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TYLOPHORA INDICA : A BIOTECHNOLOGICAL APPROACH

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Present review article describes the classification, taxonomic description, medicinal use, habitat and distribution of *Tylophora indica* ,an important indigenous plant used for many deseases like asthama, inflammation, anticancerous and immunomodulatory .The article contain the efficient protocols of indirect organogenesis and secondary metabolites production *in vitro* tissue culture of *T. indica*.In addition to this, the study of antioxidant activity, cytotoxicity and anti-inflammatory activity of *T. indica* extracts are also included in this review. The molecular characterization of regenerated micro shoots of *T. indica* was carried out by using the RAPD and ISSR analysis.

Keywords : Anti-inflammatory activity; Antioxidant acitivity; Callus; ISSR analysis; Kaempferol; Molecular characterization; RAPD; Stigmasterol; *Tylophora indica;* Tylophorin.

Production of secondary metabolites by cultured cells provides a particularly important benefit to manipulate and improve the production of desired compounds; thus biotechnological approaches to increase the concentrations of the metabolites are discussed. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated .Using this methodology a wide range of chemical compounds have been synthesized[1]. The callus initiation of *Tylophora indica* has been documented by using different medium proportion[2]. Growth inhibition is often associated with cytodifferentiation and the induction of enzymes for secondary metabolism, so dual culture system is preferred. Dual culture system involves biomass production in a medium optimum for cell proliferation followed by transfer of healthy cells to a different medium which is favorable for product yield. This strategy was used by Zenk *et al.*[3], for the production of indole alkaloids by *Catharanthus roseus* cells. In the present study, this strategy was used to enhance the production of stigmasterol in *Tylophora indica* tissue culture.

Tylophora is a genus of slender climbing perennial plants which has about 60 species from various parts of the world.This name has been derived from two ancient greek words - 'Tylos' meaning "knot" and 'phoros' meaning "bearing". It was earlier placed in Asclepiadaceae which has now been sunk into Apocyanaceace .This review article describes *in vitro* organogenesis as well as the production and enhancement of secondary metabolite in tissue culture of *T. indica,* which is an anti-asthamatic, anti-inflammatory climber plant.The review is divided into several parts as follows.

1.Classification- Kingdom - Plantae, Order -Gentiales, Family - Apocynaceae, Subfamily-Asclepeadaceae, Genus-*Tylophora,* Species-*indica.*

2.Vernacular names: Bengali- Antamul; Hindi- Antmool, Janglipikvan; Kannada-Adumuttada, Nepala; Malayalam- Vallippala; Marathi- Kharaki-rasna, Anthamul, Pitmari; Oriya- Mendi, Mulini; Tamil-Koorinja, Peyppalainadu; Telugu- Verripala, Kukka-pala.

3.Taxonomic description: Perennial, small, slender, much branched pubescent twining or climbing herbs or under shrubs; sap yellowish, found in the sub-himalayan tract from Uttar Pradesh to Meghalaya and in the central and peninsular India, ascending up to 1,260 m. Rootstock 2.5-5 cm thick, roots long, fleshy, with longitudinally fissured light brown, corky bark; leaves 6.0-10.5 x 3.8-6.0 cm, ovate-oblong to elliptic-oblong, acute to acuminate, cordate at base, thick, pubescent beneath when young, glabrous above; petioles up to 12 mm long; flowers minute, 1-1.5 cm across, in 2 to 3-flowered fascicles in axillary umbellate cymes; calyx divided nearly to the base, densely hairy outside; segments lanceolate, acute; corolla greenish-yellow or greenish-purple; lobes oblong, acute; Fruit a follicle, up to 7 x 1 cm; ovoid-lanceolate, tapering at apex forming fine mucro, finally striate, glabrous. Seeds 0.6-0.8 x 0.3-0.4

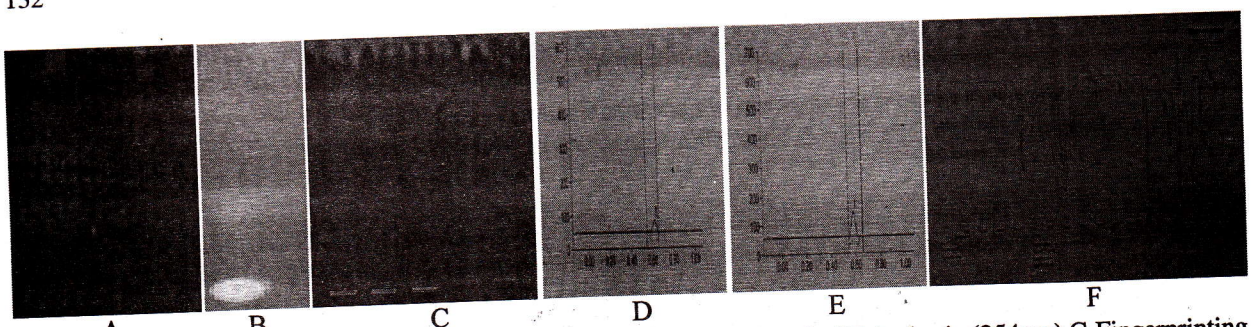

Fig.1. A -Preparative Thin Layer Chromatography (at 254 nm) ,B- Standard of Tylophorin (254nm),C-Fingerprinting of High Performance Liquid Chromatography of methanol: ethanol(8:2) extract of *Tylophora indica* leaves,D-HPTLC Chromatogram of methanol: ethanol(8:2) extract of *T. indica* leaves, E-Standard, F-Infra Red spectral studies of isolated Tylophorin with standard.

cm, broadly ovate or ovate-oblong, flat, brown, dark coloured in centre; coma 2.0-2.5 cm long[1-6].

4.Medicinal use-Its antitumor, immunomodulatory, antioxidant, antiasthmatic, smooth muscle relaxant, antihistaminic, hypotensive, antirehumatic activities are scientifically proven. In Ayurveda, the plant has been used in treatment of asthma , dermatitis and rheumatism[1,6]. Although the leaf and root of this plant are widely used for treating jaundice in Northern Karnataka, there is a paucity of scientific evidence regarding its usage in liver disorder[3]. The other reported activities, include cytotoxic effect[7] immunomodulatory activity[8], anticancer activity and antiamoebic activity[9].

5. Habitat- Found in the plains, forests, and hilly slopes and outskirts of the forest[10]. Forms dense patches in the forest in moist and humid conditions, in open hill slopes and narrow valleys, also cultivated for its medicinal uses. The plant shows stunted growth in the areas with lesser rainfall.

6. Distribution- The plant inhabits up to an elevation of 1,260 m in the sub-Himalayan tract and in the central and peninsular India. It also occur with in Eastern, North-East and Central India, Bengal and, parts of South India[11]. Except throughout plains of India, it also harbor in Ceylon, Malay island and Borneo[12].

7. Propogation - Tylophora is conventionally propagated through the seeds. The seeds show good germination percentage, but fruit set is rare. Seeds start germination in 10 days and the germination will complete in 3 weeks.After germination, the 3 months old plantlets are ready to transplanting in the field but the transplantation should be done in rainy season and plant distance should also be maintained. The annual rainfall required for *Tylophora* plant is 1000 -1500mm.The plant prefers partial shade condition of the forest and soil rich in humus. It needs the support of host for climbing to a sunny location. For its cultivation, loamy soil to clay and supplemented with farmyard manure, ambient conditions of temperatures and sunlight are desirable.

8. Phytochemicals - Tylophora plant has been reported to contain 0.3-0.4% of alkaloids *viz* Tylophorine, Tylophorinine, Tylophorinidine, Septicine, Isotylocrebrine, Tylophoricine, sterols, flavanoids, wax, resins and tannins[5]. Actually,the major constituent of *Tylophora* is Tylophorine, responsible for astrong inflammatory action. The active constituents of *Tylophora indica* Barephenanthroindolizidine, alkaloids. Recently some rare alkaloids namely tyloindicines A, B, C, D, E, F, G, H, I and J, desmethyltylophorine, desmethyltylophorinine, isotylocrebrine, anhydroustylophorinine, anhydrous-dehydrotylophorinine, γ-fagarine, skimmianine, 14-hydroxyiso tylocrebrine, 4,6- desmethylisodroxy-o-Methyltylophorinindine have been reported. Presence of tannin, saponin and terpenoid in aqueous extract revealed more pharmacological activity[13].

9. Bioassay

9.1Anti-inflammatory activity-Despite the progress made in the medical science chronic inflammation is still considered as major health problem thus this area need new drugs and further research. Many plants of Asclepeadaceae family have been evaluated for the anti-inflammatory activity .The plant has been explored biotechnologically as well as phytochemically[14-23]. The anti-iflammatory property of the plant has been reviewed by many workers[24-28]. Chaturvedi *et al.*[29] have extracted, isolated and characterized the tylophorin from leaf part of the plant by using the techniques like TLC,HPTLCand IR along with the standard reference compound of tylophorin.The extraction of tylophorin was carried out by using methanol and ethanol (8:2) solvent system.The tylophorin isolated by Preparative Thin Layer

Table 1. The anti-inflammatory % activity of tylophorin from *Tylophora indica* leaves and comparison with DFS, a standard compound. SD ± mean of three replicates significant p value p>0.01 (Chi square method).

Concentrations mg/ml	% Haemolysis (DFS)	% DFS Activity	% Haemolysis (Tylophorin)	% Tylophorin activity
Positive (+) control				
0.001	89.20 ±0.016	10.79±0.017	85.09±0.052	14.90±0.096
0.01	87.04±0.056	12.96±0.005	84.23±0.063	15.76±0.056
0.1	77.32±0.051	22.67±0.009	80.99±0.025	19.06±0.082
1.0	76.67±0.071	23.26±0.078	71.70±0.067	28.29±0.008
10	75.37±0.059	24.62±0.019	54.85±0.012	45.14±0.051

Table 2. Antioxidant activity of *Tylophora indica* callus.

Sr.no.	Concentration (µg/ml)	% Inhibition of Aq. extract of *T. indica* callus	% Inhibition of methanolic extract of *T. indica* callus	% Inhibition of Ascorbic acid
1	10	19.31±0.032	22.91±0.142	24.73±0.096
2	20	21.17±0.121	26.98±0.087	29.98±0.067
3	30	32.82±0.142	29.74±0.043	32.65±0.094
4	40	39.61±0.143	31.98±0.064	45.66±0.064
5	50	41.62±0.043	37.54±0.095	57.87±0.063
6	60	58.97±0.085	64.072±0.054	67.55±0.086

Table 3. Effect of different growth regulators on Growth Index of *Tylophora indIca* (GI).GI (Growth Index) = Final weight - Initial weight /Initial weight. Mean S.E.± of Three replicates.

Sr.No.	IBA (ppm)	NAA (ppm)	BAP (ppm)	Kinetin (ppm)	GI
A	-	2	0.2	-	5.69 ± 0.011
B	-	1	0.1	0.1	3.11 ± 0.052
C	-	1	0.1	0.2	3.98 ± 0.041
D	-	2	-	0.1	5.12 ± 0.032
E	0.5	-	0.2	-	2.11 ± 0.011
F	1.0	-	0.1	-	2.18 ± 0.019

Table 4..Showing the effect of different hormone on bud formation.- NIL ;+ Poor ;++ Good ;+++ Better;+++++ Excellent.

Sr.no.	BAP (ppm)	Kinetin	Bud formation
1.	1	-	+
2.	-	1	++
3.	1	1	++++++
4.	1.5	1	+++
5.	1	1.5	+++
6.	1.5	-	++
7.	-	1.5	-
8.	1.5	1.5	+++

Table 5. Showing the effect of different hormone on shoot formation.

Sr no.	BAP (ppm)	Kinetin (ppm)	% Shoot formation	Shoot formation /gram
1	0.5	1	63	8±0.054
2.	1	0.5	75	16±0.063
3.	1.5	-	84	21±0.025
4.	2	-	90	26±0.12
5.	1	1	68	15±0.053
6.	1.5	0.5	47	7±0.014

Chromatography (PTLC) was evaluated for anti-inflammatory activity by using HRBC *in vitro* method with standard compound DFS, 10 mg/ml concentration of tylophorin has given the significantly good result (45.14%) (Table1) among all the concentrations used.The isolated tylophorin from *T. indica* of Kelkar Farm house Mulund,Mumbai showed significant anti-inflammatory activity. This study suggests that the plant collected from Kelkar farm house might be used in the herbal anti-infammatory formulations.

9.2 Antioxidant activity- The anti-asthamatic activity of the plant is attributed to the presence of phenanthroindolizidine alkaloids[29-33]. The antioxidant activity of *Tylophora indica* leaves has been evaluated[34].It became necessary to examine the antioxidant activity and cytotoxicity of callus to evaluate it's pharmacological potential.Extract from the leaves of *T. asthmatica* were investigated for antioxidant activity. The methanolic extract of *T. asthmatica* had a 2, 2 diphenyl 1-1-picryl hydiazyl (DPPH) scavenging activity of 84.6% at 250 µg/ml and a reductive potential of 0.77% at 100 µg/ml. These values were comparable with those of gallic acid, 91.4% at 250 µg/ml and ascorbic acid, 0.79% at 60 µg/ml as standards for DPPH scavenging activity and reductive potential, respectively. These findings suggest that the rich phytochemical content of *T. asthmatica* and its good antioxidant activity may be responsible for its popular and wide traditional use. The experiment was carried out with the leaves of the selected medicinal plants. The results are discussed with the available literature[35]. The aqueous and methanolic extracts of *Tylophora indica* callus were examined for cytotoxicity on V cells by using MTT assay and antioxidant activity by using DPPH radical scavenger method.

In cytotoxity experiment the CC_{50} of aqueous extract was maximum (31.83mg/ml) than methanolic extract (25.49 mg /ml). The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured 40.81 µg /ml, 60.43 µg /ml and 38.51µg/ml, respectively. The IC_{50} value of the aqueous and methanolic extract of *T. indica* and ascorbic acid was found to be at 40.81 µg /ml,60.43 µg/ml and 38.51µg/ml, respectively. The IC_{50} value of aqueous extract was comparable to the standard compound ascorbic acid[36] (Table 2).

10.Biotechnological approach-The *in vitro* raised plantlets with well developed shoot and roots were acclimatized successfully and grown in greenhouse by using2,4,5 T, IBA and kinetin[37]. Chaturvedi *et al.*[22] have established the media for indirect organogenesis of *T. indica in vitro* by trying several media (Table3,4,5).

Indirect organogenesis has been reported by Chaturvedi and Chowdhary[21].The green compact callus was grown in MS media supplemented with 2ppm of NAA and 0.2 ppm of BAP from tender leaf explants. The initiation of callus was seen after 20 days of inoculation.Subculturing the developed callus into MS media supplemented with 2ppm of BAP was done. The shoot buds were initiated within 15 days.This protocol for organogenesis in *T. indica* with 26±0.032 /gram of callus capacity is not well documented.

11.Secondary metabolites production-Tylophora indica contain many secondary metabolites such as alkaloids, steroid flavonoids etc. Chaturvedi *et al.*[22] have evaluated the callus of *T. indica* callus for these secondary metabolites and enhanced their content by using different protocol.

11.1 Production of alkaloid(tylophorin) in tissue culture of T. indica-Dried extract was subjected to the Thin Layer Chromatographic analysis (TLC) by using the solvent system of Toluene: Ethyl acetate: Diethyl amine (14:2:2) corresponding with that of standard reference compound of tylophorin (Alexis Co. New Delhi). Developed plates

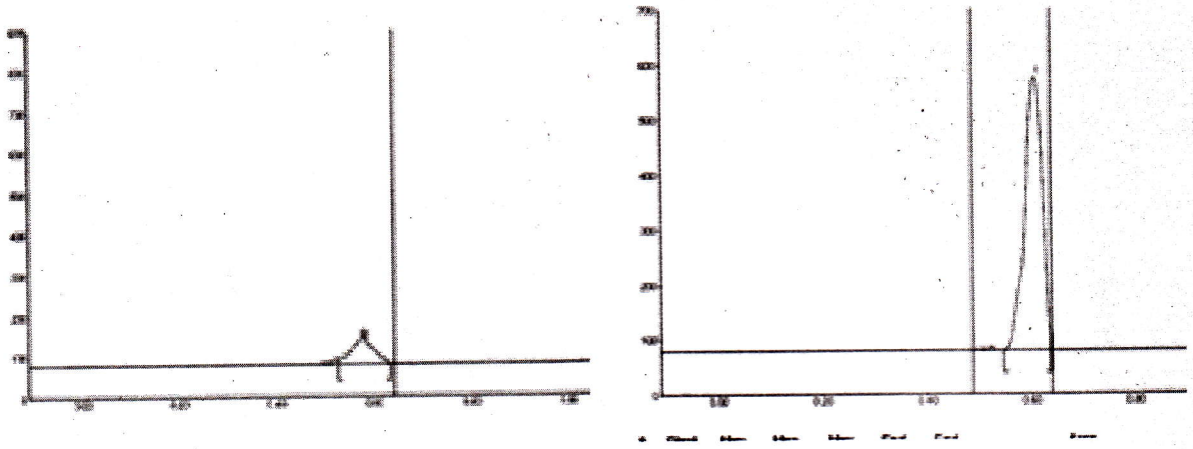

Fig.A,B. Graph of HPTLC results of four wks old callus of *Tylophora indica* with standard reference compound of Stigmasterol.

Table 6. The effect of different compounds on production of tylophorin in *Tylophora indica.* ± represent the mean value s.e. of three replicate.

SNo.	Culture	Media	Compound	Sugar	Tylophorin content %(4 wk)	Tylophorin content%(6 wk)
1.	Callus	MS	0.1 ppm DIPA		0.09±0.122	0.13±0.043
2.	Callus	MS	0.2ppmDIPA		0.104±0.62	0.19±0.045
3.	Callus	MS	0.1Kinetin		0.15±0.321	0.21±0.034
4.	Callus	MS	0.2Kinetin		0.16±0. 453	0.25±0.025
5.	Callus	Zank	Tyrosin 40mg/100ml	50 g/l	0.36±0.032	0.39±0.052
6.	Callus	Zank	Tyrosin 60mg/100ml	gluco.	0.28±0.054	0.42±0.051
7.	Callus	zank	Ornithine 40mg/100ml		0.19±0.042	0.21±0.041
8.	Callus	Zank	Ornithine 60mg/100ml		0.28±0.053	0.31±0.048
9.	Suspen.	Zank	Salicyclic acid 10 mg/100ml		0.11±0.043	0.19±0.012
10.	Suspen.	Zank	Salicyclic acid 20 mg/100ml		0.21±0.064	0.29±0.045
11.	Suspen.	Zank	nil	30 g/l sucros.	0.146±0.054	0.31±0.012
12.	Suspens.	MS	nil		0.19±0.012	0.25±0.041
13.	Suspens.	Zank	*Aspergillus niger* 0.7mg/l	15 g/lglucos	0.11±0.031 in 20days	0.14±0.012 in 30 days
14.	Callus	MS	Phe.al. f25mg/100ml	30g/l succro	0.154±0.02	0.136±0.054
15.	Callus	MS	Phe. fed 50mg/100ml	30g/l sucros	0.198±0.02	0.241±0.058
16.	Callus	MS	Phe. fed 75 mg/100ml	30g/l sucros	0.251±0.04	0.292±0.023
17.	Callus	MS	Phe. fed 100mg/100ml	30g/l sucros	0.253±0.02	0.267±0.076
18.	Callus	MS	BAP1ppm	30g/l sucros	0.173±0.04	0.195±0.032
19.	Callus	MS	BAP1.5 ppm	30g/l sucros	0.165±0.02	0.198±0.076
20.	Callus	MS	BAP 2 ppm	30g/l sucros	0.174±0.32	0.273±0.043
21.	Callus	MS	BAP 2.5 ppm	30g/l sucros	0.185±0.23	0.211±0.086
22.	Callus	Zank	two weeks old	50g/l sucros	0.183±0.41	0.175±0.032
23.	Callus	Zank	four weeks	50g/l sucros	0.179±0.84	. 198±0.053
24.	Callus	Zank	six weeks old	50g/l sucros	0.216±0.64	0.241±0.087
25.	Callus	Zank	eight weeks	50g/l sucros	0.165±0.32	196±0.065
26.	Leaves				0.31±0.032	
27.	Callus				0.004±0.087	

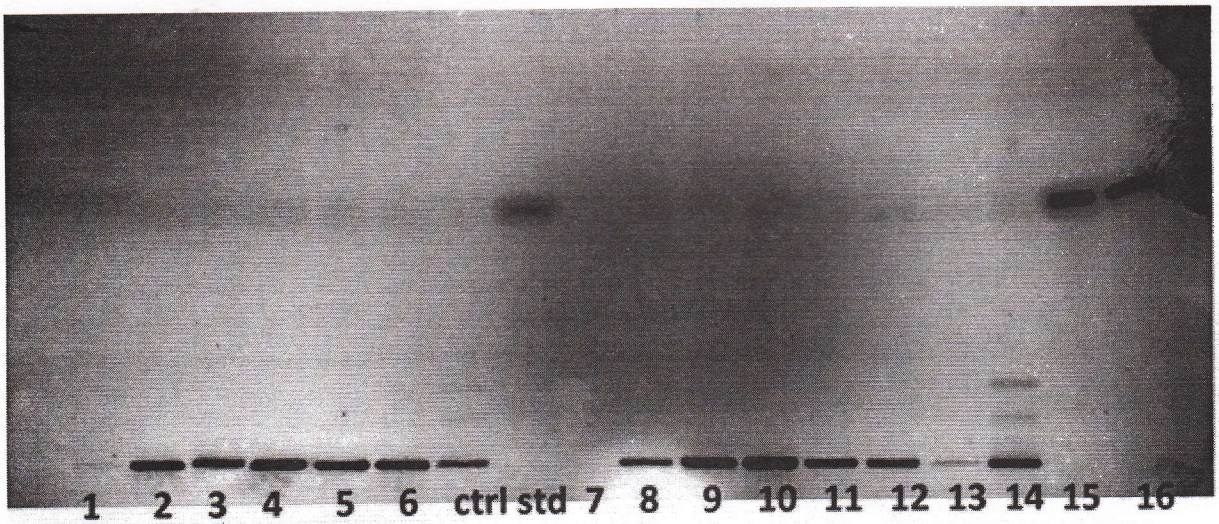

Fig. HPTLC fingerprinting of *Tylophora indica* callus(4 wks old callus) 1-galactose, 2-mannitol,3-xylose,4-fructose, 5-glucose,6-sucrose ctr-control,std six wks old 8-mannose,9-galactose ,10-xylose,11-fructose,12-glucose, 13-sucrose,14-sorbitol,15-control,16-std.

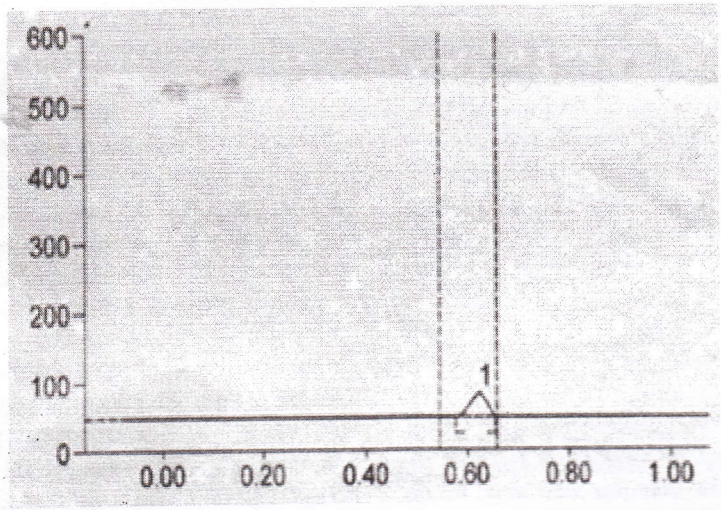

Fig.2. Showing the HPTLC analysis at 254 nm of leaves(A) with standard reference compound of tylophorin(B).

Table 7. Effect of different compounds on production of kaemmpferol in callus of *Tylophora indica.* ± represent the mean value s.e. of three replicate.

S.No	Compound	Kaempferol content in Static culture(%)		Kaempferol content in Suspension culture(%)	
		10 mg/100ml	20 mg/100ml	10mg/100ml	20 mg/100ml
1	Salicyclic acid	0.21±0.076	0.18±0.096		
2	Cinnamic acid	0.47 ±0.056	0.12±0.054		
3	Ornithine	0.15±0.064	0.93±0.058		
4	Tyrosin			0.54±0.047	1.49±0.048
5	Phenylalanine			0.153±0.078	0.239±0.038
6	Callus	0.096±0.076			
	Leaves	0.1609±0.054			

Table 8. Effect of different sugars on stigmasterol production in *Tylophora indica* callus. ± represent the mean value s.e. of three replicate.

Sugars(30g/l)	Stigmasterol (%) four wks old callus	Stigmasterol(%) six wks old callus
Mannitol	0.017±0.007	0.022±0.041
Galactose	0.021±0.008	0.018±0.007
Fructose	0.02±0.095	0.011±0.071
Glucose	0.023±0.087	0.023±0.093
Sucrose	0.025±0.065	0.018±0.081
Sorbitol	0.02±0.063	0.019±0.085
Xylose	0.0110.047	0.01±0.071
Control	0.009±0.073	

were sprayed with Dragendorff's reagent. A characteristic brick red color corresponding to standard compound showed the presence of tylophorin alkaloid. The Rf value was calculated (0.52) .

HPTLC Analysis (High Liquid Chromatography)- HPTLC analysis was carried out in Anchrom Test Lab Pvt. Ltd using silica gel plates (60F254 Manufacturer E. MERCK KGaA). Sample application was carried out on CAMAG Linomat 5 Instrument (CAMAG Linomat 5 "Linomat5_080222" S/N 080222).Inert gas was used as spray gas. Sample solvent type was methanol. Dosage speed was 150nl/s and syringe size was 100µl and the analysis wave length was 430 nm. Toluene: Ethyl acetate was used as mobile phase.

Enhancement of tylophorin in the callus of T. indica- The callus, used in this experiment, was maintained as mentioned above. Various type of compounds were used to enhance the tylophorin content in somatic cells. Static as well as suspension cultures were used for this purpose. Precursor and intermediate compound of biosynthetic pathway (Phenylalanine, Tyrosin. Salcyclicacid, Ornithine) and biotic elicitor (Auxin (Dipa),cytokinin (kinetin, BAP) were used in different sets of experiment. Various experiments have been documented regarding the enhancement of alkaloid in tissue culture of medicinal plants. Zank media was used separately and in combination with these compounds. In some experiments the type of sugar was changed from sucrose (3%) to glucose (5%)[22] (Table 6).

11.2 Production of flavonoid(Kaempferol) in tissue culture of T. indica- The production and enhancement of kaempferol was carried out in callus of *T. indica*.The accumulation of kaempferol was evaluated in undifferentiated callus of *T. indica* through TLC, HPTLC analysis with standard reference compound.. Kaempferol is a strong antioxidant and help to prevent oxidative damage to our cells, lipids and DNA. In the present investigation, we have enhance the kaempferol content in *T. indica* tissue culture by using precursors like sallicyclic acid, ornithine, cinnamic acid,tyrosin and phenylalanine in different concentrations (10 and 20 mg/ 100 ml). Here we have used static as well as suspension culture to enhance the kaempferol concentrarion. The callus of *T. indica* was initiated and maintained on MS (Murashige and Skoog's) medium supplemented with 3% of sucrose, while Zenk production media was used as production media[22].

HPTLC Analysis-HPTLC analysis was carried out in Anchrom Test Lab Pvt. Ltd using silica gel plates (60F254 Manufacturer E. MERCK KGaA),Sample application was carried out on CAMAG Linomat 5 Instrument (CAMAG Linomat 5 "Linomat5_080222" S/ N 080222).Inert gas was used as spray gas. Sample solvent type was methanol. Dosage speed was 150nl/s and syringe size was 100µl and the analysis wave length was 430 nm. Toluene: Ethyl acetate (14:2) was used as mobile phase. A remarkable enhancement in kaempferol content was obtained by using 20 mg /100 ml of tyrosin (1.49% dw ;control -0.096%dw) in suspension culture ,which is more than tenfold increase (Table 7). The enhancement of kaempferol in callus of *Tylophora indica* by using these amino acids was reported for the first time[22] (Table 7).

11.3 Production of phytosterol(stigmasterol) in tissue culture of Tylophora indica-Production of secondary metabolites by cultured cells provides a particularly important benefit to manipulate and improve the production of desired compounds; thus biotechnological approaches to increase the concentrations of the metabolites are discussed.

HPTLC Analysis-HPTLC analysis was carried out in Anchrom Test Lab Pvt. Ltd using silica gel plates

(60F254 Manufacturer E. MERCK KGaA),Sample application was carried out on CAMAG Linomat 5 Instrument (CAMAG Linomat 5 "Linomat5_080222" S/N 080222).Inert gas was used as spray gas. Sample solvent type was ethanol. Dosage speed was 150nl/s and syringe size was 100µl and the analysis wave length was 430 nm.Hexane:Acetone (8:2) was used as mobile phase.

The enhancement of stigmasterol ,a precursor of hormones *in vitro* tissue culture of *T. indica*.was carried out .Chaturvedi *et al.*[23] have examined the effect of different kinds of sugars (mannitol, galactose, fructose, glucose, sucrose, sorbitol, xylose) in 30g/l concentration separately with Zenk basal medium for the production of stigmasterol in the tissue culture of *T. indica.* The initiation and maintenance of callus was carried out on MS basal media supplemented with 0.2 ppm of BAP and 2ppm of NAA, for leaf explant ,while Zenk medium was used as a production medium.The sucrose has given the best results in four weeks old callus, which was highest among all test samples used (0.025%) and shown highly significant increase which was ten times higher than control (0.009%).These results suggests that sucrose may be involved in the production of stigmasteol.This study also suggests that the content of Zank media in addition to sucrose may favors the production of stigmasterol in callus of *T. indica.* This prototocol for stigmasterol enhancement in *T. indica* was not documented till date. Statistical data showed that the results were significant (P value >0) (Table 8).

Molecular characterization of regenerated plants by RAPD (Random Amplification Polymorphic DNA) and ISSR (Inter Simple Sequence Repeat) analysis-The main objective was to evaluate the homogeneity of the regenerated microshoots of *T. indica* to its mother plant. Regenerated microshoots were developed from leaf explants *via* indirect organogenesis using specific culturing conditions. Work was carried out to develop microshoots of *T. indica* and then the regenerated micro shoots were subjected for the RAPD and ISSR analysis to scrutinize for their polymorphism with the mother plant. Due to overexploitation, plant tissue culture techniques has been used to conserve this extinct species. DNA was extracted from regenerant and subjected to RAPD and ISSR analysis separately for molecular characterization. In the case of RAPD, the bands obtained were all monomorphic and hence it was proved that the regenerants are true-to-type within themselves as well as to the mother plant. Whereas in ISSR analysis, banding pattern results showed 45.71% polymorphism in the regenarants with mother plant, thus giving only 54% clones, thus, the protocol used for culturing can be used for clone production as well as for the selection of elite varieties[38].

Present study reveals that the incorporation of different compound in the growth media of cell culture enhace the content of secondary metabolite,which are medicinally important compounds. These compounds may be precursor either intermediate compound of biosynthetic pathway of secondary metabolite,or abiotic or biotic elicitor. The effect of these compounds on production of secondary metabolite might be due to a stress which in turn enhance the biosynthetic capacity of a cell .The further work on molecular level is required to fulfill the today's need. The Express Sequence Tag (EST) analysis and Barcoding of *Tylophora indica* will fuilfill this requirement.

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