

Ethno-Eco-Chem-Medico and Tissue Culture Knowledge of the Asthma Climber-Antmool (*Tylophora Indica*)

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Review Article

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Abstract

Antmool (*Tylophora indica* (Burm. F.) Merrill) is a highly potent medicinal plant for asthma from sub-Himalayan tract which extends from Uttar Pradesh to Meghalaya. This contains many active chemicals viz., tyloindicines, tylophorinine, isotylocrebrine, tylophorine etc. This covers information concerning its various medicinal values viz., anti-inflammatory, antitumor, antiallergic, antimicrobial, anti hyperglycaemic, anti hyperlipidemic, antioxidant, immunomodulatory, anti-stress activity, anti-plasmodium activity and free radical scavenging properties. The *in vitro* studies confirmed that this have broad-spectrum anti-microbial activity and also inhibitory effect on HIV. It is useful in Alcohol induced anxiety, myocardial damage. It is hepatoprotective and has diuretic activity. It can inhibit cellular immune responses.

It is the famous remedy in Indian folk medicine since time immemorial for solving health issues viz., asthma, whooping cough, bronchitis, cold, cough, sore throat, snake bite, lucorrhoea and food poisoning. This has high medicinal recognition, so harvested on a large scale in an uncontrolled manner from its wild habitats. This review records various biotechnological approaches for large scale propagation of *T. indica* under *in vitro* conditions. This demonstrates the growth regulator synergy and even effect of explanting season. This also records results of culture studies and planting substrate used. The study carried out demonstrates that *in vitro* raised plant and callus produces better alkaloidal yields in comparison to the field plants. Novel antibacterial compounds can also be isolated from *in vitro* culture of explants. The review covers assessment of clonal fidelity, future of Ri T-DNA integrated rol Genes.

Keywords: *T.Indica*; Tylophorine; Bronchial Asthma; Myocardial Damage; Hepatoprotective

Introduction

Antmool (*Tylophora indica* Burm. F. Merrill), a member of family Asclepiadaceae, is an indigenous plant of

northern plains of India. It grows as light green delicate creeper. This also grows naturally in other sub-tropical regions of the world viz., Egypt, South Africa, United States, Cyprus, Iran etc. It is commonly called as 'Indian ipecac' in

English, 'Jangali pikvan' in Hindi and 'Antmool' and 'Anthrapachaka' in Sanskrit. It grows as dense patches in the forest in moist and humid conditions on open hill slopes/narrow valleys. It grows well in sandy soils. It shows reduced growth in areas with less rainfall ranging 30 to 40 cm. It is a perennial twining or climbing herb. Leaves come in the range of ovate to elliptic oblong (Figure 1). It bears knotty and fleshy roots. The plant has long and twining stems and semi shrubby. Its mesophyll has 6-8 spongy parenchyma layers and 2-3 layered palisade tissue. It bears rosettes of calcium oxalate crystals [1]. Flowers are small 1 to 1.5 cm and fruits are 7 x1 cm long which is ovoid-lanceolate and tapering at apex. Flowers and fruits are formed in August to December [2,3]. It can be propagated through seeds. But it has low rate of germination. To propagate it through stem cuttings is not easy as it fails to produce roots. So it needs *in vitro* micropropagation for growing plantlets for cultivation [4].

In central part of India fresh leaves are taken to cure bronchial asthma [5]. Leaves are effective in rheumatism, dermatitis, inflammation, allergies and bronchitis [6-8]. The leaves and roots are taken in hydrophobia. The dried leaves have diaphoretic, emetic and expectorant properties. Leaves and roots of plant bears 0.2-0.46 % therapeutically useful alkaloids viz. Tylophorinine, tylophorine and tylophorinidine [9,10]. Due to these this has many medicinal values which solve various human diseases. Its main alkaloid tylophorine shows anti-tumor,

anticandidal, antiamebic and anticancerous, but also antiinflammatory immunosuppressive properties [11-14].

Now in present century the Tylophorine has been identified in the *Asclepiadaceae* and *Moraceae* family and their claimed medical uses include the treatment of cancer, lupus, and inflammation [15-19]. Wu, et al. [20] found alphenanthroindolizidine, tylophorine in the leaves of *F. septica*.

Research investigations have been done to explore the phytochemical, medicinal and tissue cultural aspects of plant. But the data available is quite scattered far and widely. So an effort carried out to compile the phytochemistry, pharmacological profile and tissue cultural approaches used for its mass multiplication of this plant.

Ecology

T.indica is an indigenous plants from Indian plains. It grows up to an elevation of 1260 m in the sub Himalayan portion. It grows well in Central, North East Hills, Eastern plains, Bengal and parts of W Ghats/ S.plateau India [21]. Besides it is also found in Borneo, Ceylon and Malay islands [3]. It is now cultivated (Figure 1) for its medicinal uses. Nadkarni [21] mentioned that it grows well on a wide range of drained fields and prefers sandy soils.



Figure 1: *T.indica* under cultivation at AUH (supported by bamboo sticks or iron rods).

Phytoconstituents

The researchers have studied *T.indica* time to time in

order to find out presence of phytochemicals in its various parts (Table 1).

Source	Phytochemicals	Reference
Plant body	Septicine, Tylophorinidine, Tylophorine, Tylophorinine, Isotylocrebrine, Tylophoricine, resins, tannins, sterols, flavanoids, wax and resins	Rao, et al. [22]
	0.2-0.46% alkaloids viz. tylophorinidine, (+)-septicine, isotylocrebrine	Govindachari, et al. [23]
	Tylophorine, tylophorinine	Phillipson, et al. [24] Gopalkrishnan, et al. [14]
	Tylophorine derivatives (PBTs) N(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-5-aminopentanol and N-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-1-2-peridinemethanol	Gopalakrishnan, et al. [25]
	Tyloindicines	Bhutani, et al. [13]
	Phenanthroindolizidine alkaloids known as tyloindicines A-E, 14-hydroxyisotylocrebrine, 4,6-desdimethylisotylocrebrine, Tylophorine, 6-desmethyltylophorine, tylophorinidine and 5-hydroxy-O-methyltylophorinidine	Ali and Butani [26]
	Phenanthroindolizidine alkaloids such as tyloindicines A-E	Ali and Butani [26]
	Tylophorinicine, sterols, flavonoids, wax, resins and tannins	Rastogi and Mehrotra [27]
	Anhydrous dehydrotylophorinine, Tylophorindine, desmethyltylophoridine, desmethyltylophorinine and desmethyltylophorine	Gupta [28]
	Septicine, O-Methyl tylophorinidine and simple aliphatic acid	Reddy, et al. [29]
	Quercetin, α - and β -amyrins, Kaempferol, quercetin, desmethyltylophorine, 4,6-des-methylisodroxy-o-methyltylophorinidine, Tyloindicines H, I and J, desmethyltylophorinine, isotylocrebrine, non-alkaloidal compounds - octaosanyl octacosanoate, tetratriacontanol, β -sitosetrol, sigmasterol, tyloindane, cetyl-alcohol, wax, resin, coutchone, pigments, tannins, glucose, calcium salts, potassium chloride	Gupta, et al. [1]
	Phenanthroindolizidine alkaloid, 3-O-demethyl tylophorinidine (VI)	Dhiman, et al. [30]
	Alkaloids, flavonoids, phenols, saponins, steroids and terpenoids	Ranemma, et al. [31]
	Tylophorine	Umamaheswari, et al. [32]
Leaves	Tylophorine, tylophorinine, tylophorinidine and septidine	Mulchandani, et al. [33]; Bhutani, et al. [13]
	Septidine, tylophorinine (C ₂₃ H ₂₅ O ₄ N), Tylophorine (C ₂₄ H ₂₇ O ₄ N), tylophorinidine (C ₂₂ H ₂₂ O ₄ N)	Bhutani, et al. [13]
	Alkaloids, carbohydrates, steroids, saponins and triterpenes	Gujrati, et al. [34]
	Flavanoids, glycosides, saponins, carbohydrates, proteins and aminoacids, tannins, terpenoids and alkaloids	Meera, et al. [35]
	Tannins, saponins, flavonoids, carbohydrates and alkaloids	Balasubramanian, et al. [36]
	Alkaloids, glycosides, tannins and flavonoids	Raut, et al. [37]
	3-O-demethyl tylophorinidine and phenanthroindolizidine	Dhiman, et al. [30]
	Alkaloids, flavonoids, glycosides and Terpenoids	Sathyabama and Kingsley [38]
	Phenanthroindolizidine alkaloid, 3-O-demethyl tylophorinidine	Dhiman, et al. [39]
	Tylophorinidine	Manikoth, et al. [40]

	Tylophorine	Umamaheswari, et al. [32]
Roots	Tylophorine (C ₂₄ H ₂₇ O ₄ N), tylophorinine (C ₂₃ H ₂₅ O ₄ N), tylophrinidine (C ₂₂ H ₂₂ O ₄ N) and septidine	Mulchandani, et al. [33]; Bhutani, et al. [13]
	Tylophorine (C ₂₄ H ₂₇ O ₄ N), tylophorinine (C ₂₃ H ₂₅ O ₄ N), tylophrinidine (C ₂₂ H ₂₂ O ₄ N) and septidine	Bhutani, et al. [13]
	Tylophrinidine, paramethoxysalicyldehyde and essential oil	Ghani [41]
	phenanthroindolizidine alkaloid, 3-O-demethyl tylophorinidine (VI)	Dhiman, et al. [30]
	Tylophorine	Umamaheswari, et al. [32]
Callus	Kaempferol	Chaturvedi and Chowdhary [42]

Table 1: Phytochemical constituents reported from the asthma climber -*T.indica*.

Medicinal Attributes

It has been found that *T.indica* is a useful medicine for various respiratory clinical conditions like cough/asthma, constipation, diarrhoea, earache, headache, rhinitis, fearful dreams, and lumbago. It has also relieved people from vertigo, flatulence, profuse perspiration, bitter taste in mouth etc [43]. The medicinal attributes are as follows-

Bronchial Asthma

Bronchial Asthma means inflammation of lungs in which the patient complains recurrent attacks of breathlessness. The wheezing takes place because of narrowing in airways in lungs. The cause of asthma may be genetic or allergic response. But the disease develops and persists as a result of changes in the environment, food and life style. A study revealed moderate to complete relief in asthma symptoms if 150 mg of the leaf by weight of *T.indica* is chewed, swallowed in early morning daily [44]. If taken alcoholic extract 40 mg of *T.indica* twice daily up to six days can control symptoms of asthma [44]. A study recorded amount of oxygen in the lungs increased upon using the leaf powder [45].

In a study a group of asthmatic volunteers in the early morning at 5 AM one freshly plucked *T. indica* leaf was given to each person for a period of 5 consecutive days. The patients were asked to chew the leaf slowly and go to bed. In 80% of the cases the patients got relief from brachial problem with treatment of *T. indica*. However long and plentiful use of the leaves caused slackness and weakness to the patient [32].

Alcohol Induced Anxiety

Alcohol consumption has been big challenge to health since ancient times. Ethanolic extract *T.indica* can give tested in alcohol induced anxiety in Wistar albino rats [40]. They reported that alcohol induced anxiety may be prevented through ethanolic extract of *T.indica* in rats

(Wistar albino). It showed increase in conc of Dopamine in the brains of rats due to *T. indica*. Tylophorinidine have role in anxiolytic activity which mitigates lethal pressure of alcohol in central nervous system.

Anti-Inflammatory

T. indica has been used locally for control of inflammatory activities and rheumatism. Anti-inflammatory study of phenanthroindolizidine alkaloids showed suppression of lipopolysaccharide (LPS)/interferon (IFN) induced nitric oxide production in RAW264.7 cells. Phenanthroindolizidine alkaloids, tylophorine and ficuseptine-A showed powerful suppression of production of nitric oxide and have no cytotoxicity [46,47]. Found significant anti-inflammatory activity in ethanolic extract when administrated @100,200 and 400mg/kg in dose dependent manner in Albino wistar strain rats.

Myocardial Damage

Basheeruddin and Sowmya [48] studied the effect of hydroalcoholic extract of *T. indica* (HETI) on experimentally-induced myocardial infarction (MI) in rats. The propranolol 10 mg/kg (PRO-10), HETI @ 100 mg/kg, (HETI-100) or 200 mg/kg (HETI-200) were given to Albino rats up to 30 days orally. Their subcutaneous application of isoprenaline (IPL) @150 mg/kg induced MI in two consecutive days. HETI-200 and PRO-10 produced myocardium protection against IPL damage. It showed reduction in lactate dehydrogenase (LDH) and creatine phosphokinase-MB (CK-MB) in serum but an increase in activities of enzymes in heart tissue homogenate (HTH). The HETI-200 increased endogenous antioxidants (SOD and catalase) activities in comparison to IPL. This protection may be due to presence of flavanoids. This shows antioxidant activity through inhibiting the release of oxygen free radicals (OFR).It can also be due to formation of endogenous antioxidants such as catalase and SOD in IPL-induced cardiotoxicity [48].

Antitumor

Sometimes existing therapeutic treatments fail to cure cancer so there is a need of new and efficient plant drugs. Plant based drugs show no side effects. It shows no chances of drug resistance. In this connection Tylophorine have been found to possess antitumor activity with a newer mode of action. Polar phenanthrene-based tylophorine derivatives (PBTs) N(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-5-aminopentanol and N-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-1-2-peridinemethanol have been proved to be potentially cytotoxic against A-549 human cancer cells [25].

Tylophorine can check cAMP response in HepG2 lung carcinoma through treatment of forskolin, TPA and TNF α respectively. Tylophorine may reduce S-phase progression and arrest growth at G1 phase in HONE-1, HepG2 and NUGC-3 of carcinoma cells [49]. The phenanthroindolizidine alkaloids pergularine and tylophorinidine can retard activity of thymidylate synthase and dihydrofolate reductase which shows anticancer activity [50,51].

From leaves and stem parts of *T.indica* 3-O-demethyl tylophorinidine (VI) and phenanthroindolizidine were extracted which showed anticancer activity against various cancer cell lines. It showed anticancer activity at IC50 value which ranges 0.89-1.40 μ M [30].

Antiangiogenesis targeting of vascular endothelial growth factor receptor 2 (VEGFR2) is an important tool in cancer treatment. Tylophorine reduced VEGF-induced angiogenesis such as proliferation, migration and even tube formation in endothelial cells. It can directly check downstream signaling pathways, VEGFR2 tyrosine kinase activity and also Akt, Erk and ROS of endothelial cells. Tylophorine creates anti-angiogenesis effects through vascular endothelial growth factor receptor 2(VEGFR2) signaling process. Thus it may be a potential of *T.indica* doing anti-angiogenesis and anticancer therapies [52]. Effective anticancer activity of *T.indica* acetone and ether extracts on BHK-21 cell line was reported [53]. At 300 μ g/ml chloroform leaf extract of *T.indica* (TICLE) disclosed 79.97% ($p < 0.01$) and TIELE (*T.indica* ethanolic leaf extract) revealed 65% antiproliferation on MCF-7 cells with IC50 values 90 μ g/ml and 131.94 μ g/ml respectively [54].

The ethanolic extracts of *T. indica* for anti-proliferative effect were studied against HCT-15 colon cancer cell lines. In vitro free radical scavenging studies showed potent antioxidant activity [55]. There was an increased cell death and membrane damage in HCT15 cells treated at IC50

value of 40 microgram /ml of crude extracts of *T.indica*. Increased LDH leakage produced increased damage of membrane when treated with ethanol fractions of *T.indica*.

Antiallergic

The antiallergic activity of tylophorine was studied and compared with that of disodium cromoglycate. This was done by observing fluctuations in volume of the perfusate/minute. For preparation of aqueous extract (5%) of *T.indica*, the dried leaf powder was soaked in water for 24 h and filtered. The aqueous extract @0.5 ml/100 g was injected intraperitoneally to 6 Rats for three days prior to sensitization of rats. The use of extract intraperitoneally @5 mg/kg enhanced the flow from 7.65 to 19.55 ml/min in lung. This may be due to membrane stabilizing, immunosuppressive and bronchodilatory property of *T.indica* [56].

Hepatoprotective

Gujrati, et al. [34] investigated hepatoprotective activity in alcoholic and aqueous leaf extracts of *T.indica*. Ethanol extract caused increase in liver weight and volume. This resulted an increase in serum aspartate and alanine transaminase, alkaline phosphatase, total bilirubin, cholesterol and triglycerides. There was reduction in total albumin and protein levels, damage to hepatocytes and thiopentone induced sleeping time. This evidences that ALLT and AQLT have hepatoprotective activity. The alcoholic extract has greater hepatoprotective activity in comparison to the aqueous extract.

The methanolic extract of *T.indica* leaves was also tested for hepatoprotective role in carbon tetrachloride posed hepatotoxicity in albino rats. The protection was estimated on basis of serum glutamate oxaloacetate transaminase and pyruvate transaminase, protein and serum bilirubin. In silymarin @25 mg/kg treated animals the hepatoprotective activity of methanolic extract @ 200 mg/kg and @300 mg/kg body weight was compared. *T.indica* leaf extracts @200 and 300 mg/kg showed reduction of serum hepatic enzymes in comparison to rats treated with carbon tetrachloride. The histopathological after methanolic extract treatment showed recovery in hepatocytes [57].

Antimicrobial

For antibacterial and antifungal properties of the extracts of *T.indica* were studied. Use of crude extracts of leaf had higher antibacterial activity in comparison to root and shoot against *Mycrococcus luteus*, *Staphylococcus*

aureus and *P. aergenosa* and *Bacillus subtilis*. *Escherichia coli* were not inhibited even at higher doses of either crude or solid extracts of *T.indica*. But crude and solid extract recorded antifungal action against *Trichoderma viride*, *Aspergillus niger* and *A.fumigatus*. The solid extract showed higher antifungal activity in comparison to crude extracts [29,58].

The methanolic leaf extract of *T.indica* showed bacterial inhibitory activity against *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *E. coli*, *P.vulgaris* and *S. typhi* [29,58]. The *in vitro* raised *T.indica* plants showed antibacterial activity against *Streptococcus agalactiae*, *S.pyogenes* *Staphylococcus aureus*, *S.epidermidis*, *Enterococcus faecalis* and *Bacillus* species. Aqueous leaf extract is effective against *S. Epidermidis* [59]. Sangeetha, et al. [60] observed significant activity of leaf extract of *T. indica* against *S. aureus* but none against *P. aeruginosa* and *E. coli*.

Raut, et al. [37] studied antibacterial potential of root extracts of *T. indica* against *Escherichia coli*, *Micrococcus roseus* and *Pseudomonas flavescens*. They reported that methanol root extracts of *T. indica* shows inhibition of all bacterial strains producing 15mm zone of inhibition against *P. flavescens* and 4mm zone of inhibition against *M. roseus*.

Agar well diffusion method was used to find out antibacterial activity in *T.indica* and for comparison with *in vitro* grown plants and callus. *T.indica* alcoholic leaf extract showed activity against gram negative bacteria [61,62]. MIC was in range of 3.05 to 12.0 µg/ml against gram positive bacteria but 1.53 to 24.0 µg/ml against gram negative bacteria. So it may be used in the treatment of various infections [61,62].

Siraj, et al. [63] studied *in vitro* raised callus for antimicrobial activity against gram negative, gram positive and even resistant bacteria having *bla* genes. The MIC values of the alcoholic extracts of *in vitro* raised callus was 1.53 -24.0 µg/ml against gram positive and those harbouring *bla* genes was in the range of 12.0 -98.0 µg/ml.

Khatoon, et al. [64] studied antifungal activity in *in vitro* raised plant. MIC of *in vitro* raised plant was in the range of 1.53 to 49.0 µg/ml against fungi viz., *Candida albicans*, *Candida krusei*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Fusarium* spp., but MIC of parent plant was in range of 12.0 to 98.0 µg/ml. This records a conclusion that *T. indica* contains good antifungal activity which may be useful in various fungal

infections .So *in vitro* cultivation of the explants can be used for getting novel antifungal compounds [64].

Susceptibility of bacteria to the phytochemical extracts of *T. indica* was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, (gram negative) and *Staphylococcus aureus* (gram positive). The screening showed broad spectrum of antibacterial activity [38]. Ranemma et al. [31] found higher antibacterial activity in *T. indica* against *S. aureus* when compared to *E. coli*, *P. aeruginosa* and *K. pneumonia*.

Anti-Hyperglycemic and Anti-Hyperlipidemic Activity

Swathi, et al. [65] found effect of methonolic extract of leaves of *T.indica* on blood glucose in alloxone @120 mg/kg. induced diabetic rats. This was given @ 200,300,400mg/kg. up to 28 days. *T.indica* showed decrease $p < 0.001$ in blood glucose. It showed alteration in lipid metabolism, triglyceride, serum total cholesterol and low density lipoprotein cholesterol levels but an increase in high density lipoprotein cholesterol conc in diabetic rats $p < 0.001$. This showed lowering of bilirubin, creatinine and lipid profile of treated diabetic rats showing that *T. indica* has anti diabetic potential.

Checking Cellular Immune Response

Ganguly and Sainis [66] studied the effect of Tylophora alkaloids mixtures and found them inhibiting delayed hypersensitivity reaction in rats. The alkaloids inhibited contact sensitivity to dinitrofluorobenzene in mice. Lymphocytes obtained from sensitized mice if treated with tylophora alkaloid may suppress delayed type of hypersensitivity response.

Diuretic Activity

The diuretic activity was studied in aqueous and alcoholic extracts of *T.indica* leaves in rats. There was increase of cation and anion excretions and urine volume. Na^+ /K^+ ratio for aqueous and alcoholic extract were 2.04 and 2.18 respectively. The normal value for Na^+ /K^+ ranges 2.05 -2.83. The aldosterone secretion gets decreased if Na^+ /K^+ ratio falls below normal in plasma. There was an increase of Na^+ , K^+ and Cl ion excretion in aqueous and alcoholic extract treated animals [35].

Antioxidant Potential

Sharma, et al. [8] reported antioxidant activity in *T. indica*. This was done by using 2, 2-diphenyl 1-picrylhydrazyl (DPPH). Leaf extract resulted strongest

antioxidant activity of 59.25 $\mu\text{g/ml}$. The DPPH radical scavenging were 63.03 % for ascorbic acid, 12.85 % for root and 17.24 % leaf extract, at concentration of the 200 $\mu\text{g/ml}$. Ascorbic acid exhibited higher DPPH scavenging activity than the compound at all concentrations. Ascorbic acid, root and leaf extract @700 $\mu\text{g/ml}$ showed scavenging action 89.85%, 80.15% and 64.12% respectively [31].

Immunomodulatory

Immunomodulatory activity of *T.indica* alkaloids was studied for crude extract of the leaves of *T.indica* which produced delayed hypersensitivity reaction in rats. The alkaloid mixture checked sensitivity for dinitrofluorobenzene in mice when introduced prior to or after contact sensitization [67]. Recently Vadlamani, et al. [68] recorded moderate immunostimulant action in this plant.

Anti-Stress Activity

Antistress activity in aqueous extract of *T. indica* @100, 250 and 500 mg/kg was studied using chronic cold restraint stress model in Wistar rats [69]. They mentioned that stimulation of hypothalamus pituitary adrenal axis under stressful situation changes biochemical parameters viz, glucose, plasma corticosterone, proteins and triglyceride which affects spleen weight and adrenal gland.

Antiplasmodium Activity

In tropical countries a large rates of mortality occurs due to Malaria. The emergence of drug resistant *Plasmodium* parasite strains has prompted to search newer effective and antiplasmodial agents which have minimal side effects. George, et al. [70] studied the role of extract of *T.indica* *in vitro* against *P. falciparum* strains 3D7 and RKL-9. The Hydroalcoholic was found to be active and hence it may be effective antiplasmodial agents against RKL-9 drug resistant strains.

Tissue Culture Studies

The vegetative field propagation of *T.indica* is not easy because of low seed viability and germination. The plants obtained from seeds show genetic variation so not fit for commercial purpose [71]. But it needs large scale demand. Tissue culture studies have been conducted in *T.indica* from explants. The embryo like structures was developed through somatic cells which give rise to normal plantlets when cultured on media [72].

The callus cultures obtained through stem explants produces a broad spectrum regenerative potential and

tissue differentiation producing bipolar embryos, roots, shoots even plantlets [73].

The somatic embryos were obtained on MS medium from leaf explant when supplied with BA 1-2 mg/l or kinetin 1-5mg/l [74]. From 100 mg of embryogenic callus within 60 days 30 somatic embryos were obtained at an optimum 2mg/l of BA. The embryoids were transplanted to sterile vermiculite for hardening. After two weeks of hardening the plantlets were transferred in green house which showed 90% survival [75].

Chaudhuri, et al. [75] studied a new micropropagation system for *T. indica* by using root explants cultured on MS medium having 6-benzyladenine or 2- isopentyladenine which produced organogenic nodular meristemoids in 4 weeks. 88-96% survived then transferred to the field.

It has been recorded that callus induction and transformed roots depend on the explant type, bacterial strain and inoculation site. They found induction when explants were infected with strain A4 *A. rhizogenes*. They also noticed culture medium harboured tylophorine up to 9.78+/-0.21 mg l(-1) [76]. Thomas and Philip [71] reported organogenesis through immature leaf pieces on MS medium supplemented with 1.5 μM 6-benzyladenine and 7 μM 2,4-dichlorophenoxyacetic acid. 92% of explants produced callus. When hormone 8 μM thidiazuron was used the shoot buds were formed in 100% cultures. This was an average of 66.7 shoots per culture. 3 μM indole-3butyric acid was used for roots formation in half strength MS-medium. These plants were transferred to soil with 92% survival.

Faisal, et al. [77] investigated a method for plant establishment of *T.indica* with high frequency of shoot regeneration from petiole derived callus. They produced callus from explants in MS basal medium with 2.5 μM thidiazuron and 10 μM 2,4- dichlorophenoxyacetic acid. The *in vitro* raised plantlets with well-developed shoot and roots got successfully established in garden soil earthen pots.

The sections from leaf of *T.indica* were cultured in MS medium enriched through 2,4-dichlorophenoxy acetic acid and thidiazuron. The dose 0.5 μM TDZ and 1.5 μM 2,4 D showed effectiveness in inducing somatic embryos. Plant got regenerated through *in vitro* somatic embryos on semisolid medium [78].

Sheng, et al. [79] investigated the impact of abscisic acid, sugars, gibberellic acid in somatic embryogenesis in

internodal explants obtained callus of *T. indica*. They reported that internodal explants formed embryogenic calli and MS medium added 4micromol/L 2, 4-Dichlorophenoxyacetic acid (2, 4-D) proceed the best. They found that 200mmol/L sucrose with 2micromol/L ABA , 200mmol/L sucrose with 10micromol/L GA3 and 200mmol/L sucrose with 6micromol/L Kn improved somatic embryogenesis significantly in *T. indica* while glucose alone or with sucrose had inhibitory action. The embryos obtained got converted into plants easily.

Dennis and Thomas [80] got regeneration of plants from calli. The calli formed shoot buds in 3-4 weeks. The frequencies of calli forming shoots ranged 5 to 44%. There was optimum regeneration of shoot on MS medium when added 0.4 μ M NAA and 5 μ M TDZ. The 44% cultures produced an average of 12 shoots per callus. Kaushik, et al. [81] found a combination of IBA and NAA @ 2 and 4 mg/l respectively proved the best for root initiation.

Rani and Rana [82] studied in vitro propagation of *T.indica* on parameters such as influence of explanting season, growth regulator synergy, culture passage and planting substrate. From nodal segments bud break (85%) and multiple shoot formation got induced when it was cultured on MS medium having 2.0mg/l BAP. They recorded rooting of the excised shoots in secondary or subsequent cultures were best activated in ½ strength MS medium having 0.5 mg/l IBA. For hardening vermi compost was the most useful planting substrate which ensured 96% survival of regenerated plantlets.

Chaturvedi and Chowdhary [42] studied the accumulation of kaempferol in undifferentiated callus. Rajavel and Stephan [83] described an effective protocol for fast *in vitro* multiplication of *T. indica* using various carbon sources, viz. AR grade sucrose, white refined sugar (table sugar), and unrefined brown sugar, jaggery and sugarcane juice. The explants were cultured initially in MS medium along with Naphthalene acetic acid and 6-Benzylamino purine. NAA was useful in shoot development. Selected concentrations were used for adding carbon sources in different sugars (2% and 3% w/v) as an alternative ingredient for the MS medium composition. The study revealed that the percentage of response was high in AR grade sucrose 95.2% dose followed by white refined sugar 94.8%, sugarcane juice 76.8%, unrefined brown sugar 73.8% and jiggery 67.6% respectively. So alternative carbon source can be a cheaper alternate in place of MS media for propagating *T. indica*.

Sharma, et al. [84] reported *in vitro* formation of plantlets by indirect organogenesis in *T.indica*. Calli were formed from *in vivo* leaves of *T. indica* in MS medium when added Indole-3-butyric acid 0.5 mg l⁻¹ and 6-Benzylaminopurine 2.0 mg l⁻¹. The multiple shoots 12.00 \pm 1.50 emerged and formed on MS medium when fortified through Thidiazuron 0.1 mg l⁻¹.

Nayeem, et al. [85] used adventitious roots of *T. indica* as explants and found. They reported that Maximum shoot regeneration occurred when roots cultured having IBA at 2.0 mg/L concentration. Shoot bud elongation was obtained on MS full strength having BAP at 0.1 mg/L and rooting occurred on MS half strength medium having IAA at 0.2 mg/L concentration.

Soni, et al. [86] reported an enhancement in tylophorine level in *T.indica* by precursor feeding and micropropagation. Nodal segments and leaf were kept on MS medium by adding doses of auxins and cytokinins to get standard micropropagation method. The best dose for regeneration of leaf was MS+0.5mg/l IAA+1mg/l Benzyladenine and node was MS+0.5mg/l Indole-3-acetic acid+2mg/l Kinetin. Tyrosine which is a precursor of tylophorine was added in different doses at different time intervals. The highest tylophorine content of 27.71 μ g/g DW was obtained in presence of tyrosine.

Studies on Clonal Fidelity

Sharma, et al. [84] studied tissue cultured plantlets to find clonal fidelity by Inter simple sequence repeat markers. A total of 71 clear and distinct bands were formed when 6 primers were used. The banding pattern of each primer showed uniformity when compared with mother plant having 93% homology. The ISSR analysis studies indicated genetic stability.

Role of Ri T-DNA Genes In Regeneration

Roychowdhury, et al. [87] studied the role of integrated Ri T-DNA genes in regeneration. The Fifty root lines studied resulted integration, expression of four rol genes of TL-DNA. They found spontaneous regeneration from root lines. There was stable integration and expression of rol genes in embryogenic callus lines, root lines and even in spontaneously induced somatic embryos. Variant Ri transformed plants resulted in highest tylophorine content. They found the effects of T DNA genes on tylophorine content, growth and morphology of the Ri-transformed plants was stable for a long term.

Agrobacterium Mediated Transformation

Alagumanian, et al. [88] carried out *in vitro* culture for transformation by co-cultivation of *T.indica*. The maximum growth in terms of fresh and dry weight was observed in case of 2, 4-D @ 5mg/l and the minimum growth was observed @ 0.5mg/l. But IAA @ 5mg/l showed growth promotion in nodal explants of *T.indica*. Among different explants of *T. indica*, nodal explants showed maximum growth response in culture.

Enhancement of Compound

Chaturvedi and Chowdhary [42] reported an increase in kaempferol content in *T.indica* by applications of salicylic acid, ornithine, cinnamic acid, tyrosin and phenylalanine @10 and 20 mg/100 ml. The callus of *T. indica* cultured on MS medium with addition of 3% sucrose had much increase in kaempferol content when used @20 mg /100 ml of tyrosin in suspension culture.

Antmool in Folk Medicine

Antmool leaves act as expectorant hence useful in respiratory infections and bronchitis and even whooping cough. This has analgesic property [89].

“Antmool” or Indian ipecac is mainly used in treatment of allergies of respiratory tract, bronchitis, asthma and hay fever. The home remedies using *T.indica* help in whooping cough, common respiratory infections, cold etc. as follows;
For Respiratory Problems, Asthma And Cough: Take 3 to 4 fresh dust free leaves of Antmool chew empty stomach followed by a glass of lukewarm water.

Whooping Cough and Bronchitis: *T.indica* helps in removing cough and bronchitis in children. Take one fourth of fresh Antmool leaf. Grind to paste and mix with 1 gram honey and feed to the child up to 5 days.

Cold and Cough: Take 2 fresh leaves of *T.indica* and Basil leaves each along with clove(1g) and ginger(1g) boiled in 200 ml of water to make decoction. Filter it to drink 1 spoonful two times daily up to 10 days to cure cold and cough.

Snake Bite: For this take root of *T.indica*, section in small pieces and chew 10 g twice in a day for ten days. Prashantkumar and Vidyasagar [90] reported that for snakebite, handful of leaves is crushed in urine of person with snakebite and the extract (2-3 drops) is dropped into nostrils.

Lucorrhoea: Boil a few leaves and root (10 g) of *T. indica* together for 5 min in 200ml of water. Strain it and allow the decoction to get cooled and refrigerated. Take a dose of 20ml once a day in morning up to a week.

Food Poisoning

For food poisoning extract of five leaves of *T. indica* is taken, initially vomiting occurs and later it gives relief [90].

Side Effects

The side effects produced due to use of *T. indica* are sleepiness or giddiness and mouth pain, decreased taste and upset stomach [91,92]. Patient on tylophora consumption experiences soreness in mouth, nausea, vomiting and loss of taste for salt. It has been found that extracts are safe in smaller doses [93]. At larger doses the dried leaves may result in poisoning. Tylophorine and tylophorininine may cause skin eruption and redness. *T.indica* have toxic role for *Paramecium caudatum* at concentration of 1 in 50,000 [94]. This should not be taken in pregnancy because it may cause abortion.

Formulations

This has a great demand worldwide because it is effective against asthma. Sabina Corporation, an ayurvedic nutraceuticals, a U.S. company are producing standardized extract of *T. indica* which contains 0.1% alkaloid. This is useful in respiratory disorders. Tylophora Plus capsules are marketed by Ayush Herbs Inc. as an Ayurvedic herbal formulation which is useful in lungs problems. It supports body immune system when used in combination of *Embllica officinalis*, Piper longum and Ginger. Geriforte Aqua is also prepared by Himalaya group of companies which is used to delay hypersensitivity.

Conclusion

For developing new drug formulations *T. indica* has immense potential because of phytoconstituents present which show various therapeutic effects.

The *in vitro* developed plants have better antibacterial activity in comparison to the parent natural field grown plants. This has antibacterial activity against many gram negative and gram positive bacteria such as *S. aureus* and *S. epidermidis*. It is useful in curing infectious disease causing organisms, which now show resistance to the common antibiotics. Antibiotic resistance in microorganisms is increasing these days due to their excessive use for treatment of simple infections. Although synthetic antibiotics have been developed yet they have many side effects, hence alternative plant derived medicines are now becoming popular. The extract of *T.indica* also has diuretic potential which supports its ethnopharmacological uses.

Present review clearly reveals *T. indica* extract has remarkable antibacterial potential. It can be used to derive novel antimicrobial agents in treatment of infections viz., pneumonia, diarrhoea, urinary tract infections and wounds.

The explants from node, leaf and stem grown on MS medium added with different hormones like NAA, IAA, IBA and 2,4-D summarises that nodal explants have maximum morphogenetic potential than explants of *T. indica*. It can be concluded from tissue culture studies that further research is still required to explore various carbon sources on *in vitro* *T. indica* plant regeneration. It also needs to search an effective regeneration protocol for large scale multiplication and propagation. It needs more study how to enhanced level of tylophorine in this endangered species.

Studies are also required to enhance kaempferol in callus of *T. indica*. It is evident from review T-DNA genes have a potential effect on tylophorine content. So in this context more studies will have to be tried for all compounds present in this plant.

Conflict Of Interest Statement

We declare that we have no conflict of interest.

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