INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

COMPARATIVE PHYTOCHEMICAL ANALYSIS OF *IN-VIVO* AND *IN-VITRO* DERIVED *COSTUS PICTUS* D. DON PLANTS BY HPTLC

Radha Devi G.M *
Research Scholar, Department of Botany, Bangalore University, Bangalore, Karnataka, India
*Corresponding Author Email: radha.kle@gmail,com

Article Received on: 12/05/19 Approved for publication: 19/07/19

DOI: 10.7897/2230-8407.1009277

ABSTRACT

The present study has been conducted for micro propagation followed by the investigation of phytochemical constituents by preliminary phytochemical screening and chromatographic analysis through TLC (Thin Layer Chromatography) and HPTLC (High Performance Thin Layer Chromatography) from the leaf extract of *in-vivo* and *in-vitro* derived *Costus pictus* plants. The nodal explants with axillary buds of *Costus pictus* were cultured in B5, MS, SH basal nutrient media supplemented with various concentration of plant growth regulators (PGR's) such as BAP, 2,4-D, IBA 1 mg to 10 mg/l, different combination of TRIA 1 µg/l to 10 µg/l. The best response was observed in 25 µg/l TRIA within 4 weeks of inoculation, complete plantlet was obtained by 8 weeks, multiple roots up to 20 cm long with pseudo stem and 6-7 board leaves. The main aim of this work was to elucidate the various secondary metabolites such as alkaloids, flavonoids, saponin and phenols in the Hexane, Di-chloro-methane, Chloroform, Ethyl acetate, Methane and Aqueous extracts of *Costus pictus* leaves. Initially by preliminary phytochemical screening, later TLC plating on specific mobile phase followed by advanced techniques like HPTLC on silica gel 60 F 254 plates as stationary phase. The linear ascending development was carried out in a twin trough glass chamber saturated with a mobile phase, HPTLC CAMAG "Linomat 5 sample applicator, TLC scanner3, Re prostar 3 and win CATS 1.3.4 software, spectro-densitometric scanning at 254 and 366 nm The HPTLC fingerprint profile displayed the presence of alkaloids, Flavonoids, Phenols and Saponins with reference to respective standards.

Keywords: *Costus pictus*, *In-vivo, in-vitro*, micro propagation, TLC, HPTLC.

INTRODUCTION

Costus pictus D. Don is a valuable and rapidly growing medicinal plant well known as "Insulin plant", fiery Costus or step ladder or spiral flag belonging to the family Costaceae, Zingiberales[1]. It is perennial rhizomatous erect pseudo stem of 20-30 cm tall, ornamental herb found commonly in Kerala and southern parts of India[2] native to south and Central America[3]. The leaves are broad elliptical arranged spirally on the aerial shoots that have a characteristic spiral monistichous (one-sided) branching spiral phyllotaxy[4]. The few fertile pseudo stems show an unique floral structures within the Zingiberales with a single fertile stamen while the remaining five infertile stamens fuses together to form a large, petaloid labellum that dominates the floral display[5], each flower is produced on cones an involucre of bracts. Each bract produces a flower at its terminal, yellow in color with orange or red striations at the tip and last for 2-3 days[6], the seeds are rudimentary, reproduces only through vegetative mode. The pseudo stem possesses dormant axillary buds at the nodal regions[7].

The leaves and rhizome of *Costus pictus* possess many therapeutic properties, recently several clinical trials have been conducted on alloxan[8], streptomycin[9] induced diabetic rats with leaf extract of *Costus pictus*; showed significant effect on reducing blood glucose level, hypoglycemic and hypolipidemic properties. It is been used as folkloric system of medicine[10], anti-helmintic[11-12], anti-bacterial[13-14], anti-oxidant, anti-proliferative[15]. The pharmacological reports of *Costus pictus* indicate that it acts as diuretic[16], anti-cancerous[17-18]. In GC-MS analysis 18 bioactive compounds were identified in leaf extracts[19], preliminary phytochemical screening shows presence of primary metabolites[20-21] and secondary metabolites, flavonoids Kaempferol 3' 4'-di-O-Me-Quercetin , 4-O-Me-Kampferol and phenolics like gentisic, 2,5-dihydroxy benzoic acid, O-coumaric, melilotic, α-resorcyclic, 3-5 di hydroxy benzoic acid, P-di hydroxy benzoic acid, cis and trans p-Coumaric acid[22]. Genomic analysis of *Costus pictus* was[23] reported the transcripts related to pathways of bixin biosynthesis and geraniol and geranial biosynthesis a major transcript from the class of iso-prenoid secondary metabolites and validated the presence of putative norbixin methyl transferases a precursor of bixin.

The tissue culture technique for medicinally important plants has shown a promising aspect in obtaining regenerates and clonal multiplication for conservation of species[24], from being exploited at large commercial scale. The micro propagation is one of the most efficient method or tools for mass propagation of pure good elite plant materials which substantially improves the quality of drug production in large scale[25]. The usage of basal growth media supplemented with specific phyto-regulators helps in regulation and enhancing the morphogenesis naturally[26-27].

The current scenarios exhibit the demand for the natural products throughout the world for pharmacologically active bio molecules, in synthesis of novel drugs. Now a day's new technology has made it possible to identify, screen and isolate these active compounds. Natural products of plant origin are widely recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological

activities. The chromatographic spectral fingerprints play an important role in the quality control of complex herbal medicines.[28] Preliminary phytochemical screening as first step to identify phytochemical compounds, followed by TLC and advanced techniques like HPTLC. HPTLC being rational for expansion of chromatographic fingerprints to determine major bioactive constituents in medicinal plants.[29] The resolution is much better and the results more reliable, reproducible than TLC. Besides the colorful pictorial representation of HPTLC image provide extra, intuitive visible color or fluorescence parameters for parallel separation of individual secondary metabolites[30]; according to guidelines of World Health Organization (WHO) for standard methodology mentioned in different pharmacopeia for herbal drugs[31]. The present work was carried out to standardize protocol for clonal production and phytochemical analysis of *Costus pictus* leaves *in-vivo* and *in-vitro* raised plants by Thin Layer Chromatography (TLC) and high-performance thin layer chromatography (HPTLC) fingerprint.

a) *Costus pictus*, b) inflorescence

MATERIALS AND METHODS

Collection of Plant Material

The plantlets and rhizome were collected from Calicut University, Calicut Kerala India the voucher specimen is preserved and documented prior identification by taxonomist[2]. The plants were maintained in green house Department of Botany, Bangalore University, Bangalore.

Required chemicals

Micro and Macro nutrients for basal media, hormones IBA, BAP, TRIA were procured from Himedia private limited, Analytical grade Hexane, Dichloromethane, Chloroform, Ethyl acetate, Methanol, standard Atropine, Quercetin, Ferulic acid, Diosgenin were purchased from Sigma, India. The HPTLC aluminium plates pre-coated with silica gel 60 F254 (20 cm x 20 cm, 0.2 mm thickness) were obtained from Merck India.

Micro propagation of *Costus pictus* by nodal explants

Preparation of Basal nutrient Media

The basal formulations like Murashige and Skoog's media[32], B5 Gamborg media (1976)[33], macro and microelements, salts and organic constituents, plant growth hormones in varying concentration of BAP (1 mg/lt to 5 mg/lt), IBA (1 mg/lt to 40 mg/lt), TRIA (1 µg/lt to 30 µg/lt), carbon source of 3% v/v sucrose and 0.8% agar is used for media. TRIA (CH_3 (CH_2)28C H_2OH) Sigma (St, Louis, MO, USA), Stock solution of TRIA was prepared by dissolving 1 mg of TRIA in 0.75 ml of Chloroform containing one drop of Tween 20 and this stock solution was gradually diluted with distilled water to the final volume of 200 ml.

Preparations of explants

The nodal buds with axillary bud derived from pseudo stem were washed thoroughly in running tap water. Then washed with distilled water, containing 4-5 drops of detergent (tween 20, India) for an hour with intermittent shaking, followed by rinsing thrice with sterile distilled water; to remove the traces of detergent under aseptic condition, later treated with 70% alcohol for 5 min, rinsed thoroughly with sterile distilled water and. Surface sterilized with 0.1% mercuric chloride for 2 min, adhering mercuric chloride was removed by washing the explants repeatedly with sterile double distilled water for 15 min. The edges of the explants were cut using a sterile blade and sliced vertically into two equal halves, the portion with axillary bud were inoculated. Cultures were incubated under the photoperiodic condition, 16 h of light duration and 8 h of dark condition, temperature at 25 ± 2°C and humidity of 96%.

Preparation of Extracts

The fresh and young leaves of both *in-vivo* and *in-vitro* derived *Costus pictus* were harvested, surface sterilized with 70% alcohol, shade dried for 5 days; dried leaves were made into fine powder of mesh size #40. The powdered plant material of *Costus pictus* was tumbled into Soxhlet apparatus and extracted serially with nonpolar and polar solvents like Hexane, Di-Chloro-methane, Chloroform, Ethyl acetate, Methane and Aqueous. Each solvent extract obtained was filtered with what man filter paper No.1, concentrated and measured, and transferred to next solvent for extraction, in each solvent extraction was carried out for 24 h at 15^0C of mantel heat and obtained solutions are condensed at 20^0C[34]. These extracts were then concentrated on the reflux condenser by recovering the solvents, later heated on a hot plate for complete evaporation. The extracts were weighed and reconstituted with respective solvents, stored in airtight containers and preserved at 4^0C for further analysis.

Preliminary phytochemical screening

The preliminary phytochemical investigation of different solvent extracts of *Costus pictus* were performed to detect the various classes of phyto-constituents such as alkaloids, flavonoids, phenols, carbohydrates, phyto sterols, glycosides, saponins and terpenoids[35].

Qualitative analysis

Screening for Alkaloid

About 0.5 g of the extract was stirred in 5 ml of 1% HCl on a steam bath and filtered. Hot distilled water was added to the residue and 1 ml of the filtrate.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide) and formation of a yellowish cream precipitation indicated the presence of alkaloids.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide) and observed. Formation of brown or reddish-brown precipitation indicated the presence of alkaloids.

Dragendroff's Test

Filtrates were treated with freshly prepared Dragendroff's reagent (solution of Potassium Bismuth Iodide) with formation of red precipitation indicated the presence of alkaloids.

Hager's Test

Filtrates were treated with Hager's reagent (saturated picric acid solution) formation of yellow colored precipitate indicated the presence of alkaloids.

Screening for Flavonoids:

To determine the presence of flavonoids in the plant samples, 5 ml of dilute Ammonia solution was added to a portion of aqueous filtrate of each plant extract followed by yellow coloration. Each extract indicated the presence of flavonoids.

Test to phytosterols

Libermann- Burchard's test

The extract is treated with 2 ml acetic anhydride. To these one or more drops of concentrated sulphuric acid is added slowly along the sides of the test tubes. An array of color changes shows the presence of phytosterols.

Test to detect carbohydrates

Fehling's test

To about 1 ml of the sample add 1 ml of each of Fehling solutions A and B then boil the solution on a water bath for few minutes. A red precipitate indicates the presence of sugar.

Test to detect glycosides

Borntrager's test

To1 ml of sample and add 3 ml of chloroform and shake well when the chloroform separates add 10% ammonia solution to it. Pink color indicates the presence of the glycosides.

Alkaline test

To a few ml of the sample add few drops of sodium hydroxide solution. A yellow fluorescence indicates the presence of the glycosides.

Test to detect terpenoids

Salkowski test

To a few ml of the extract add chloroform and concentrated sulphuric acid along the sides of the test tube. Appearance of the golden yellow color in sulphuric acid layer turns red, indicates the presence of terpenoids.

Test for Phenols

Ferric chloride test

The extract 50 mg was dissolved in 5 ml distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenol compound.

Lead acetate test

The extract 50 mg was dissolved in 5 ml of distilled water; to this 3 ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds.

Screening for Saponins

Forth Test

1 ml of distilled water was added to 1 ml of the extract and shaken vigorously. A stable persistent froth indicated the presence of Saponins.

All these above-mentioned preliminary phytochemical screening tests were carried out thrice and tabulated in the Table 1.

Development of Chromatographic fingerprint profile of all the extracts of *in-vivo* and *in-vitro Costus pictus* plants

The solvent mixtures were freshly prepared, for the separation of different phytochemical compounds; the extracts were spotted manually using capillary tubes on preparative TLC glass plate of 10 x 10 cm. The plates were activated prior to loading of the extract at 110^0C. TLC plates were eluted on the different solvent system as mobile phase for various secondary metabolites like alkaloids, flavonoids, phenols and saponins. After development the plates were heating at 110^0C for 10 min and observed in UV cabinet. The phytochemical compounds with their Rf values and chromatograms were calculated using the formula mentioned below.

Rf

Retention time Rf = Distance of centre of spot from starting point SD (Solvent distance) / Distance of solvent front from the starting point SF (Solvent front)

HPTLC Fingerprinting Analysis

Sample preparation

10 mg/ml concentration of each extracts were pipetted, dried, then re-suspended in 1 ml of HPTLC grade methanol and filtered with Whatman's filter paper and these test solutions along with standards were used for HPTLC analysis.

Development of solvent system

Different solvent system was used to develop HPTLC fingerprint profile of secondary metabolite group's viz., Alkaloids, Flavonoids, Phenols and Saponins as mentioned in Table 2.

Sample application

4 µl of sample and 4 µl of standard solution were loaded as 6 mm band length separately on pre-coated silica gel 60F254 aluminum sheet HPTLC plate 10 x 10 cm 250 µm thickness (E. Merck, Mumbai India) 8 mm from the bottom and 15 mm form the side edges after the application, using Hamilton syringe size 100 µl with the help of Linomat 5 applicator parameters as inert spray gas, methanol is used as a sample solvent type, dosage speed of 150 nl/s, pre-dosage volume 0.2 µl, sequence of syringe size 100 µl, application position Y- 8.0 mm, band length 6.0 mm, volume 10 ml, drying device oven temperature 60^0C attached to CAMAG HPTLC system which was programmed through win-CATS software.

Development of Chromatogram

The spotted plates were exposing to hot air for activation (10 min at 110^{0}C), the mobile system was saturated for 30 min with their respective mobile phase for Alkaloids, Flavonoids, Phenols and Saponins prior to development of chromatograms. The spotted plates, placed vertically in twin trough glass chamber (camag Switzerland), eluted for 15 min, up to 80% of the plate. The plates removed air dried for 5 min and placed in oven at 110^{0}C for 10 min for complete removal of solvents.

Spectro-densitometric detection

The densitometric scanning was performed with TLC scanner camag-3 equipped with win CAT 1.4.2 software (camag Switzerland) reflectance absorbance. The slit dimensions of 6 mm x 0.30 mm, scanning speed 20 mm/s, data resolution 100 µl/step, second order optical filter factor (Savitzky-galaxy 7), lamp D2. Later plate was kept in photo-documentation chamber (camag Re prostar 3) and captured the images at visible light and UV 254 nm and 366 nm the peak number with its height and area peak display and peak densitogram were identified, as represented in figures 3-6, densitograhic images of both *in-vivo* and *in-vitro* depicted in Figures 7 and 8.

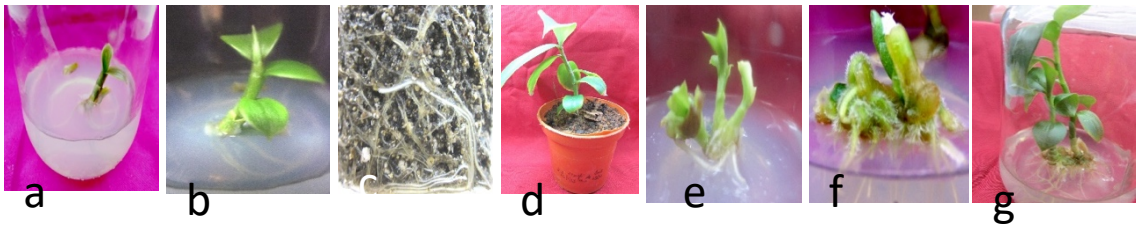

Figure 2: a) and b) B5 + TRIA 25 µg/l c) rooting d) acclimatization e) MS + BAP, f) and g) MS + IBA 20 mg/l

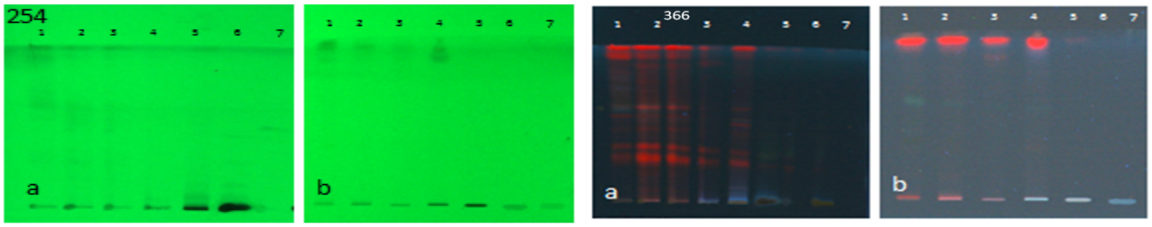

Figure 3: Alkaloids profiling a) *in-vivo* plant b) *in-vitro* plant, 1-Hexane, 2-Dichloro methane, 3-Chloroform, 4-Ethyl acetate, 5- methanol, 6- aqueous, 7-std Atropine

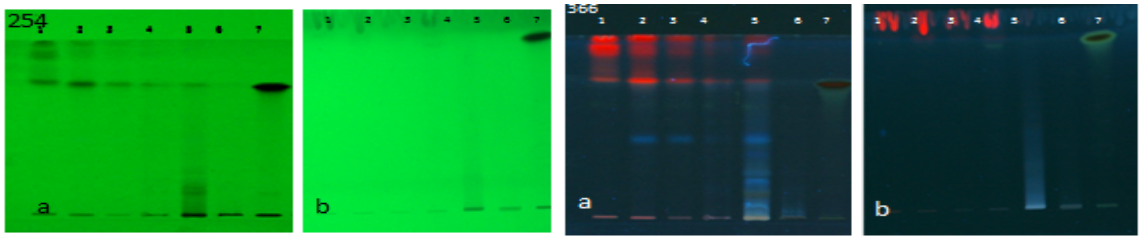

Figure 4: Flavonoids profiling a) *in-vivo* plant b) *in-vitro* plant, 1-Hexane, 2-Dichloro methane, 3-Chloroform, 4-Ethyl acetate, 5- methanol, 6- aqueous, 7-std Quercetin

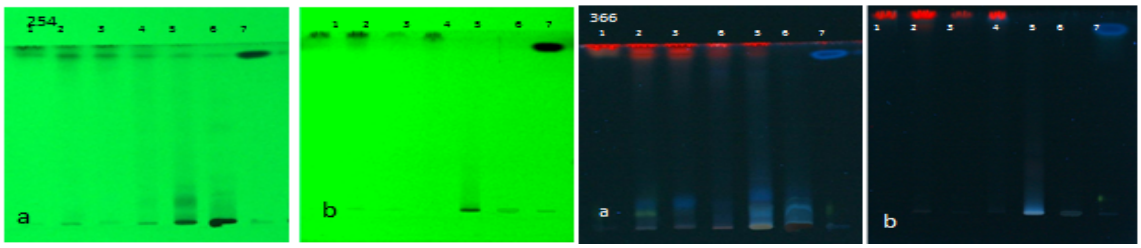

Figure 5: Phenols profiling a) *in-vivo* plant b) *in-vitro* plant, 1-Hexane, 2-Dichloro methane, 3-Chloroform, 4-Ethyl acetate, 5- methanol, 6- aqueous, 7-std Ferulic acid

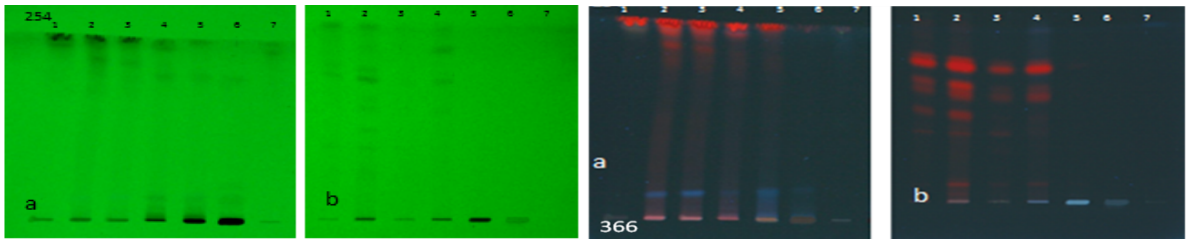

Figure 6: Saponin profiling a) *in-vivo* plant b) *in-vitro* plant, 1-Hexane, 2-Dichloro methane, 3-Chloroform, 4-Ethyl acetate, 5- methanol, 6- aqueous, 7-std Diosgenin

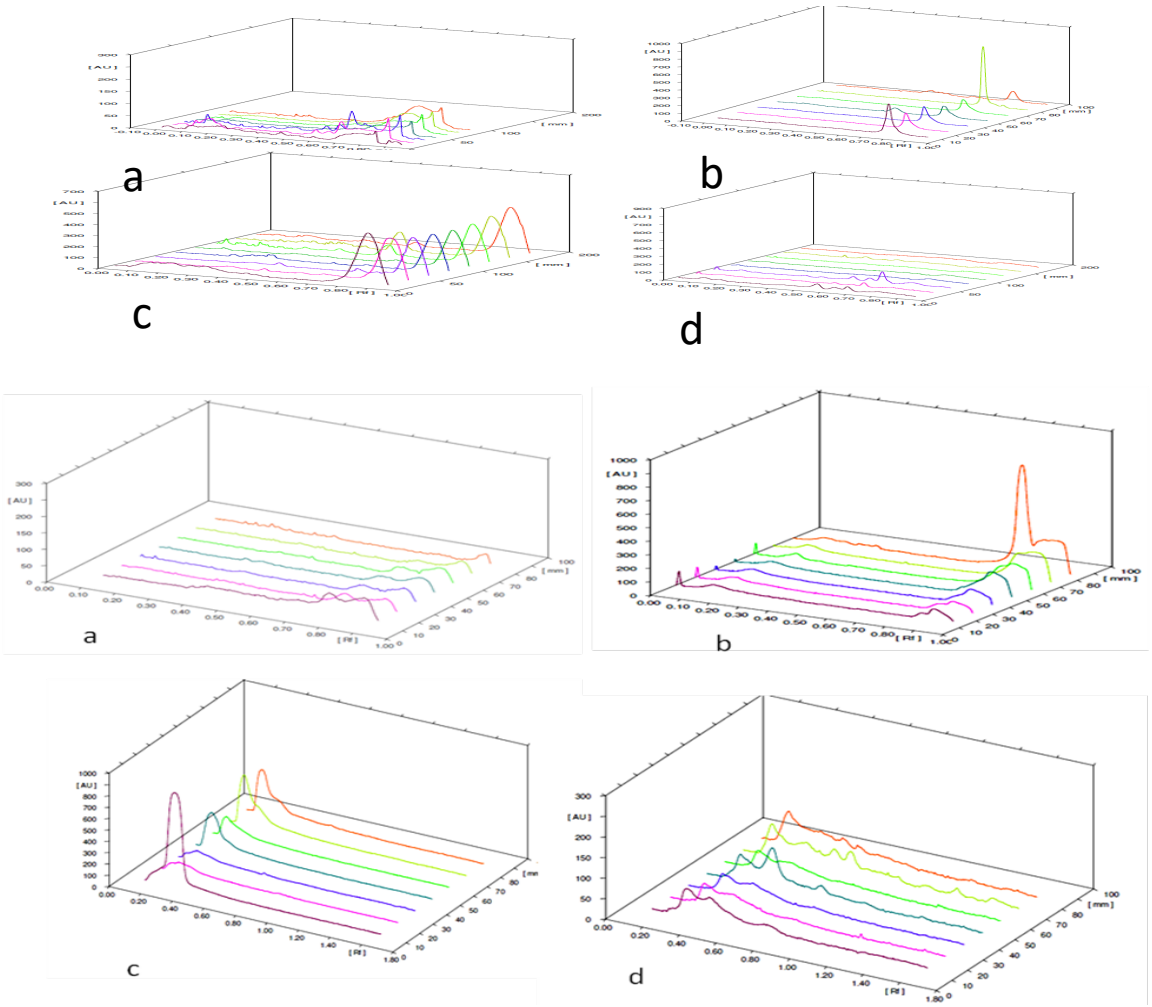

Figure 7 and 8: *in-vivo* and *in-vitro* Densitometer, a) Alkaloids b) Flavonoids c) Phenols d) Saponins

Table 1: Preliminary phytochemical screening

S. No.	Phytochemicals	Test conducted	Observation	*In-vivo* plants	*In-vitro* plants
1	Alkaloids	Mayer's test	Yellowish cream Precipitate	+	+
		Wagner's test	Reddish brown precipitate	+	+
		Dragendroff's test	Red precipitate	+	+
		Hager's test	Yellow precipitate	+	+
2	Flavonoids	Alkaline reagent test	Yellow coloration	+	+
3	Phyto-sterols	Liberman- Burchard's test	Array of colour changes	+	-
4	Carbohydrates	Fehling's test	Red precipitate	+	+
5	Glycosides	Borntrager's test	Pink colour	+	-
		Alkaline test	Yellow Fluorescence	+	-
6	Terpenoids	Salkowsk's test	Golden yellow colour	+	-
7	Phenols	Ferric Chloride test	Dark green colour	+	+
		Lead acetate test	Bulky white precipitate	+	+
8	Saponins	Froth test	Stable persistent froth	+	+

Table 2: Mobile phase for determination of secondary metabolites

Mobile phase	compound
Toluene: Ethyl acetate: Diethyl amine (70:20:10)	Alkaloids
Toluene: Acetone: Formic acid (40.5:40.5:10)	Flavonoids
Toluene: Ethyl acetate: Glacial Acetic acid: Formic acid (4:9:4:1)	Phenols
n-Hexane: Ethyl acetate (7:3)	Saponins

Table 3: Rf values of *in-vivo* and *in-vitro* derived plants in different extracts of *Costus pictus*

Secondary Metabolite	Extracts	*In-vivo* samples	*In-vitro* samples
Alkaloids	Hexane	0.10, 0.23, 0.45,0.73,0.82,0.88	0.40, 0.76,0.86
	Dichloromethane	0.07,0.13,0.55,0.60,0.64,0.83	0.66, 0.75, 0.83, 0.87
	Chloroform	0.07,0.42,0.54,0.59,0.64,0.69,0.83	0.75, 0.87
	Ethyl acetate	0.10,0.70,0.83	0.75,0.87
	Methanol	0.56, 0.73,0.83	0.74, 0.87
	Aqueous	0.52,0.72,0.74,0.82	0.74,0.87
Flavonoids	Hexane	0.75	0.01,0.13,0.84
	Dichloromethane	0.74	0.01,0.13,0.14,0.24
	Chloroform	0.73	0.13
	Ethyl acetate	0.10,0.14,0.46,0.51,0.66,0.73	0.13
	Methanol	0.73	0.14,0.22
	Aqueous	0.01,0.73,0.82	0.12,0.45
Phenols	Hexane	0.13,0.20,0.27,0.32,0.46,0.49,0.86	0.32
	Dichloromethane	0.33,0.45,0.64,0.87	0.24
	Chloroform	0.43,0.69,0.75,0.79,0.87	0.24,0.67
	Ethyl acetate	0.21,0.27,0.29,0.32,0.34,0.54,0.87	0.23,0.48
	Methanol	0.11,0.35,0.64,0.68,0.87	0.21
	Aqueous	0.05,0.08,0.11,0.14,0.16,0.18,0.20,0.22,0.23,0.31,0.43,0.59,0.63,0.87	0.22
Saponins	Hexane	0.12,0.17,0.42,0.55,0.62,0.66,0.78,0.87	0.32,0.44,0.75
	Dichloromethane	0.02,0.12,0.55,0.62,0.66,0.73,0.77	0.32, 0.47
	Chloroform	0.03,0.12,0.37,0.43,0.55,0.62,0.66,0.78	0.31,0.47
	Ethyl acetate	0.04,0.78	0.31,0.48,0.75
	Methanol	0.77	0.30,0.47
	Aqueous	0.05,0.79	0.28,0.47,0.62,0.71,0.97

RESULTS AND DISCUSSION

Effect of PGR on shoot multiplication and elongation

The shoot buds well proliferated in MS + 2 mg BAP, but shoots elongated only after repeatedly sub-culturing on same regeneration media for 16 weeks. In MS + IBA 20 mg/l 2 shoots were obtained within 8 weeks of culture. However, B5 media containing varying concentration of TRIA significantly showed rapid proliferation of roots and pseudo-stem within 4 weeks.

Rooting of Nodal explants

Rapid rooting was observed within 2 weeks of inoculation in B5 +TRIA 20-25 µg/l concentration measured up to 20-30 cm. In MS + BAP 2 mg/l rooting initiated after 4 weeks, similarly in MS + IBA concentration roots regenerated within 4 weeks. The roots regenerated in the basal nutrient medium were relatively strong and thick and durable during all the stages of growth and development.

Acclimatization of well-developed plantlets

The well-developed plant lets of length 5 cm to 7 cm were removed from the culture bottles, transferred to soil rite and covered over by polythene cover for 3 to 4 weeks. Later transferred to soil and sand mixture of 1:1 ratio; 100% survival rate along with formation of micro rhizome after one month of potting were noticed after the acclimatization process as depicted in Figure 1.

Phytochemical analysis

The preliminary qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phyto-sterols, carbohydrates, glycosides, terpenoids, phenols and saponins Table 2. Later the preparatory TLC studies helpful in standardization of mobile solvent system those were ideal for each group. The HPTLC plates observed 254 nm and 366 nm, compared with standards. The spectral densitometric patterns of extracts were demonstrated with the corresponding peaks, for alkaloids, flavonoids, phenols, saponin in both *in-vivo* and *in-vitro* raised plants. The spectral bands corresponding to the sample and standard peak were noticed and matched, in all extracts of *in-vivo* and *in-vitro* grown plant samples tabulated in Table 3 a chromatograph of all the extracts TLC plates are provided in Figure 2 to Figure 6.

CONCLUSION

In the present observation of *Costus pictus* nodal explants, micro propagation protocol for rapid, reproducible and standardized method for direct regeneration were noticed in B5 media with Triacontanol hormone instead of breaking the bud dormancy and later sprouted buds as a secondary explant[36]. The vertical incision to the nodal explant and inoculating the explant having axillary bud only shows significant results. The preliminary screening TLC and HPTLC analysis, *in-vivo* and *in-vitro* extracts showed the presence of metabolites content. In our investigation we have found the presence of secondary metabolites from the leaves of micro-propagated plants similar to natural plant as it is important that plants derived from the plant tissue culture are free from contamination and diseases free and useful in the production of various secondary metabolite, thus this protocol serves as a basic

idea for utilization of plants in large scale as the demand for this plant is increasing day by day thus can avoid over exploitation of the species from its natural habitat.

ACKNOWLEDGEMENT

The author is thankful for Mr. Jilani S.M and Dr Roopashree T S, Government Pharmacy College Bangalore India for providing HPTLC facility for analysis of the plant analysis.

REFERENCES

1. Nayagam R Justin. Compendium on *Costus pictus*. A medicinal spiral ginger, LAP, Lambert. Academic Publishing, Germany; 2015.
2. Sabu M. Zingiberaceae and Costaceae of South India. Indian Association of Angiosperm Taxonomy. Calicut. India; 2006.
3. Prakash K Hedge, Harini A Roa and Prasanna N Roa, A review on Insulin plant (*Costus igneus*). Pharmacognosy Reviews 2014; 8(15): 67-72.
4. Kirchoff and Rutishauser. The phyllotaxy of *Costus* (Costaceae). Botanical gazette, Volume 151. p. 88-105.
5. Kirchoff, B.K. Floral Ontogeny and evolution in the ginger group of the Zingiberales; 1988. p. 45-46.
6. Merina Benny. Insulin Plant in gardens. Natural product Radiance 2004; 3(5): 349-350.
7. K. Punya Pani, J.G Sharma. Micro propagation and micro-rhizome in *Costus pictus*. D. Don using *in-vitro* and *ex-vitro* nodal segments as explants. Noctulae Scientia Biologicae 2012; 4(2): 72-78.
8. Nandha Kumar. Anti-diabetic activity of Methanolic leaf extract of *Costus pictus* D. Don in alloxan induced Diabetics rats. Journal of Health sciences 2007; 53 (6): 655-663.
9. Jayasri MA, Gunasekaran S. Radha A. Mathew TL. Anti diabetic effect of *Costus pictus* leaves in normal and streptozotocin- induced diabetes rats. International .Journal of Diabetes and Metabolism 2008; 16: 117-122.
10. Joshi SG. Medicinal plants Oxford and IBH Publishing Co. Private Limited. Janapath New Delhi 190.
11. Saju Thomas D.R B Seetha Devi. Phytochemical and *in vitro* anthelmintic studies of hydro alcoholic extract of *Costus pictus* D. Don. International Journal of Pharmacy and Pharmaceutical sciences 2013; Vol 5 Issue 3.
12. Jeneth Berlin Raj, Rama Krishna Kalaivani. Comparative *in vitro* evaluation of anthelmintic property of leaves and rhizome of *Costus pictus* D. Don against albenzole. National Journal of Physiology, Pharmacy and Pharmacology 2016; 6(5): 438-441.
13. L. Joji Reddy and Beena Jose. Evaluation of antibacterial activity of the leaf essential oil *Costus pictus* D. Don from South India. International Journal of Current Pharmaceutical Research 2010; 2(3): 68-70.
14. Ramya R. Dhamotharan R. Antibacterial activity and screening of anti-bacterial compounds of *Costus pictus* D. Don using GC-MS. International Journal of Science and Research (IJSR) 2016; 5(6): 810-814.
15. Prejeena V, Suresh SN and Varsha V. Phytochemical screening, anti-oxidant analysis and anti-proliferative effect of *Costus pictus* D. Don leaf extracts. International Journal of Recent Advances in multi-disciplinary Research 2017; 4(03): 2373-2378.
16. Comargo MEM, Wajera C.R, Torres RS, Alderate MEC. Evaluation of the diuretic effect of the aqueous extract of *Costus pictus* D. Don in rat, Poc. West Pharmacol Soc 2006; 49: 72-74.
17. Nadumane VK, Rajashekar S, Narayana P, Adhinarayana S, Vijayan S, Prakash S *et al*. Evaluation of the anticancer potential of *Costus pictus* on fibro sarcoma (HT-1080) cell line, Journal of Natural Pharmacy 2011; 2: 72-76.
18. Malairaj Sathwan *et al. In vitro* anti-oxidant and Anti-cancer potential of Bark of *Costus pictus* D. Don; Asian pacific Journal of Tropical Biomedicine 2012: 741-749
19. Annie George, Thankamma, V.K Rema Devi and Annette Fernanddez. Phytochemical investigation of Insulin plant (*Costus pictus*). Asian Journal of Chemistry 2007; 19(5): 3427-3430.
20. Urmila Kumawat and Shraddha Shimpi. Pharmacognostic studies of the *Costus pictus*. D. Don; Journal of Herbal Medicine and Toxicology 2009; 3(1): 127-130.
21. Ajithadas Aruna, Ramaraj Nandhini Venkatachalam. Karthikeyan Pandi Bose, Shanmuganthan Jagadeesh, Kannappan Vijaya Lakshmi P. Insulin Plant (*Costus pictus*) leaves; Pharmacognostical standardization and phytochemical evaluation. American Journal of Pharmacy and Health Research 2014; 2(8): 107-119.
22. Remya R Daniel M. Phytochemical and Pharmacognostic investigation of anti diabetic *Costus pictus* D. Don. International Journal Pharmaceutical Biomedical Research 2013; 3 (1): 30-39.
23. Annadurai *et al*; Next generation sequencing and de-nova transcriptome analysis of *Costus pictus* D. Don a non model plant with potent anti-diabetic properties. BMC Genomics 2012; 13(663): 1-15.
24. Vijayasree N, Udayasri P, Aswani KY, Ravi BB, Phani KY, Vijay VM, Advancements in the production of secondary metabolites. Journal of Natural Products 2010; 3: 112-123.
25. Ramachandra SR, Ravi Shankar GA. Plant cell culture; Chemical factories of secondary metabolites. Biotechnology Advances 2002; 20: 1001-153.
26. Bhojwani SS and M K Razdan. Plant tissue culture, theory and Practice, Development in Crop Sciences 1996; volume 5, Elsevier Amsterdam.
27. Razdan MK. Introduction of plant tissue culture 2nd edition, Science Publisher's; Inc, New Hampshire; 2003. p. 132.
28. Nazarath Agrockiamary S, Vijaylakshmi K. Chromatographic separation of Bioactive compounds *Ipomea batatas* Lam (Sweet Potatoes) by column, High-Performance Thin Layer Chromatography and Gas Chromatography-Mass spectrum analysis techniques. Asian Journal of Pharmaceutical and Clinical Research 2014; 7(4): 4-8.
29. N Malliga Elangovan, MS Dhanarajan, I Elangovan. Preliminary phytochemical screening HPTLC fingerprinting profile of leaf extracts of *Moringa oleifera* and *Phyllanthus emblica*. International Research Journal of Pharmaceutical and Biosciences 2015; 2(2): 32-40.
30. Mythili Sathiavelu, Sathiavelu Arunachalam. High Performance Thin Layer Chromatography profile of *Cassytha filiformis*. Asian Pacific Journal of Tropical Biomedicine 2012: S1431-S1435.
31. World Health Organization. Quality control method for medicinal plant material. Geneva World Health Organization; 1998.
32. Murashige T and F Skoog. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiology plant 1962; 15: 473-497.
33. Gamborg, OL T. Murashige, T.A Thorpe and Vasil. Plant tissue Culture Media. Journal of the tissue culture association. 1962; 12: 473-478.
34. Harborne JB. Phytochemical methods. A Guide to Modern Techniques of Plant Analysis third edition; 1998, published by Chapman and Hall Publishers London U.K.
35. Trease G E, Evans WC. Pharmacognosy 15th Edition, London, Saunders Publishers; 2002.

36. K Punya Pani, JG Sharma. Micro propagation and micro-rhizome in *Costus pictus* D. Don using *in-vitro* and *ex-vitro* nodal segments as explants. Noctulae Scientia Biologicae 2012; 4 (2): 72-78.

Cite this article as:

Radha Devi G M. Comparative phytochemical analysis of *in-vivo* and *in-vitro* derived *Costus pictus* D. Don plants by HPTLC. Int. Res. J. Pharm. 2019;10(9):167-174 http://dx.doi.org/10.7897/2230-8407.1009277

Source of support: Nil, Conflict of interest: None Declared