani and Rana, 2010) [39]
10.	Leaf segments	BA, TDZ, 2,4-D or 2,4,5-T.	embryogenic calli	(Gantait *et al*., 2017a) [12]
11.	Leaf	NAA, IBA, IAA, kinetin, BA	Callus formation	(Anand *et al*., 2012) [2]

3. Somatic embryogenesis

Somatic embryogenesis is the development of embryo from cluster of somatic cells. It follows two paths one directly from explant and another involves callus stage. Somatic embryogenesis is used for growth of plant at large scale. Plant regeneration through somatic embryogenesis is considered as more efficient than shoot multiplication (Roberts *et al*., 1995) [42]. Plant regeneration via leaf callus was first time reported in 2001 by using RAPD technique and somatic embryogenesis (Jayanthi *et al*., 2001) [22]. The effects of T-DNA genes on morphology, growth, and tylophorine content of Ri- transformed plants duringregeneration by somatic embryogenesis (Roychowdhury *et al*., 2015) [43].

4. Synthetic seed production

In plant biotechnology synthetic seed production is an advanced and fast growing method. At the present time, it has become an attractive approach for conservation of mass multiplication and transportation of germplasm (Gantait *et al*., 2015) [11]. This technology uses the meristematic tissue of plant material (nodes, somatic embryos, shoot tips) and therefore decreasing dependence on conventional methods of propagation and micropropagation. The technology uses nodes, somatic embryos/buds/ shoot tips plant propagules, which mimic seed and develop into a plantlet. The plant material used is embedded into matrix which works as an endosperm, containing all the necessary components (carbon source, growth regulators, nutrients, antimicrobial

agents). In 2011, it was shown that production of synthetic seeds using somatic embroids which induced from leaf explantsand that leaf explants were incapsulated in calcium alginate (Devendra *et al*., 2011) [7].

5. Agrobacterium mediated genetic transformation

Transfer DNA (T-DNA) of *Agrobacterium rhizogenes* a root inducing plasmid is used for the transformation process in plant genome (Roychowdhury *et al*., 2013) [44]. There are many other methods to transfer DNA directly through microinjection, electroporation, liposome fusion. However, *Agrobacterium* mediated transfer has major advantages over other systems. Transformed roots was induced with the bacterium culture which grew in axenic culture then transformed plant showed distinct morphological features which include short internodes, more branches, numerous plagiotropic roots, short internodes and small wrinkled leaves and it was also resulted accumulation of biomass and tylophorine production (Chaudhuri *et al*., 2006) [6]. Transgenic hairy root culture is another way to increase the production of secondary metabolites. In comparison to callus culture and suspension cultures, hairy root cultures are known for their biochemical, morphological and cytogenetical stability. The stability of transformed roots has been maintained in *in vitro* conditions of *T. Indica* (Roychowdhury *et al*., 2015) [43]. The effect of *crypt* gene on secondary metabolites was studied by the transformation of *cryptogein*gene with *T.indica* via *Agrobacterium* mediated, this effect has been widely studied (Basu *et al*., 2017).

6. Role of endophytes for the production of bioactive compounds in *Tylophora indica*

Endophytes are ubiquitous microorganisms which include bacteria and fungus, it colonized in most of the plants (Nair and Padmavathy, 2014) [33]. Endophytes does not cause any harm to the host plant, thus, they showmutualism and antagonism behaviour with host plants (Carroll, 1988). Endophytic fungi produce many bioactive metabolite which is used to protect plant that can be called as defense activator for plant. Theendophytic fungi *viz*., *Dothediomycetes*sp., *Alternaria tenuissima*, *Thielaviasubthermophila*, *Alternaria* sp., *Nigrosporaoryzae*, *Colletotrichum truncatum* and *Chaetomium sp.*has been isolated from leaves and stem of *T. Indica* and their activitywas checked against plant pathogenic fungus. This activity has been widely studiedby Kumar *et al*., 2011[26].

7. Extraction and purification of Tylophora

Several methods have been reported for efficient extraction and purification of tylophorine from *Tylophora* leaves. Maceration method was used for tylophorine extraction and obtained extract was purified by acid-base purification technique. Another method was reported for the extraction of tylophorine by HPTLC technique. In this study extraction oftylophorine was done fromleaves of regenerated plants withthe solvents (chloroform, hexane and dichloromethane), this extraction has been widely studied (Kaur *et al*., 2011b) [41].

Conclusion and Future Prospects

It can be concluded that *T. indica* is an important medicinal plant. It has many medicinal and pharmacological properties. The plant having the great potential to develop the new drug. A lot of research has been done on phytochemical, medicinal and biotechnological approaches of the plant but further more method for purification of tylophorine yet to be discovered for the mass multiplication and rapid growth of the plant. In the current state of knowledge, *T. indica's* toxicological aspects are still in the exploratory stage. Acute toxicology studies conducted in different clinical and preclinical settings cannot provide any substantial answers. It is therefore necessary to conduct a detailed acute and chronic toxicity study on *T. indica* extracts and isolated compounds in order to establish their safety and toxicological profiles. This will facilitate the use of tract information in the medical field. Hence, more research should be carried out on *T. indica* extracts and their bioactive constituents in order to understand their mechanism of action, structure-activity relationship, pharmacokinetics, and dose determination in various diseases. Novel gene discovery and pathway involved in the function of gene is not yet reported. Furthermore, different methods have to be discovered for the enhancement of tylophorine in this endangered plant species. So, by using various described techniques the plant can be save from extinction and secondary metabolites of this plant can also be used positively.

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