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Callus Induction Studies and Active Components and Antioxidant Activity Investigation from Leaves and Callus of *Tecoma stans* L. Juss. Ex Kunth.

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ABSTRACT

Tecoma stans is a tropical flowering plant which is used in herbal medicine treatment for diabetes, digestive problems etc. The present investigation was carried out to study the effect of different plant growth regulators on callus induction from *Tecoma* leaves and to compare the antioxidant activity from the leaves and in vitro generated callus. Qualitative analysis of phytochemicals from the leaves of *T. stans* were done using different solvents. Callus culture were initiated from leaf explants on Murashige and Skoog medium supplemented with different concentrations and combinations of hormones like 2,4-D, NAA, BAP and Kinetin for rapid initiation of callus and biomass production. To study the antioxidant activity the effect of leaf extract on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical was measured. The best efficiency of callusing was observed when 2,4-D and BAP in 2mg/l concentration each were used which was followed by 2,4-D and Kinetin. Methanolic and ethanolic leaf extract indicated higher antioxidant activity than the callus extract. In vitro studies and production of biomass and extraction of bioactive compounds may contribute in conservation management of naturally grown plants.

Keywords: *Tecoma stans*, antioxidant, plant hormones, callus culture

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INTRODUCTION

Plants have been used in virtually all cultures as a source of medicine. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). Genetic biodiversity of traditional medicinal herbs and plants is continuously under the threat of extinction as a result of growth-exploitation, environment-unfriendly harvesting techniques, loss of growth habitats and unmonitored trade of medicinal plants [1].

Tecoma stans (L.Juss.ex Kunth) or Yellow bells, from family Bignoneacea, is a semi-evergreen ornamental tropical shrub which is used traditionally for reducing blood glucose. Almost all the parts of *Tecoma stans* are of medicinal importance and used traditionally for the treatment of various ailments. The *Tecoma stans* leaves, barks and roots have been used as muscle relaxant, mild cardiogenic and chloretic activity. As pharmacological uses *Tecoma stans* have been used in herbal medicine treatment for diabetes[2], digestive problems, control of yeast infections, as powerful diuretic, vermifuge and tonic[3]. Flower and leaves have some medicinal value for the treatment of various cancer[4]. Its leaves are used traditionally in Mexico to control diabetes[5,6].

Various phytochemicals present in *T. stans* are responsible for the medicinal value of the plant. Explant of the plant, cultured *in vitro*, has been found to retain the capacity to synthesis compounds identical to that in the intact plant. Callus culture can facilitate optimization of alkaloids production and subsequent isolation[7]. Tissue grown as callus masses can sometimes yield high amount of secondary metabolites. Plant derived phenolic compounds exhibit a considerable antioxidant activity. Polyphenols are of medicinal importance due to their free-radical scavenging activity. The antioxidant property can be determined by their reactivity as hydrogen-or electron-donating agents, the stability of the resulting antioxidant derived radicals, their reactivity with other antioxidants and their metal chelating properties[8]. The present study was aimed at the preliminary phytochemical screening of *T. stans* plants by using aqueous and organic solvent extracts of the leaves. *In vitro* culture of leaves was carried out for callus induction and the antioxidant activity was tested from leaves and leaf derived callus.

MATERIALS AND METHODS

Healthy and young leaves of *Tecoma stans* were collected from the campus of Dr. D.Y. Patil Institute of Biotechnology and Bioinformatics, Pune for the phytochemical and *in vitro* studies.

Preliminary screening of secondary metabolites

Shade dried leaves of *T. stans* plants were powdered using sterile mortar and pestle along with desired solvent. Powdered samples were extracted individually with methanol, ethanol, ethyl acetate and water, and used for preliminary screening of phytochemicals . Qualitative analysis was carried out for alkaloids, glycosides, phenolics, anthraquinones,

tannins, saponins and flavonoids by following the method described by Kokate[9] and Sofowora[10].

In vitro culture of explants for callus induction

Leaf explants were used for the present studies. Washed explants were prepared and surface sterilized with Bavistin (1% w/v) for 3 minutes and with mercuric chloride (0.1% w/v) for 10 minutes followed by washings with sterile distilled water for several times to remove the traces of HgCl₂. The explants were inoculated on Murashige and Skoog[11] basal media fortified with 3% sucrose and supplemented with various combinations and concentrations of auxins 2, 4-Dichlorophenoxyacetic acid (2, 3, 4 mg/lit) and cytokinin, kinetin (2, 3, 4 mg/lit) or 6-Benzyladenine (2, 3, 4 mg/lit). pH of the media was adjusted at 5.8 before gelling the medium with 0.8% agar- agar type (Himedia). The cultures were incubated at 25 ± 2°C with a photoperiod of 16 h at 3000 lux light intensity of cool white fluorescent light. All the experiments were repeated twice with 10 cultures per treatment. Data were taken after 4-6 weeks by visual observation of the culture.

Determination of total antioxidant activity

Free radical scavenging activity of plant samples and callus was checked against stable 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH). The measurement of the DPPH radical scavenging activity was performed according to methodology described by Lee *et al* [12]. Positive control was prepared by mixing 4 mL of ascorbic acid (0.05 mg/mL) and 1 mL of DPPH (0.4 mg/ml). Negative control was prepared by mixing distilled water with 1 mL of DPPH. 4 mL of the extract (a final concentration of 20 mg/mL) were added to 1 mL DPPH. The mixture was gently homogenized and left to stand at room temperature for 30 min. Absorbance was read using a UV-VIS Spectrophotometer, at 520 nm. % scavenging of the DPPH free radical was measured using the following equation:

$$\% \text{ DPPH radical-scavenging} = \frac{[(\text{absorbance of control} - \text{absorbance of test Sample}) / (\text{absorbance of control})] \times 100.}$$

RESULTS AND DISCUSSION

Medicinal plants are the most exclusive source of life-saving drugs for majority of the world's population. The result obtained in the phytochemical screening of the *Tecoma stans* plants (Table 1) varied according to the solvents used for the extraction of the leaves. Methanol and ethanol extracts of the leaves showed the presence of all the secondary metabolites studied- saponins, flavonoids, tannins, phenols, anthraquinones, alkaloids and glycosides which would be the active principles of the plant. The presence of flavonoids, glycosides, carbohydrates, proteins, triterpenoids and tannins using methanolic extracts are previously reported in *Lantana indica*[13]. Ethyl acetate extracts indicated the presence of saponins, tannins and phenols whereas aqueous extracts showed saponins, flavonoids phenols and alkaloids from leaves of *T. stans*. Saponins are glycosides occurring widely in plants. Saponins possess hypocholesterolemic and antidiabetic properties[14]. Plant glycosides were reported to exhibit antidiabetic characteristics[15]. The presence of these phytochemicals may be responsible for the antidiabetic properties of *Tecoma* plant.

Flavonoids have been referred to as nature’s biological response modifiers, because of their inherent ability to modify the body’s reaction to allergies and virus. Alkaloids are the most efficient therapeutically significant plant substances. The present studies indicated saponins and phenolics by using all the extraction solvents used in studies and tannin was found to be absent in aqueous extracts. Phenolic compounds are one of the largest and most ubiquitous groups of metabolites in plants [16]. Solvent system plays an important role in the solubility of phytochemical components in the crude extracts. The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites.

Table 1: Phytochemical analysis of the different solvent extracts of *T. stans*.

Phytochemicals	Solvent used for extraction			
	Methanol	Ethanol	Ethyl acetate	D/W
Saponin	+	+	+	+
Flavonoid	+	+	-	+
Tannin	+	+	+	-
Phenols	+	+	+	+
Anthroquinones	+	+	-	-
Alkaloids	+	+	-	+
Glycoside	+	+	-	-

+ present, - absent

Plant tissue culture technology is an alternative for the mass production of plant derived compounds. The present study showed that various combinations of auxin 2, 4 D with cytokinins BAP and kinetin produced callus with variable response (Table 2) and significant differences in frequency and degree of callusing was observed. The observations were taken from 4-8 weeks and was noted that 2, 4 D with BAP in a concentration 2mg/l each indicated 100 % callus induction (Fig 1), whereas the same concentration of 2,4-D+Kinetin indicated the lowest frequency of callus induction in shoot apex of *T. stans*. Studies on leaf callus induction in *Bupleurum* showed that lower concentration of 2, 4, D is more beneficial and increase in 2, 4, D concentrations did not improved callus induction[17]. Early callus induction was observed in leaf explants in comparison to shoot apex when the given hormonal combinations used in *T. stans* and growth of the callus increased significantly with the incubation period from 4-8 weeks in both the explants. Increase in concentration of 2, 4 D and kinetin in the *in vitro* culture of *Tecoma* shoot apex showed an increasing frequency of callus induction and growth. Highest percentage of callus induction from shoot tips of *Cassia obtusifolia* L. was reported by using a combination of 2, 4 D and kinetin[18]. Calli obtained from all explants were brown in color, which imparts the influence of phenols, and compact in nature. The development of *in vitro* cell lines of *T. stans* with antioxidant compounds provides the possibility of generating uniform material cultured under conditions independent from environmental factors[19]. *In vitro* callus growth is influenced by the explants and plant growth regulators used in the culture medium. The plant *Tecoma stans* by *in vitro* results appears as interesting and promising and may be effective as potential source for novel antioxidant drugs.

Considerable attention has been focused on medicinal plants with antioxidant properties. Anti oxidant activity of the ethanolic and methanolic extracts of plant leaves and the callus obtained from leaves are given in fig 2. The results indicate that both methanolic

and ethanolic fractions of the leaves exhibit higher scavenging activity than the *in vitro* callus. The ethanolic leaf extract indicated highest antioxidant activity at 30 minutes of incubation. The extraction by using ethanol and methanol as solvent showed moreover similar results in terms of % scavenging activity. The present results are in agreement with the report of Tanwar *et al*²⁰ that antioxidant activity of methanolic extract of the *in vivo* grown *Spilanthes acemella* is higher than the *in vitro* grown callus. The presence of hydroxyl groups of plant phenolic compounds is responsible for their free radical scavenging ability. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers[21]. The pharmacological uses of *Tecoma stans* in herbal medicine can be attributed to the phenolics and other secondary metabolites from the plant.

Table 2: Effects of explants and plant growth hormones 2,4-D, Kinetin and BAP on callus Induction

Explant	PGR's and conc. (mg//l)		Callus frequency	Remarks
Leaf Explant	2,4-D+BAP	4+4	80%	Brown, compact callus
	2,4-D+BAP	3+3	78%	
	2,4-D+BAP	2+2	100%	
Leaf explant	2,4-D+Kinetin	4+4	70%	
	2,4-D+Kinetin	3+3	68%	
	2,4-D +Kinetin	2+2	71%	
Shoot Apex	2,4-D+Kinetin	2+2	56%	
	2,4-D+Kinetin	2+4	64%	
	2,4-D+Kinetin	4+2	67%	
	2,4- +Kinetin	4+4	72%	

(The experiments were repeated twice, each experiments consisting of 8 replicates.)

**Figure 1 - Callus induction in *T. stans* leaves: a. Callus initiation
b. Callus growth in 28 days, c. Callus growth in 45 days,
d. callus proliferation after 56 days**

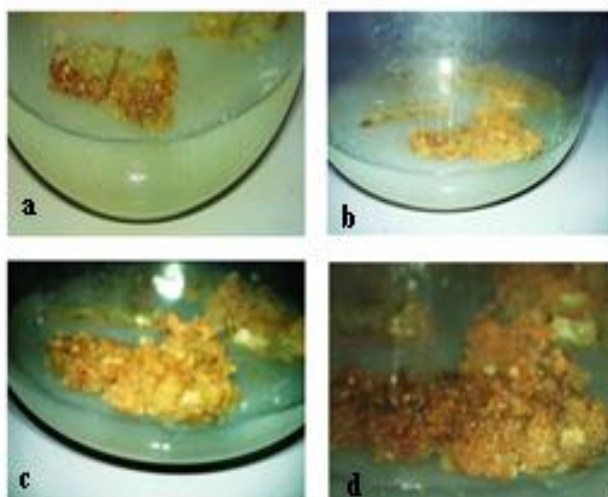
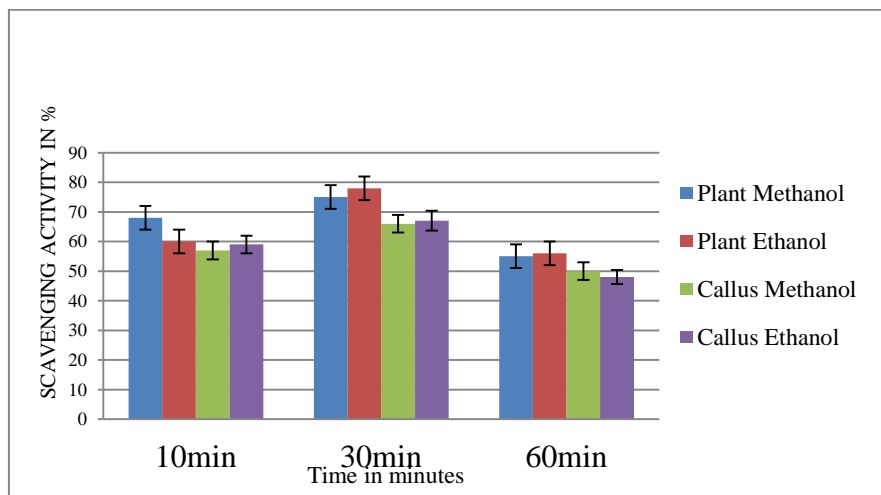


Figure 2: Comparison of antioxidant activity in leaves and callus of *T. stans* by using different solvents



Sustainable management of medicinal plant species is important due to their value as a potential source of new drugs. The present study reveals that antioxidant properties are indicated by the plant leaves and undifferentiated callus culture of *Tecoma stans*. Plant Biotechnology techniques can be refined to optimize the production of medicinally important phytochemicals *in vitro* which may contribute for the conservation of the natural plant resources.

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