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RESEARCH ARTICLE

IN VITRO PROPAGATION OF *TYLOPHORA INDICA* (BURM. F.) MERR. VAR. *INDICA*: A THREATENED MEDICINAL CLIMBER

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SUMMARY A reliable and organized procedure for indirect shoot organogenesis from *Tylophora indica* leaf explants has been developed. Several dosages of 2,4-D (0.5–2.0 mg/l) were utilized both independently and in conjunction with Kn (0.4–0.6 mg/l) for callus induction from leaf explants. A maximum callus induction response of 96% was seen in MS medium enriched with a blend of 1.5 mg/l of 2,4-D and 0.5 mg/l of Kn. An optimal shoot regeneration was achieved on MS medium enriched with 1.0 mg/l of BAP, 0.6 mg/l of Kn and 0.5 mg/l of IBA with 85% response rate and an average of 16.8 shoots and 2.23 cm shoot length per culture. Half-strength MS medium fortified with 1.0 mg/l IBA yielded the best rooting results, producing 100% rooting frequency and an average of 5.2 roots and 2.6 cm root length per shoot. The transplantation of rooted plantlets into soil demonstrated a high survival rate of 90%. The methodology can be used for the large scale propagation of this endemic medicinal plant.

Keywords: in vitro propagation, *Tylophora indica*, leaf explant, callus.

INTRODUCTION

Tylophora indica (Burm. f.) Merr. var. *indica* is a perennial woody climber indigenous to the plains and hilly woodlands of eastern and southern India, with a distribution range extending up to 900 m altitude (Anonymous 1976). The plant is recognised by various synonyms, including *Vincetoxicum indicum* (Burm. f.) Mabb. var. *indicum* (Apocyanaceae- APG system), *Cynanchum indicum* Burm. f., *Tylophora asthmatica* (L. f.) Wight & Arn., *Asclepias asthmatica* L. f., and *A. prolifera* Rottler. ex. Ainslie. Its common English name is 'emetic swallow-wort' (Sasidharan 2004). It holds a significant historical role in Ayurveda (Chopra et al. 1986) and Siddha (Ram et al. 2009) medicinal traditions, notably for addressing bronchial asthma and thus, the species name *T. asthmatica* (Shivpuri et al. 1972). Traditionally, *T. indica* has been in use for treating whooping cough, bronchitis, dysentery and diarrhoea (Kirtikar & Basu 1991, warrier et al. 1994) and has gained recognition for its

antibacterial (Balasubra-manian et al. 2010), antipsoriasis (Sarma & Misra 1995), antidiabetic (Swathi et al. 2012), anti-neuroinflammatory (Gupta et al. 2020) and hepatoprotective (Gujrati et al. 2007) properties. The therapeutic attributes of *T. indica* is ascribed to its diverse phyto-chemical composition, encompassing alkaloids such as tylophorine (Ratnagiriswaran & Venkatachalam 1935), tylophorinine (Govin-dachari et al. 1961), tylophorinidine (Dhiman et al. 2013), (+) septicine, isotylocrebrine, and tylophorinicine (Gopalakrishnan et al. 1979), along with flavonoids, resins, sterols, tannins and wax (Govindhari et al. 1975). Despite its medicinal significance, *T. indica* faces the threat of endangerment in India due to its extensive utilization for medicinal purposes (Faisal & Anis 2003). The plant primarily reproduces through seeds and the very low seed viability and suboptimal germination constrains its natural proliferation (Faisal et al. 2007). Attempts at vegetative propagation through stem cuttings have proven to be challenging (Chandrasekhar et al. 2006). To address the escalating demand for *T. indica* and to alleviate reliance on wild populations, there is an imperative need to develop alternative propa-gation techniques with heightened multiplication rates (Kunene & Misarirambi 2018).

Tissue culture emerges as a viable and economical strategy for conservation by offering a means to generate substantial number of genetically identical plants in a brief timeframe. This approach holds promise for contributing to the conservation and sustainable utilization of *T. indica*, ensuring its accessibility for future generations (Sharma & Chandel 1992). The present study aims at optimising the culture media and conditions for mass multiplication of *T. indica* by callus organogenesis from leaf explants.

MATERIAL AND METHODS

Leaf explants of medium maturity from high-quality plants were collected from Nambiakulam, Kottayam district of Kerala state, India (Fig. 1). The leaves were surface sterilized by soaking for approximately 30 sec in 70% ethanol (u/v) followed by 5 min in 0.1% mercuric chloride solution (u/v) and finally washing at least thrice with doubledistilled water. The growth medium utilized was MS medium (Murashige & Skoog 1962), supplemented with 3% sucrose and solidified with 0.8% agar (w/v). Phytohormones were incorporated as necessary, and the pH was maintained at 5.8. The culture media and equip-ments were autoclaved for 20 min at 121° C and 15 lbs pressure. The cultures were maintained at 22° C under a 16 h illumination and 8 h darkness cycle using 40-watt fluorescent light tubes. Each experiment was replicated thrice, with each treatment comprising 24 tubes. The presentation of data involved mean values with standard deviation (mean ± SD), and a multiple range test

(DMRT) at a significance level of 0.05% was applied to compare mean values.

To induce callus formation, segments of leaves of medium maturity, each measuring 2 cm^2 with midrib were cultured on MS medium having varying amounts of 2,4-D (0.5 to 2.0 mg/l), both independently and in conjunction with Kinetin (Kn) at concentrations that ranges between 0.4 to 0.6 mg/l. The objective was to investigate the initiation of callus. Following a 45 d cultivation period, the success of the culture was assessed by enumerating the calli to the total number of uncontaminated explants. Subsequently, the obtained calli were recurrently subcultured every 45 d onto MS medium enriched with 1.5 mg/l of 2,4-D. The yellowish and friable calli were transferred to MS medium enriched with varying combinations and doses of 6-benzyl aminopurine (BAP) (1.0 mg/l), Kn (0.4-0.6 mg/l), Indole-3-butyric acid (IBA) (0.4-0.6 mg/l), Indole-3-acetic acid (IAA) (0.4-0.6 mg/l) and Naphthalene acetic acid (NAA) (0.4-0.6 mg/l) for callus regeneration. Subsequent subcultures were conducted at regular 45 d intervals. The resultant shoots generated from the calli were moved to MS medium enriched with BAP (0.1 to 0.5 mg/l) for elongation.

Shoots upon reaching a height of 3 to 4 cm were moved to half-strength MS medium augmented with IAA, IBA or NAA to facilitate the rooting process. Following a 45 d cultivation period, the response percentage, the average length and the number of roots on each shoot were counted and recorded. Following removal from the tubes, the plantlets underwent a sterile double-distilled water rinse to eliminate any residual medium and the rooted plantlets were carefully transplanted to polycups packed with a combination of autoclaved soil and vermicompost in a 1:3 proportion. To prevent wilting, they were shielded under high humidity conditions and covered with polythene bags for the first 3 wks. Regular irrigation with a quarter-strength MS medium was provided and the survival percentage was determined after 3 months of acclimatization.

Every experiment was carried out thrice with 24 cultures retained for every treatment. ANOVA (analysis of variance) was performed to analyze the data using SPSS software version 24.0. (Armonk, NY: IBM Corp). Duncan's multiple range test (DMRT) was used at a significance level of $p \geq 0.05$ to determine significant variations between the mean values. This rigorous statistical approach ensured the reliability and validity of the experimental results.

OBSERVATIONS

For callus induction, several doses of 2,4-D (0.5 to 2.0 mg/l) both alone and in combination with Kn (0.4 to 0.6 mg/l) was tried. Variations in the percentage of response was observed with variations in the concentration and combination of phytohormones tested. MS basal medium and all the tested phytohormones at various doses and

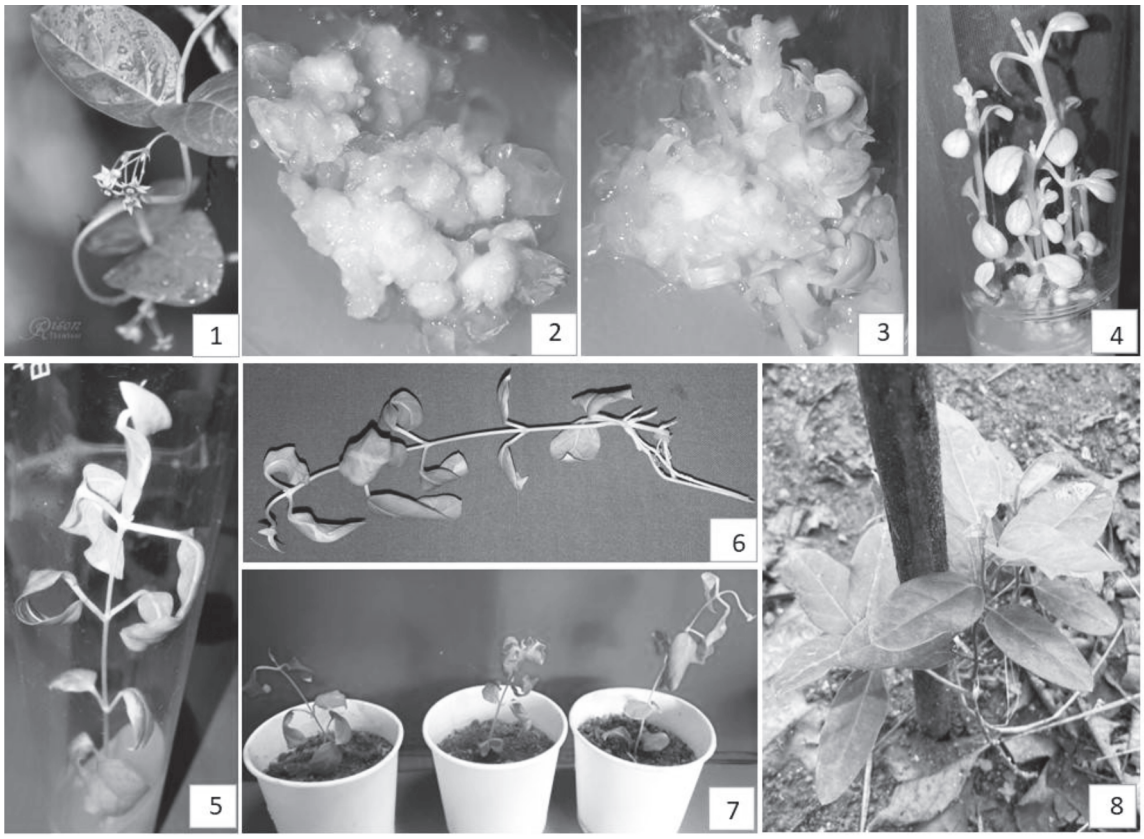
combinations showed callusing with variations in the percentage of response. Callus induction was observed within one wk of culture initiation from the cut ends of the explants that eventually displayed a greenish-yellow and nodular appearance. The percentage of callus induction and the callus mass in terms of both fresh and dry weights were observed at moderate rates when 2,4-D alone was employed. Among the various doses of 2,4-D applied for callus induction, 1.5 mg/l 2,4-D exhibited an optimum response of 58% callus induction with a mean 1247 mg and 123 mg fresh and dry weights respectively, after 45 d (Table 1). An increase in callus induction and calli mass percentage was observed when a combination of 2,4-D and Kn was applied. A maximum callus induction response of 96% with a mean 2829 mg fresh weight and 182 mg dry weight was obtained after 45 d with the combinatorial effect of 1.5 mg/l 2,4-D and 0.5 mg/l Kn (Table 1, Fig. 2). Thus, an increase in the percentage of callus was accompanied by a corresponding increase in the fresh and dry weights of the callus. The calli formed were then further subcultured for multiplication.

TABLE 1: Effect of various PGRs on organogenic callus induction from leaf explants of *T. indica* after 45 d.

Plant growth regulators (mg/l)		Callus induction (%)	Fresh weight (mg)	Dry weight (mg)
2,4-D	Kn			
0.0	0.0	31	748 ± 2.6^{e}	69 ± 3.7^{e}
0.5		41	961 ± 4.03^{d}	99 ± 7.2^{d}
1.0		45	987 ± 5.06^{c}	107 ± 4.6^{c}
1.5		58	1247 ± 3.8^{a}	123 ± 5.4^{a}
2.0		54	1018 ± 3.6^{b}	111 ± 5.8^{b}
0.5	0.4	57	1046 ± 4.6^{d}	119 ± 4.8^{d}
1.0	0.4	62	1567 ± 3.7^{c}	129 ± 5.0^{c}
1.5	0.4	83	2339 ± 5.3^{a}	162 ± 2.7^{a}
2.0	0.4	70	1931 ± 5.4^{b}	141 ± 3.3^{b}
0.5	0.5	81	2241 ± 3.6^{d}	154 ± 2.6^{d}
1.0	0.5	87	2494 ± 4.5^{c}	172 ± 4.1^{c}
1.5	0.5	96	2829 ± 2.5^{a}	182 ± 4.4^{a}
2.0	0.5	93	2708 ± 4.4^{b}	174 ± 3.5^{b}
0.5	0.6	58	1460 ± 3.6^{d}	126 ± 5.9^{d}
1.0	0.6	66	1794 ± 3.3^{c}	130 ± 4.8^{c}
1.5	0.6	87	2538 ± 4.1^{a}	174 ± 5.9^{a}
2.0	0.6	79	2158 ± 4.8^{b}	153 ± 5.8^{b}

Mean ± SD of three replicates with each replicate having 24 explants. Mean values having the same letters are not significantly different from each other according to Duncan's multiple range test ($p \geq 0.05$).

The yellowish and friable calli were then moved to MS medium enriched with different combinations and doses of BAP, Kn, IBA, IAA and NAA. The emergence of the first vegetative domes was observed within 10 d (Fig. 3). Caulogenesis results were assessed after 45 d. A maximum shooting response of 85% with a mean shoot number of 16.8 and shoot length of 2.23 cm for each culture was obtained with a combination of 1.0 mg/l BAP, 0.6 mg/l Kn and 0.5 mg/l IBA. Caulogenesis was noticed across all tested combinations and concentrations (Table 2, Fig. 4). Upon further transfer to a shoot elongation medium, regenerated shoots displayed an



Figs 1–8: *T. indica* 1. A twig of the mother plant. 2. Callus induction from leaf explant on MS medium fortified with 2,4-D (1.5 mg/L) and Kn (0.5 mg/L) 3. Emergence of shoot buds 4**.** Proliferation and elongation of shoots after 6 wks on MS medium enriched with BAP (1.0 mg/L) + Kn (0.6 mg/L) + IBA (0.5 mg/L). 5. Healthy elongated shoot after 8 wks on MS medium augmented with 0.1 mg/L BAP. 6. Rooting of shoot on half strength MS medium supplemented with 1.0 mg/L IBA. 7. Plantlets transferred to polycups 8. An acclimatized plant after 6 months.

increasing trend in shoot elongation with decreasing BAP concentration. The maximum shoot elongation was attained on MS medium supplemented with 0.1 mg/l of BAP. (Fig. 5).

Shoots after reaching a height of 3– 4 cm were shifted for rooting onto half-strength MS media fortified individually with different doses of IAA (0.5–2.0 mg/l), IBA (0.5–2.0 mg/l) and NAA (0.5–2.0 mg/l). The strongest rooting response was observed on half-strength MS medium enhanced with 1.0 mg/l IBA with 100% response rate and a mean root number of 5.2 and root length of 2.6 cm. Whitish roots appeared within 10 d and all the tested media, exhibited rooting responses at varying degrees (Table 3, Fig. 6). Well-rooted plantlets were subsequently

TABLE 2: Effect of various PGRs on shoot induction from leaf derived callus of *T. indica* after 45 d.

Plant growth regulators (mg/l)					Percentage of response	Mean No. of shoots per explant*	Mean shoot length (cm)
BAP	Kn	IBA	IAA	NAA			
0.0	0.0	0.0	0.0	0.0	47	3.3 ± 0.6^{e}	0.76 ± 0.15^{g}
1.0	0.4				54	3.8 ± 0.83^{e}	0.93 ± 0.05^{g}
1.0	0.5				59	7.4 ± 1.14^{d}	1.36 ± 0.11^{ef}
1.0	0.6				63	8.0 ± 1.0^{e}	1.60 ± 0.1^{c}
1.0	0.6	0.4			69	14 ± 1.58^{b}	1.93 ± 0.11^{b}
1.0	0.6	0.5			85	16.8 ± 1.48^{a}	2.23 ± 0.15^{a}
1.0	0.6	0.6			67	11.4 ± 1.14^{c}	1.73 ± 0.11^{c}
1.0	0.6		0.4		66	8.6 ± 1.14^{d}	1.56 ± 0.05^{cd}
1.0	0.6		0.5		73	15 ± 1.5^{ab}	2.2 ± 0.1^{a}
1.0	0.6		0.6		66	10.4 ± 1.14^{c}	1.7 ± 0.1^{c}
1.0	0.6			0.4	68	12.0 ± 1.0^{c}	1.93 ± 0.05^{b}
1.0	0.6			0.5	60	7.60 ± 1.34^{d}	1.4 ± 0.1^{de}
1.0	0.6			0.6	55	7.2 ± 1.30^{d}	1.2 ± 0.1^{f}

Mean ± SD of three replicates with each replicate having 24 explants. Mean values having the same letters are not significantly different from each other according to Duncan's multiple range test ($p \geq 0.05$).

transferred to polycups packed with a 1:3 combination of sterilized soil and vermicompost for acclimatization (Fig. 7). The survival percentage was recorded after 3 months, revealing that out of the 30 plantlets transferred for acclimatization, 27 survived, resulting in a transplantation survival rate of 90%.

DISCUSSION

In vitro regeneration of plants is influenced by many factors including the source of explant, composition of the medium and the plant growth regulators. The majority of tissue culture studies are focused on the effect of plant growth regulators at various stages of plant regeneration (Li et al. 2018). In our study, in vitro regeneration of *T. indica* was accomplished by callus induction from leaf explants. Leaf explants have proven to be excellent for indirect shoot regeneration by callus formation in many plants. There were earlier reports of successful indirect regeneration through callus organogenesis from leaf explant in many plants such as *Melothria maderaspatana* (Baskaran et al. 2009), *Justicia gendarussa* (Bhagya et al. 2013), *Chirita swinglei* (Chen et al. 2016), etc. Callus induction was achieved by the

TABLE 3: Effect of various PGRs on rhizogenesis after 45 d.

Plant growth regulators			Percentage of response	Mean No. of roots	Mean root length (cm)
IAA	IBA	NAA			
0.0	0.0	0.0	52	2.1 ± 0.5^{f}	1.1 ± 0.9^{f}
0.5			65	3.2 ± 0.7^{c}	1.3 ± 0.2^{h}
1.0			71	4.2 ± 0.6^{b}	1.5 ± 0.2^{gh}
1.5			77	3.4 ± 0.8^{c}	1.8 ± 0.2^{cde}
2.0			63	2.6 ± 0.5^{c}	1.5 ± 0.1^{fg}
	0.5		93	4.2 ± 1.03^{b}	1.6 ± 0.2^{efg}
	1.0		100	5.2 ± 1.2^{a}	2.6 ± 0.2^{a}
	1.5		96	4.8 ± 0.7^{ab}	1.9 ± 0.2^{bcd}
	2.0		91	4.6 ± 0.8^{ab}	1.7 ± 0.1^{cdef}
		0.5	64	3.0 ± 0.8^{c}	1.9 ± 0.2^{bc}
		1.0	70	2.9 ± 0.7^{c}	2.1 ± 0.3^{ab}
		1.5	74	3.2 ± 0.7^{c}	1.9 ± 0.2^{cde}
		2.0	59	2.6 ± 0.5^{c}	1.7 ± 0.1^{defg}

Mean ± SD of three replicates with each replicate having 24 explants. Mean values having the same letters are not significantly different from each other according to Duncan's multiple range test ($p \geq 0.05$)

application of various phytohormones for stimulating cell division and differentiation, following a methodology supported by Mwaniki et al. (2019). A balanced application of auxin and cytokinin is very necessary for callus induction (Bourgaud et al. 2001). Among auxins, 2,4-D is widely used for callus induction as it can initiate de-differentiation of explant cells and among cytokinins, Kn is commonly used as it can activate cell division and morphogenesis of cells (George et al. 2008). In our study, MS medium supplemented with 2,4-D alone and in combination with Kn at various concentrations was used for callus induction and a maximum response of 96% was observed with a combination of 1.5 mg/l 2,4-D and 0.5 mg/l Kn, with an average of 2829 mg fresh weight and 182 mg dry weight of callus. In our study, the effect of the combination of BAP and Kn, along with different auxins (IBA, IAA and NAA) on shoot induction was examined. All tested combinations demonstrated a good response with a combi-

nation of 1.0 mg/l BAP, 0.6 mg/l Kn, and 0.5 mg/l IBA exhibiting the highest response of 85% and an average number of 16.8 shoots per explant and a mean shoot length of 2.23 cm. A similar synergistic effect of auxin and cytokinin on shoot induction has been reported in *Gymnema sylvestre* (Reddy et al. 1998). The combined action of auxin and cytokinin has been widely utilized in various systems to achieve a higher rate of regeneration (Deepa et al. 2018).

In the current study, half-strength MS medium enriched with varying doses of IAA, IBA, and NAA was employed for root induction. Out of the 3 auxins tested for rooting, half-strength MS medium supplemented with IBA at a concentration of 1 mg/l demonstrated superior result with a 100% response with a mean number of 5.2 roots and a mean root length of 2.6 cm. Similar reports of successful rooting on half-strength MS medium supplemented with IBA have been documented in other plants, including *Passiflora foetida* (Shekhawat et al. 2015) and *Ruta graveolens* (Faisal et al. 2005). Immediate steps should be taken to ensure the conservation and sustainable utilization of *T. indica*, thereby safeguarding its availability for future generations. This may necessitate the implementation of conservation initiatives, cultivation strategies, and sustainable harvesting practices to mitigate the risk of extinction and preserve both the plant's ecological integrity and its medicinal significance.

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Declaration

The authors have no conflict of interest.

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RESEARCH ARTICLE

QUALITATIVE PHYTOCHEMICAL, HPTLC AND HPTLC ANALYSES OF LEAF AND BARK EXTRACTS OF *DENDROPHTHOE GAMBLEI*

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SUMMARY The present work is to investigate the phytochemical constituents of ethanol, ethyl acetate and chloroform extracts of leaf and bark. Preliminary phytochemical screening of extracts showed the presence of different bioactive substances viz., carbohydrates, glycosides, phenolics, terpenoids, alkaloids, flavonoids, tannins, steroids, oils, gums and mucilage. HPTLC and HPLC analyses of the leaf and bark extracts of *D. gamblei* revealed the presence of quercetin and kaempferol.

Keywords: *Dendrophthoe gamblei,* HPTLC, HPLC, phytochemical analysis.

INTRODUCTION

A great deal of research investigations evaluating novel drugs have turned to medicinal plants due to their multivalent activity and lower risk of side effects. Since the time immemorial, man has been fascinated towards a peculiar group of angiosperms known as parasitic flowering plants. These are a bizarre group of dicotyledons that continues to baffle the botanists all over the world. Parasitic angiosperms are distributed in 20 distinct recognized families. The largest family of parasitic angiosperms is Loranthaceae popularly called mistletoes. According to Parker & Riches (1993), mistletoe refers specifically to woody shoot parasite of Loranthaceae and Viscaceae.

Dendrophthoe gamblei, a parasitic plant belongs to the family Loranthaceae. It was recently discovered phanerogam found in the Indian peninsula (Singh et al. 2020). The genus *Dendrophthoe* comprising 30 species from tropical Africa to Australia with its center of diversity in Western Malaysia and 7 species are reported from India (Patel et al. 2012).

Numerous ethnomedical plants are vetted based on their phytochemical analyses, which reveal the essential components. The ability to identify different chemical substances has various limitations. HPLC is a versatile, robust and widely used technique for the isolation of natural products; it is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture (Cannell 1998, Piana et al. 2013). The HPTLC can be utilized for quantitative analysis of plant material and quality

control analysis since it is easy to use, quick, accurate, repeatable, selective and affordable (Palani et al. 2011).

Nevertheless, very little information is available on *Dendrophthoe* with reference to the occurrence of the chemical compounds and their biological function. As *D. gamblei* is a recently discovered species, no investigations on its phytochemical, HPTLC and HPLC analyses have been reported. Hence the present study.

MATERIAL AND METHODS

Field trips were undertaken to Siddara betta, Tumkur district, Karnataka to collect the fresh leaves and bark of the material. They were washed thoroughly with running tap water and dried under shade, and separately powdered using blender and stored in air tight container for further analysis. 10 g powder of each was weighed and extracted individually using 50 ml of 3 different solvents viz., ethanol, chloroform and ethyl acetate for 4 h in a water bath at 60˚ C by decoction method. The contents were filtered through the Whatman No.1 filter paper. The extracts thus obtained were allowed to evaporate the solvents in a hot air oven. The condensed extracts were stored in Eppendorf vials at 4˚ C till further use.

Qualitative phytochemical screening tests of the leaf and bark extracts were performed according to the standard protocols for detecting the presence of various phytochemicals (Harborne 1998, Khandelwal 2008).

HPTLC is an important analytical tool in the separation, identification and estimation of various classes of natural phytoconstituents. HPTLC studies were carried out following the method of Harborne (1998).

1.0 g of *D. gamblei* leaf powder extract was taken and dissolved in 30 ml of methanol for 30 min on water bath at 70º–80º C. Filtered, combined the filtrates and concentrated to 5 ml. Proceeded for spotting.

Extracted 10 mg of quercetin reference standard with 10 ml 50% methanol for 15 min on water bath at 70º–80º C. Filtered and concentrated to 5 ml. Proceeded for spotting.

1.0 g of bark powder extract was taken and dissolved in 30 ml methanol for 30 min on water bath at 70º–80º C. Filtered, combined the filtrates and concentrated to 5 ml. Proceeded for spotting.

Mobile phase: Toluene: Ethyl acetate: Formic acid [2:5:1.5]

10 ml of mixture of toluene: ethyl acetate: formic acid (2:5:1.5) was transferred to the chromatographic tank. Placed a Whatman Filter paper disc and closed with the lid for faster saturation of the tank with the solvent system. Allowed the tank to saturate for 30 min. 5 µl of sample(s) (as 10 mm bands separated by a distance of 15 mm; at 10 mm from the base) was applied on a HPTLC silica plate using a Linomat HPTLC applicator. The plate was left in fume hood to let the solvent to evaporate. Placed the plate in the tank as near vertical as possible

ensuring that the line of application is well above the solvent level. Replaced the lid tightly and allow the solvent to ascent to 1.5 cm below the top of the plate. The plate was removed and let it to air dry in fume hood.

Thereafter, anisaldehyde sulphuric acid reagent was sprayed and viewed under UV 366 nm.

The mobile phase consisted of 2 solvents, A and B at a flow rate of 1.5 mL/min. Solvent A was 0.05% acetic acid and solvent B was acetonitrile.

For preparation of quercetin solution, 10 mg of quercetin reference stan-dard was taken and dissolved it in 50 ml of methanol. Heated on water bath for 15–20 min at 70º–80º C and made up the volume to 50 ml with water, filtered and injected the solution.

For preparation of kaempferol solution, 10 mg of kaempferol reference standard was dissolved in 50 ml of methanol. Heated on water bath for 15–20 min at 70º– 80º C and made up the volume to 50 ml with methanol, filtered and injected the solution.

400 mg of bark and leaf powder was taken and extracted in 50 ml of 50% methanol separately. Heated on water bath for 15–20 min at 70º–80º C, collected and combined the filtrates separately. Made up the volume to 50 ml with 50% methanol, filtered and injected the solution. Injected 20 µl of samples and standards separately. Calculated the quercetin and kaempferol peak area of the standard as compared to samples and calculated the assay by employing the following formula:

$$\frac{\text{Standard weight}}{\text{Sample weight}} \times \frac{\text{Sample area}}{\text{Standard area}} \times \%\ \text{of standard assay} = \%\ \text{of sample assay}$$

OBSERVATIONS

Qualitative phytochemical analysis of the leaf and bark extracts with 3 different solvents such as

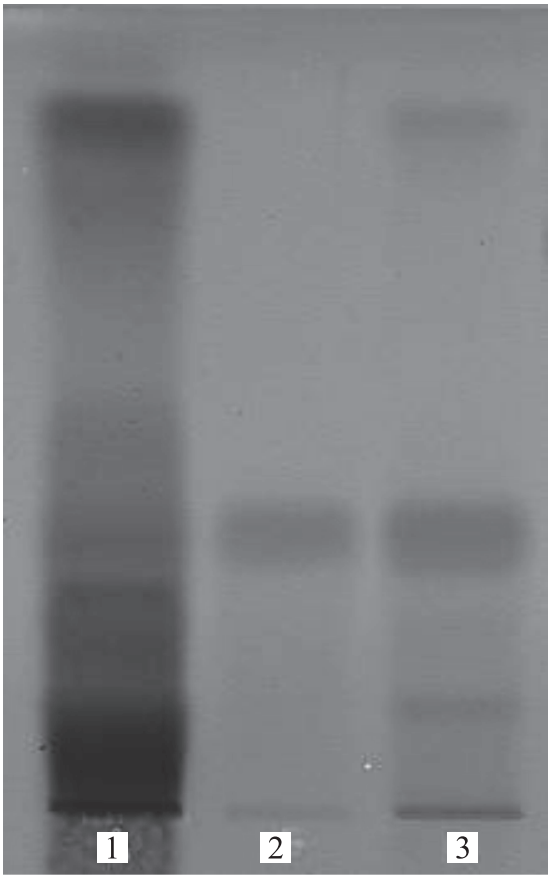

Fig. 1: Spectrogram of *D. gamblei* extracts and the standard quercetin analyzed by HPTLC 1. Leaf powder 2. Quercetin reference standard 3. Bark powder.

TABLE 1: Phytochemical screening of leaf and bark extracts of *D. gamblei.*

Phytochemical constituents	Ethanol		Chloroform		Ethyl acetate	
	Bark extract	Leaf extract	Bark extract	Leaf extract	Bark extract	Leaf extract
Carbohydrates	+	+	+	+	+	+
Proteins	+	+	+	+	+	-
Glycosides	+	+	-	+	+	+
Saponins	+	+	-	-	-	-
Alkaloids	+	+	+	+	+	+
Phenolics	-	+	+	-	+	+
Flavonoids	+	+	+	-	+	+
Terpenoids	-	-	-	+	+	-
Tannins	+	+	-	-	+	+
Steroids	+	+	+	+	+	+
Volatile oils	-	+	+	-	+	+
Non-volatile oils	+	+	+	-	+	+
Gums and mucilage	+	+	+	+	+	+

Presence (+) or absence (-) in different extracts.

TABLE 2: HPLC analysis of leaf and bark extracts of *D. gamblei* and standard quercetin.

Sample	Sample wt.mg in 50ml	Sample conc. (mg/ml)	Sample RT for quercetin (min)	Sample area for quercetin (µAU)	Quercetin std conc. (mg/ml)	Quercetin standard area (µAU)	Quercetin (%)
Bark	401	8.02	5.652	14997	0.20	86743	0.42
Leaf	403	8.06	5.648	25352			0.70

TABLE 3: HPLC analysis of leaf and bark extracts of *D. gamblei* and standard kaempferol.

Sample	Sample wt.mg in 50 ml	Sample conc. (mg/ml)	Sample RT for kaempferol (min)	Samplearea for kaempferol (µAU)	Kaempferol std conc. (mg/ml)	Kaempferol standard area (µAU)	Kaempferol (%)
Bark	401	8.02	11.172	2870	0.2	91358	0.08
Leaf	403	8.06	11.281	1435			0.04

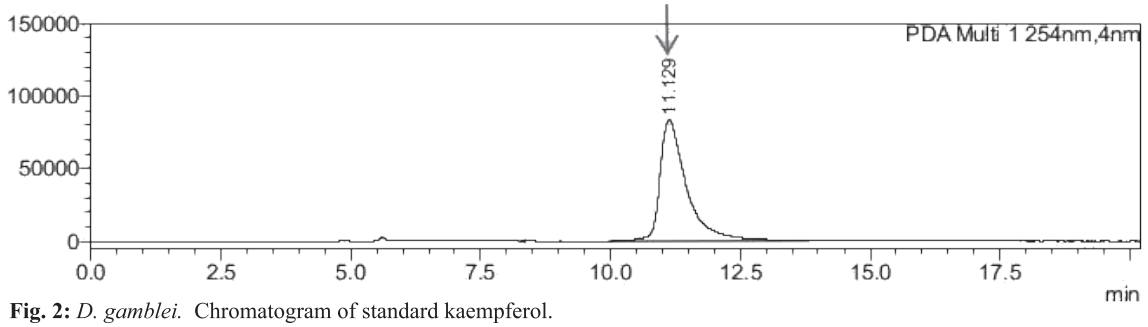

Fig. 2: *D. gamblei.* Chromatogram of standard kaempferol.

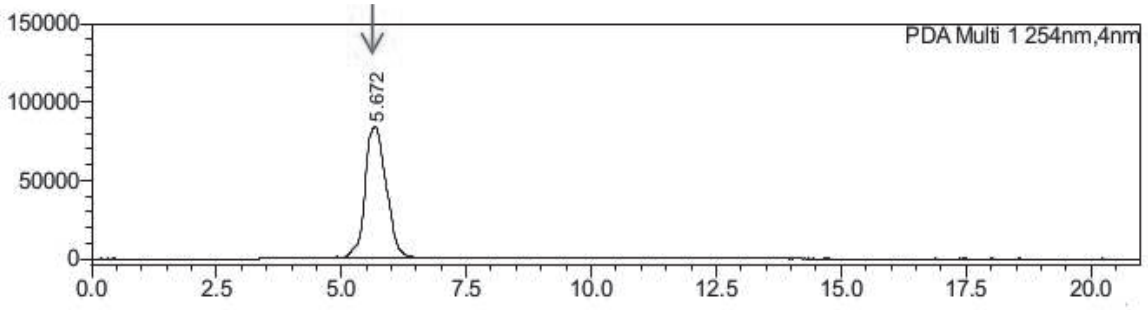

Fig. 3: *D. gamblei.* Chromatogram of standard quercetin.

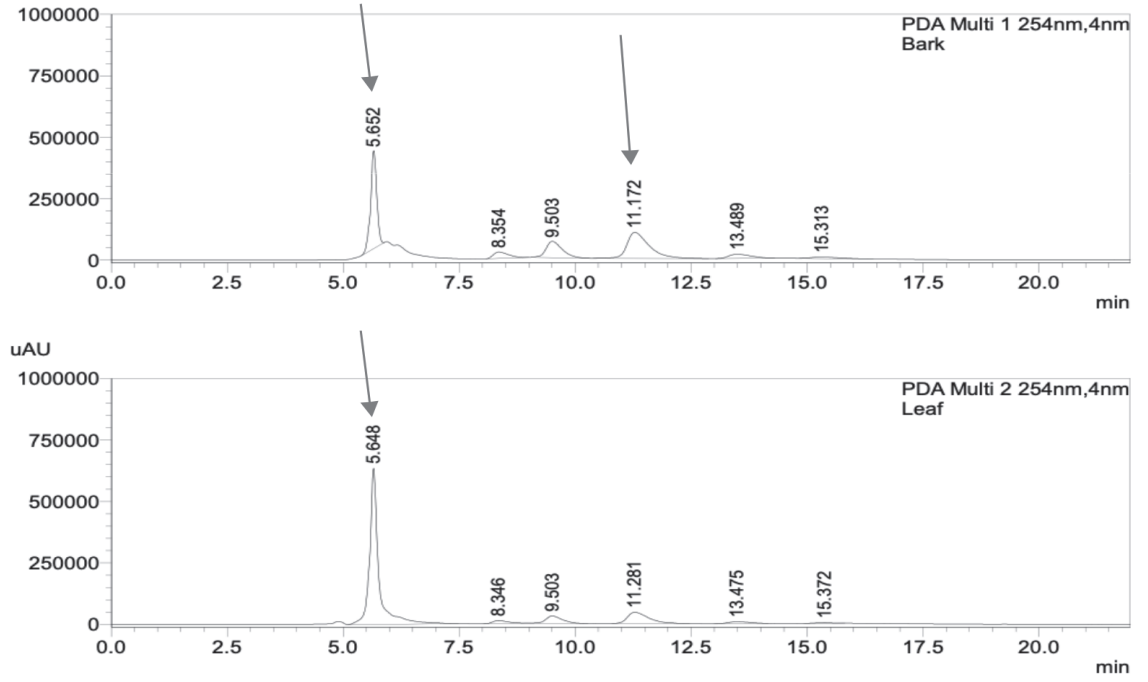

Fig. 4: *D. gamblei.* Chromatograms of bark and leaf samples.

ethanol, chloroform and ethyl acetate solvents revealed the presence of various phytoconstituents (Table 1).

Fingerprint analysis by HPTLC is one of the most powerful tools to link the botanical identity of the chemical constituent profile of the plant. The results from HPTLC fingerprint scanned at wavelength 366 nm for *D. gamblei* leaf and bark methanolic extracts showed the presence of quercetin (Fig. 1). From the chromatogram, it is clear that a band corresponding to quercetin is visible in both reference and test solution tracks. Plate showed the presence of band of quercetin (which was used as standard) in the standard lane and in the sample at the same position as the standard lane a band had appeared which confirms the presence of quercetin in the methanolic leaf and bark extracts. Quercetin band was present in all the samples at the same retention factor value corresponding to quercetin standard.

During the present investigation of HPLC analysis, leaf and bark extracts, quercetin and kaempferol were used as the standards. The concentration of the quercetin and kaempferol were calculated. Quercetin showed a peak at Rt 5.672 min with an area of 86743 µAU (Fig. 3, Table 2) and Kaempferol at 11.129 min with an area of 91358 µAU (Fig. 2, Table 3). In the leaf sample analyzed, peaks appeared at Rt 5.648 and 11.281 min with an area of 25352 and 1435 µAU whereas in the bark sample peaks appeared at Rt 5.652 and 11.172 min with an area of 14997 and 2870 µAU (Fig. 4, Tables 2, 3) confirming the presence of quercetin and kaempferol in the methanolic plant extract. The leaf extract showed 0.70% quercetin whereas the bark extract showed 0.42% similarly, the leaf extract showed 0.04% kaempferol whereas the bark extract showed 0.08%. The graphical representation of chromatographic development of the sample also showed the presence of 4 other compounds in both the leaf and bark extracts. Among them, the first, second, third and fourth compounds in leaf extract peaks appeared at the Rt of 8.346, 9.503, 13.475 and 15.372 min, whereas in bark extract peaks appeared at the Rt of 8.354, 9.503, 13.489 and 15.313 min respectively (Fig. 4). Among the above mentioned compounds, the second compound appearing at Rt 9.503 min in leaf extract and at Rt 9.503 min in bark extract showed the highest peak and fourth compound appearing at Rt 15.372 min in the leaf extract and at Rt 15.313 min in bark extract showed the lowest peak (Fig. 4).

DISCUSSION

The *Dendrophthoe* species have not been extensively studied in terms of phytochemistry (Lim et al. 2016). Lohezic-Le Devehat et al. (2002) successfully isolated 3 flavonols from *Scurrula ferruginea*, quercetin, quercitrin and 4"-O-acetylquercitrin, and the latter acetylated derivative was uncommon in higher plants. Priyanto et al. (2014) recorded an ethanol extract

of *S. atropurpurea* consisting of flavonoid, quercetin.

Qualitative phytochemical analysis of *D. falcata* was conducted by Atun et al. (2018) revealed the presence of terpenoid, alkaloid, phenolic and saponin. Our results are in conformity with the above findings. Channabasava & Sadananda (2013) conducted phytochemical analysis of different solvents such as hexane, ethyl acetate, methanol and distilled water of *D. falcata* yielded different phytochemicals. Compared to other solvents, the aqueous solvent extracts yielded more compounds. The aqueous extracts revealed the presence of carbohydrates, cardiac glycosides, proteins, amino acids, polysterols, alkaloids, phenols, tannins and reducing sugars at higher rate. However, the hexane and ethyl acetate extracts yielded less quantity of phytochemicals. More phenolic substances were observed in aqueous extract followed by methanol extract. Similar observations were made in the present investigation also.

According to Shinde (2023), HPTLC profile of *D. falcata* fruit extract was recorded at 366 nm and showed the presence of 10 polyvalent compounds with Rf value 0.03 to 0.78. The experimental evidence of Bhagat & Kondawar (2017) from HPTLC fingerprint scanned at wavelength 366 nm for dichloromethane: methanol extract of *D. falcata* leaf powder showed the presence of 12 polyvalent phytoconstituents and Rf values ranged from 0.06 to 0.87, at 560 nm showed the presence of 9 polyvalent phytoconstituents, Rf from 0.20 to 0.94 and at 366 nm showed the presence of 9 polyvalent phytoconstituents and Rf from 0.09 to 0.90.

In the present study, overlay of the spectrograms of standard quercetin and quercetin in the leaf and bark extracts was found. The spots corresponding to the extracts leaf and bark and quercetin were scanned at 366 nm with Rf value of 0.35. From the HPTLC and HPLC analyses it is clear that not only quercetin and kaempferol, but also mixture of constituents present in the leaf and bark extracts indicate the pharmacological value of the parasite.

The study conducted by Pramestya et al. (2019) showed that though the methanolic stem extract of *pentandra* had the lowest yielded extract, but contained the most diverse compounds. Major compounds were found in the stem extract one compound Rt 9.9 min was found in both leaf and flower while two compounds Rt 9.7 and 18.5 min were found only in the leaf extract, it was also found that that the compounds with Rt of 10.4, 10.7, 11.0 and 17.9 min were the only compounds present in all *D. pentandra* organs. The compound with Rt of 10.4 min constituted the major compound in all extracts. Artanti et al. (2009) had reported that the only compound successfully isolated from *D. pentandra* extract was quercitrin that had antioxidant activity. This

suggests that the major compound Rt 10.4 min is quercitrin, it also showed that the compounds with Rt of 10.4 and 10.7 min constitute the major compounds in both flower and leaf extracts. It is interesting to note that all compounds present in the flower extract could also be found in the leaf extract, even though the stem extract had 7 compounds, Rt 2.5, 14.0, 16.0, 16.2, 16.5, 16.9 and 17.3 min that were not found in either flower or leaf extract, but one compound found in the flower extract Rt 9.9 min cannot be found in the stem extracts.

In the present investigation, quercetin and kaempferol quantification was performed through HPLC and found 6 peaks in methanolic leaf and bark extracts. These peaks were compared with the standard peak of quercetin at Rt 5.672 min and kaempferol 11.129 at 254 nm, indicating that significant amount of quercetin and kaempferol are present in methanolic leaf and bark extracts.

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Declarations

Consent to participate

We hereby certify that we have participated sufficiently in the analysis and interpretation of the data as well as the writing of the manuscript, to take public responsibility for it and have agreed to have our names listed as contributors.

Authors' contribution

With collaboration of both the authors, this project has been started. Collection of plant, experimentation and data collection was carried out by RTS and drafting and correction of the manuscript was done by LR..

Conflict of interest

The authors declare no conflict of interest.

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