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Original Article

Cytotoxic and Antioxidant Effects in Various Tissue Extracts of *Plumbago zeylanica*: Implications for Anticancer Potential

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ABSTRACT

Introduction: Complex natural products are emerging as a potent alternate and complement in anticancer therapies due to its multiple mechanisms of action and minimal effects on non-target cells/tissues. Some of the traditional herbal formulations contain *Plumbago zeylanica* L, (commonly known as Chitraka or Chitramoolam) to treat various disorders for centuries in Africa and Asia. **Methods:** The quantity of Plumbagin was detected in different tissues of *P. zeylanica* through HPTLC analysis. We evaluated and compared the alcoholic tissue extracts of leaf, stem and root of *P. zeylanica* with standard pure Plumbagin for its *in vitro* cytotoxic effects (metabolic viability; MTT assay) in A549 Lung cancer cells and antioxidant potential (DPPH free radical scavenging and total phenol content). RNA sequencing and transcriptome analysis was performed in the most potent tissues of *P. zeylanica* demonstrating efficient pharmacological activity. **Results:** The root extract containing 1.3% of plumbagin exhibited strongest scavenging potential with 50% radical scavenging (IC_{50} value of 3.99 µg) in comparison with 35.85 µg in stem (0.2% Plumbagin) and 18.06 µg in leaf with 0.00007% Plumbagin. The *in vitro* cytotoxicity in A549 cells also showed that the root extract was most potent with an IC50value of 164.5 µg as compared to extracts from stem (IC_{50}= 379.5 µg) and leaf (IC_{50}=274.9 µg). **Conclusion:** The strong antioxidant potential shown by the *P. zeylanica* extract demonstrates its ability to protect the non-target (normal) cells against oxidative stress, while the cytotoxic effects suggest that it could inhibit the growth of cancerous cells. Further, transcriptome analysis predicted the expression of potent gene transcripts coding for quinone biosynthesis in the root tissues of *P. zeylanica*.

Key words: Alcoholic Root Extract, A549 Cell Line, DPPH Assay, HPTLC Quantification, Plumbagin, Transcriptome Analysis.

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INTRODUCTION

Treatment of malignant diseases has remained a challenge to the medical community, despite advances in biomedical research, mainly due to refractoriness of tumors and limited normal tissue tolerance to therapies. Complementary and alternative therapies for the management of many diseases are spreading globally with increased use of herbal products in many countries. Recently, complex natural products have received more attention as anticancer agents, due to their potent pharmacological activity at low concentrations and minimal toxicity to normal cells.[1] *Plumbago zeylanica* Linn.(Chitraka or Chitramoolam), which belongs to the family Plumbaginaceae is associated with huge pharmacological potential to treat several diseases.[2,3] The roots of *P. zeylanica* L. have been traditionally used in the Indian system of medicine for many years and its phyto-constituents is known to possess anti-atherogenic, anticancer, cardiotonic, hepatoprotective and neuroprotective properties.[4,5] The leaf extract also exhibit anticancer, antibacterial, antifungal and anti-tumor properties and used to treat tuberculosis, rheumatic pain, syphilis and skin diseases.[6,7] Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) is the principle compound in *P.zeylanica* that has been shown to exert anticancer effects on many cancer cells.[8,9,10]

The biosynthesis and accumulation of secondary metabolites varies among populations, local races and even between different tissues of the same plant.[11,12] Huge variations in the pharmacological activity have been observed between stem, leaf, flower and root tissues of *Leucas aspera* which arise due to difference in the phytochemical constituents.[13] Similar spatial variations in phyto-constituents of medicinal plants have been observed in species of *Sorbus decora*[14]and in *Clinacanthus nutans*.[15] Hence, traditional system of medicine prefers to choose specific plant parts for mitigating different symptoms and treatment.[16,17] Therefore investigating the variation in the active principles enriched in different plant parts is an important criterion to understand chemical constituents responsible for pharmaceutical potential.

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The phytochemical and pharmacological activities of *P. zeylanica* have been studied extensively, revealing its ethano-botanical usage in traditional medicine.[3] However, the complete metabolite profile and the transcriptome analysis of this species has not been carried out to determine its pool of active gene transcripts involved in deciphering the pharmacological function of phytomedicine. In recent years, the functional genomics approach supported by targeted-metabolite and transcriptome profiling has been widely used as an efficient tool for deciphering novel gene functions in phytomedicine.[18] Owing to the rapid boom in cost effective high-throughput next generation sequencing technologies, a hot quest for sequencing the genomes and transcriptome of these life-saving medicinal plants have been instigated recently.[19] Nevertheless, large-scale bio prospection approaches in genomics and transcriptomics of phytomedicine is still its underway for exploring novel potentially important plant species.

In the present study, we investigated the antioxidant and anti-proliferative potential in different tissues of *P.zeylanica* and compared with pure Plumbagin molecule to identify the most potent tissue specific phyto-constituent exhibiting pharmacological activity for anticancer therapeutic potential. HPTLC quantification method was employed to assess the phytochemical activity through quantitative determination of the main bio-active compound plumbagin in stem, leaf and root extract of *P.zeylanica*. Further, we also carried out RNA sequencing and transcriptome analysis in the more potent tissue of *P. zeylanica* to predict potential pathways and secondary metabolite compounds with pharmacological function.

MATERIAL AND METHODS

Collection of plant material and authentication

P. zeylanica was obtained from the medicinal plant garden of Irula Tribe Womens Welfare Society (ITWWS) Thandarai, Chengalpet and authenticated by Prof. Jayaraman, Plant Anatomist Botanists, Plant Anatomy Research Centre, Tambaram (PARC/2016/3562). A voucher specimen was deposited at the Herbal and Indian medicine Research Laboratory, Sri Ramachandra University, Chennai. The stem, leaf and roots of the plant was washed thoroughly, air dried, and ground into fine powder.

Preparation of extracts

Extraction of root, stem and leaf (5g) involved cold maceration with ethanol for 24h and the filtrate was collected. It was concentrated in a rotary vacuum evaporator at 60°C.The percentage yield of stem, leaf and root was 0.44g w/w, 0.45g w/w and 0.3g w/w respectively.

Preparation of Plumbagin Standard:

Standard Plumbagin (97% purity) was purchased from Sigma-Aldrich, USA.

Estimation of total phenolic content in extracts

The total phenolic content in the extracts were estimated using the Folin-Ciocalteu method (Yu *et al*., 2002). Ethanolic extract of stem, leaf and root extract (100, 200µg/mL) were mixed with 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The final mixture was incubated at 45°C for 40 min and the absorbance was measured at 765 nm in a multi-mode reader (PerkinElmer Enspire, USA). The total phenolic content is expressed as gallic acid equivalent and experiments were assayed in triplicate.

Quantitative determination of Plumbagin through HPTLC analysis

Plumbagin content was estimated in ethanolic extracts of root, stem and leaf using Camag HPTLC system (CAMAG Scientific Inc, USA). The sample solutions were spotted as bands on pre-coated silica gel G 60 F254 (20cm×10cm with 250µm thickness, E. Merck) with Hamilton 100µl syringe plate on Camag Linomat-V applicator. The slit dimension was 6.00mm×0.45mm wherein the linear ascending development was performed in 20cm×10cm, Camag twin trough glass chamber saturated with mobile phase containing toluene: ethyl acetate (9.5:0.5) at room temperature. The mobile phase was developed by trial and error method. After the plates were air-dried, photo-documentation was performed using CAMAG REPROSTAR 3at 265nm. HPTLC plate was scanned using Camag TLC Scanner-III at 419 nm. A calibration plot was generated using the area values of peak and subjected to linear regression analysis. The extracts were spiked with the known amount of plumbagin to ascertain the presence of standard compound.

Antioxidant Potential in different tissues

The antioxidant activity of the extracts was evaluated against diphenyl-2-picrylhydrazyl (DPPH) free radicals as per method given by Srirama *et al*., 2012.[20] Different concentrations (3.65- 500µg in 20µl of ethanol) were prepared and mixed with (180µl) 50 µM DPPH. Plumbagin in different concentration (0.83-100µM) was used as the reference-standard. The reaction mixture was incubated for 30 minutes in dark at 37°C, and the absorption was measured at 517 nm using the multi mode reader (PerkinElmer Enspire, USA). The free radical scavenging activity (RSA) was measured as a percentage of DPPH discoloration with the formula:

% RSA = [(Ac–As)/Ac] ×100; where RSA: radical scavenging activity; Ac: absorbance of the negative control; As: absorbance of the plant sample.

In vitro cytotoxic activity assay

The effect of *P.zeylanica* extracts on A549 (human lung carcinoma cell line) was determined by the MTT assay.[21] A549 cell line was obtained from National centre for Cell Science, Pune and cultured in Dulbeccos Modified Eagle Medium (DMEM) with 10% Foetal Bovine Serum (FBS) supplemented with penicillin (5units/mL), streptomycin (5µg/mL). Cells ($1x10^3$cells) were seeded in96-well plate. After 80% confluence, cells were incubated with different concentrations of root, stem, leaf (15.62 to 1000 µg/ml) and plumbagin (0.83 to 100µM) for 24h.After 24h treatment, 50 µL of MTT indicator (5 mg/mL in PBS) was added to each well and incubated at 37°C for 3h. Subsequently, 150µL of DMSO was added to solubilize the purple coloured formazan crystals. The optical density was measured at 570nm using a microplate reader (PerkinElmer Enspire, USA). The IC_{50} values of test compounds were determined using Graph Pad Prism v6.0 (GraphPad, San Diego, CA).

RNA sequencing and Transcriptome analysis

Total RNA was isolated from fresh root tissues of *P. zeylancia* plant using Spectrum Plant Total RNA isolation kit (Sigma-Aldrich, USA) according to manufacturer's instructions. The quantitative and qualitative analysis of the RNA samples was performed using Nanodrop 2000 and Qubit Fluorometer (Thermo Fisher Scientific, Wilmington, DE). RNA library preparation and sequencing was performed at Genotypic Technology's Genomics facility as per Ultra Directional Library Prep Kit protocol for Illumina Multiplexed Sequencing (New England Biolabs, UK). The obtained root RNA library was sequenced using 150bp Paired-end chemistry in NextSeq 500 sequencing platform (Illumina, USA). De-novo transcriptome assembly was performed using Trinity 2.0 (default k-mers i.e 25. Trinity). Functional Annotation for clustered transcripts were analysed by the homology search against Viridiplantae dataset of the Uniprot database using BLAST-2.5.03. Pathway analysis was done using KAAS 4 Server of KEGG database to derive the secondary metabolite pathway and predict the gene transcripts involved in their biosynthesis.

Statistical analysis

All the experiments on antioxidant and cytotoxicity assay were performed in triplicate and the results were expressed as mean ± S.D. Analysis of variance (ANOVA) was used to calculate the statistical significance of

differences between groups, followed by Bonferroni test for multiple comparisons among groups using GraphPad Prism v.6.[22] P<0.05 were considered as statistically significant.

RESULTS

Total Phenolic content in extracts

The total phenolic content was determined in ethanolic extracts of *P.zeylanica* and expressed as gallic acid equivalence in 100gm of the tissue extract. The phenolic content in root extract was found to be significantly higher (17.5%w/w) in comparison to stem (2.09%w/w) and leaf tissues (1.39%w/w).The high phenolic content in the extracts of *P.zeylanica* is suggestive of its antioxidant potential.

HPTLC quantification of Plumbagin in tissue extracts

Plumbagin is one of the well-known bio-active components in *P. zeylanica* [8] and its spectral characteristics was analyzed at 265 nm and 419 nm (data not shown). Plumbagin was quantified in root, stem and leaf extracts using HPTLC where strong peak for plumbagin was observed at 419 nm. Under the separation conditions used and visualized at 265 nm, standard Plumbagin was eluted with a retention factor 0.65±0.02 (Figure 1A) and showed a good linearity (R^2=0.995) in the concentrations of 400–1000 ng/spot. The densiometric scanning followed by peak integration at wavelength of 419nm showed the presence of plumbagin at the same R_f value (R_f = 0.65) in all the three extracts of leaf, stem and root of *P.zeylanica* as shown in Figure 1B. The quantitation analysis revealed the amount of plumbagin in root, stem, leaf extracts as 1.3%w/w, 0.2%w/w and 0.00007%w/w respectively. In the present study, the maximum quantity of plumbagin was observed in the root extract (1.3% w/w), which is in agreement with the values reported earlier in *P.zeylanica.* [23,24] The amount of plumbagin increased proportionately in the extracts spiked with plumbagin, which further confirmed the presence of plumbagin in all the three tissue extracts. The quantification result revealed the tissue dependent variation in the concentration of plumbagin of *P.zeylanica,* which in turn could influence the pharmacological activity.[25] The quantification results also suggest that quantity of plumbagin in aerial part is contributed maximum by the stem tissue of *P. zeylanica* (0.2%).

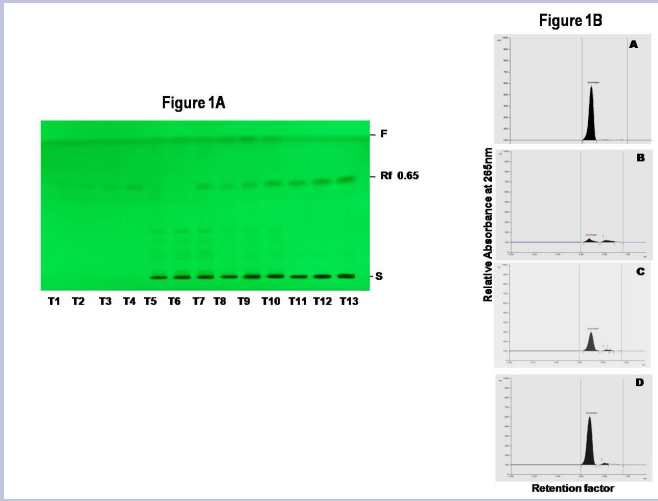

Figure 1: A. HPTLC fingerprint for Standard Plumbagin and ethanolic tissue extracts of *P. zeylanica*. Tracks: T1-T4 Plumbagin (400,600,800,1000 ng); T5-T6 Leaf extract (200, 300 µg); T7 leaf extract spiked with Plumbagin (300µg +100µg); T8-T9 stem extract (200,300 µg); T10 stem extract spiked with plumbagin (300µg +100µg); T11-T12 root extract (60, 90µg) ; T13 root extract spiked with Plumbagin (90µg+100µg). Figure 1B. HPTLC densitogram profiles showing Rf values at 0.65 for Plumbagin in A. Standard Plumbagin B. Leaf . Stem D. Root

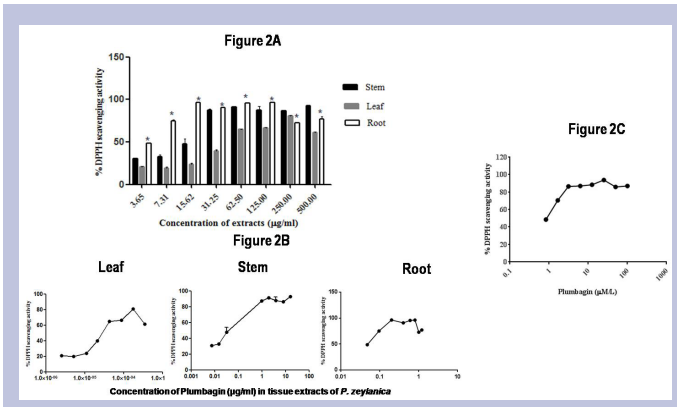

Figure 2: A. Free Radical scavenging activity in different total tissue extracts of *P. zeylanica* measured using DPPH assay. % scavenging activity values are represented as Mean ± 1SD (n=3). (*P <0.001 as compared to leaf vs root). Figure 2B. Antioxidant potential of the extracts of *P. zeylanica* plotted as a function of Plumbagin content in the extracts. Figure 2C. Free Radical scavenging activity of pure Plumbagin

DPPH radical scavenging assay

The antioxidant capacity of plant extract depends on the hydrogen donating ability of phenols and flavonoids present.[26] In the present study, all the three ethanolic extracts of *P.zeylanica* showed dose-dependent scavenging of free radicals. The total tissue extract showed a significant antioxidant (P<0.05) activity, wherein ethanolic root extract exhibited highest free radical scavenging activity (IC50 of 3.99µg/ml) in comparison with stem (IC50= 18.06µg/ml) and leaf (IC50= 35.85µg/ml) as illustrated in Figure 2A. These radical scavenging potential of the total extract was normalized with the quantity of plumbagin in extract and are plotted as function of plumbagin concentration of *P.zeylanica* (Figure 2B). The root extract containing 1.3%w/w plumbagin exhibited the antioxidant activity (IC50=0.08µg) and stem extract (0.2%w/w plumbagin) showed IC50 value of 0.05µg, while leaf extract demonstrated IC50 value of 0.000003µg. The standard pure plumbagin revealed strong antioxidant potential with an IC50 value of 0.92µM i.e. 0.16µg/ml as shown in **Figure 2C**.

The root extract with maximum phenolic content (17.5%) showed significant antioxidant activity which prompted to carry out RNA sequencing in root tissues of *P. zeylanica.* Transcriptome analysis revealed some of the gene transcripts reported for antioxidant potential are found expressing in root tissues of *P.zeylanica*. Some of the specific antioxidant enzymes namely Catalase, Superoxide dismutase and Peroxidases have been identified in root tissues of *P. zeylanica*. The functional annotation of transcripts using COG classification revealed the transcripts of the enzymes with their unique protein ID for Catalase [Cgl0256], Catalase (peroxidase I) [RSc0776], Glutathione peroxidase [RSc2674], Superoxide dismutase [SPAC1486.01] and Cu/Zn superoxide dismutase [Rv0432] as listed in Table 1. Preliminary analysis of the root transcriptome carried out here illustrates only the antioxidant transcripts expression in root tissues of *P. zeylanica*. However, the detailed analysis of these antioxidant enzymes during defense against target cell need to be explored in detail to understand the translationary effect of the expressed enzyme transcripts for antioxidant potential.

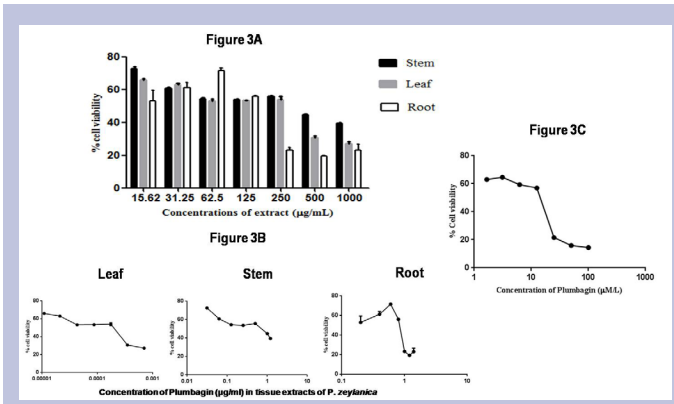

Figure 3: A. Cytotoxic effects of total tissue extracts of *P. zeylanica* studied using MTT assay. Values plotted are mean ± 1SD (n=3). Figure 3B. Cytotoxic effects of the extracts of *P. zeylanica* plotted as a function of Plumbagin content in the extracts. Values plotted are mean ± 1SD (n=3). Figure 3C. Cytotoxic effects of pure Plumbagin

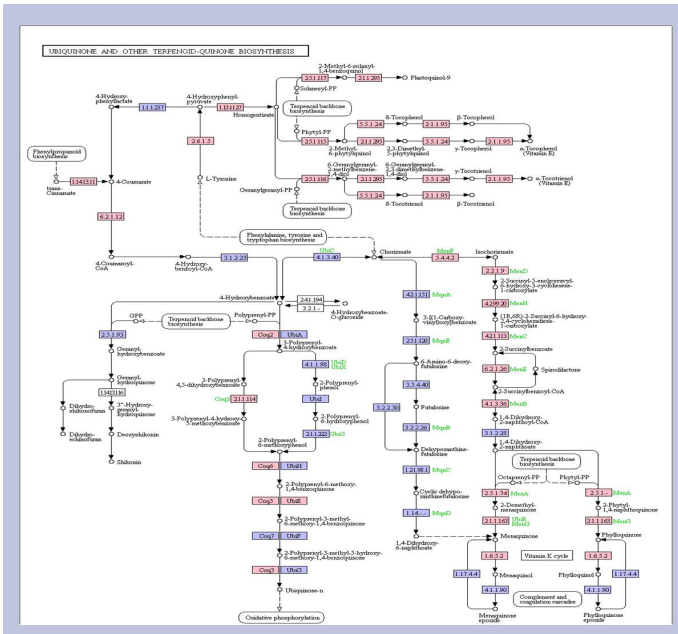

Figure 4: Terpenoid Quinone Pathway Map derived from root transcriptome of *P. zeylanica* using KAAS 4. The pathway map without color codes represents the original biosynthesis pathway obtained using KegSketch tool of KAAS database. Blue boxes represent reference pathway (KO) hyperlinked to KO entries selected from the original pathway in KEGG database and green colored genes represent organism specific pathway indicating the presence of genes in the transcriptome of P. zeylanica

Cytotoxic activity

P.zeylanica tissue extracts produced cytotoxic effect with IC50 values of 379.50µg for stem, 274.97µg for leaf and 164.5µg for root tissues on A549 human lung carcinoma cells (Figure 3A). The ethanolic root extract demonstrated more potent inhibition and dose-dependent reduction in the mitotic activity on A549 cells at <200µg/ml (50% inhibitory concentrations) in comparison to stem and leaf extract. The maximum cytotoxic effect exhibited by *P.zeylanica* root extract with high plumbagin (1.3%) on A549 cells clearly substantiates anticancer properties of plumbagin as reported in other cancer cell lines.[27,28] Comparative analysis on the cytotoxic effect normalized to plumbagin content in all the three extract predicted IC50 values for stem extract at 0.70µg, leaf extract at 0.00023µg and 0.20 µg in root extract as indicated in Table 2 (Figure 3B). The standard Plumbagin revealed 50% inhibitory concentration at 13.64µM i.e.,2.56 µg and exhibited similar plateau effect as in root tissue at higher concentration (Figure 3C) indicating that it is necessary to determine optimum concentration of plumbagin for evaluating cytotoxicity.

RNA sequencing Analysis in root tissues of P.zeylanica

Paired end sequencing of the root cDNA library using Illumina Next-Seq 500, generated 19 million reads with average length of 150 bp. De-novo transcriptome assembly and clustering resulted in 1,18,365 unique transcripts, out of which 67570 transcripts could be annotated. In total, 57% of transcripts were functionally annotated using BLAST-2.5.03 at high confidence level (e1-5). These transcripts were subjected to pathway analysis using KAAS 4 database. Eudicot plants in the KAAS 4 server were considered as reference organisms for pathway identification. From the root transcriptome of *P. zeylanica*, we have identified distinct secondary metabolite pathway genes mapped to the reference pathway in KAAS database. Many secondary metabolite pathways have been derived, out of which terpenoid-quinone biosynthesis pathway is shown in Figure 4 that represent enzymes and transcripts involved in phenyl propanoid and quinine metabolism. Plumbagin, a naphthoquinone is one of the important secondary metabolites in *P. zeylanica,* synthesized in the roots and demonstrate wider pharmacological activity.[6] The quinone pathway in plants is also reported to have role in antioxidant defense.[29] The colored codes on the pathway map have been computationally generated, where white boxes are hyperlinked to KO, ENZYME, and REACTION entries in metabolic pathways. The enzymes marked green in the terpenoid-quinone pathway map (Figure 4) represents genes (MenA-MenH and UbiE) involved in the biosynthesis of naphthoquninone. Menaquinone epoxide and Phylloquinone epoxide are some of the key gene transcripts identified that leads to metabolism of terpenoids and polyketides, which are reported to be unique to the plant specific secondary metabolite biosynthesis pathway.[30]

DISCUSSION

It is well established that among the composition of phyto-constituents, secondary metabolites of the plants play a vital role in determining the bio-efficacy of medicinal plants.[31,6] Further, the isolation of secondary metabolites are known to vary with the extraction process and tissue parts of the plants.[12,13] Comparative phytochemical evaluation in different tissue extracts of *P.zeylanica* revealed that the quantity of phyto-constituents varied in different parts of the plant (Figure 1A and Table 2). In this study, quantification of plumbagin in three distinct tissues of *P.zeylanica* by HPTLC revealed that root extract contain maximum quantity, in comparison to leaf and stem extract. However, this finding is at variance with the previous report, where a high plumbagin content was found in aerial part as compared to the root of *P.zeylanica,* while tissues were not discriminated clearly.[17] This discrepancy in the quantity of plumbagin could also be due to the difference in the adopted method, where the earlier study relied on estimating plumbagin through spectral absorbance (520 nm) while the present study used HPTLC quantification to resolve plumbagin precisely (419 nm).

In the present study, when we compare antioxidant potential in total tissue extracts with respective plumbagin content in extract, we could observe that plumbagin is not the sole determinant for imparting antioxidant potential in tissues of *P.zeylanica.* This could also be substantiated by the HPTLC quantification analysis where other phyto-constituents could be observed in leaf and stem tissue as shown in Figure 1A. The

Table 1: Antioxidant enzyme transcripts identified in root transcriptome of *P.zeylanica*

Gene transcripts	Protein Id	Identity with Reference Organism	Function Reported
Catalase	Cgl0256	51% Corynebacterium glutamicum	Inorganic ion transport and metabolism
Catalase (peroxidase I)	RSc0776	65.38% Ralstonia solanacearum	Inorganic ion transport and metabolism
Glutathione peroxidase	RSc2674	58% Ralstonia solanacearum	Post-translational modification, protein turnover, chaperones
Superoxide dismutase	SPAC1486.01	62.02% Corynebacterium glutamicum	Inorganic ion transport and metabolism
Cu/Zn superoxide dismutase	Rv0432	69.33%Saccharomyces cerevisiae	Inorganic ion transport and metabolism

Table 2: Comparative Analysis of estimated Phyto-constituents and bio-efficacy evaluated in different tissue extracts of *P. zeylanica*

Tissues	Total Phenolic content (Gallic acid equivalent)	Quantity of Plumbagin	IC_{50} for DPPH activity		IC50 for cytotoxicity	
			Extracts (µg/mL)	Plumbagin content in extract (µg/mL)	Extracts (µg/mL)	Plumbagin content in extract (µg/mL)
Root	17.5%	1.3%	3.99	0.08	164.5	0.20
Stem	2.09%	0.2%	35.85	0.05	379.50	0.70
Leaf	1.39%	0.00007%	18.06	0.000003	274.97	0.0002
Standard Plumbagin			0.16 µg		2.56µg	

results suggest that besides plumbagin, other phyto-constituents also play a vital role equally if not more to exert the bio-efficacy as observed in leaf tissues with negligible plumbagin (0.00007%). Although the total root extract contain maximum plumbagin and exhibited high anti-oxidant potential but the normalized plumbagin content in the root extract showed less scavenging activity. These differences suggest that other phyto-constituents in root (17.5% total phenolic compounds) might play a vital role in exerting the free radical scavenging activity.

Earlier studies have indeed shown that plumbagin in high concentration could be toxic to normal lymphocytes, whereas the ethanolic root extracts of *Plumbago rosea* possessing associated phyto-constituents in addition to plumbagin exhibited synergistic stronger anticancer effect with less toxicity.[32] Intriguingly, the cytotoxicity exhibited by leaf extract normalized with plumbagin content was multi-folds higher in *P.zeylanica* indicating that other active phyto-constituent in leaf might have synergistic action or modulatory role to exhibit desired therapeutic properties. The selective toxicity of leaf extracts in normal cells needs to be investigated further to ascertain its anticancer potential with less side effects.

Plant extracts containing phenolic compounds could exhibit antioxidant activity as well as anticancer properties.[33,34] At higher concentrations, phenolic compounds might act as pro-oxidants and alter the redox balance of tumor cells, thereby inducing apoptosis and cytotoxicity. [35] In line with this, the total root extract of *P.zeylanica* at low concentrations exhibited high antioxidant activity with an IC50 value of 3.99µg/mL, while it was clearly cytotoxic at higher concentrations, with an IC50 value of 164.5µg/mL, which correlated well with the higher level of phenolic compounds found in the root (17.5%).

Plumbagin, is one of the naphthoquinones synthesized and accumulated in the root tissue of *P. zeylanica*,[36] which is partly responsible for the multiple biological effects. The anticancer potential of Plumbagin has been demonstrated in some of the human cancer cell lines.[37,9,10] Since the root tissue of *P. zeylanica* exhibited strong antioxidant and cytotxic effects compared to other tissues, it prompted us to study the quinone biosynthesis pathway to identify naphthoquinones expressed in root tissue of *P. zeylanica* (Figure 4). Interestingly, the candidate gene transcripts of terpenoids and quinines identified in root transcriptome were found to be involved in naphthoquinone biosynthesis, which is one of the important secondary metabolite in root tissues.[38] Hence, further analysis on the other secondary metabolite pathways derived from the root transcriptome could help to bring out some of the co-expressed candidate gene transcripts necessary for biosynthesis of naphthoquinones in *P.zeylanica.*

CONCLUSION

The strong antioxidant potential shown by the tissue extracts of *P. zeylanica* demonstrates its ability to protect the non-target (normal) cells against oxidative stress, while the cytotoxic effects suggest that it could inhibit the growth of cancerous cells. These effects exhibited by total leaf, stem and root extract of *P.zeylanica* also indicates the presence of more active lead compounds in addition to active principle plumbagin for demonstrating wider pharmacological activity. Hence, the identification of other bioactive compounds in leaf and stem might reveal therapeutic effects similar to plumbagin and/or augment the action of plumbagin in a synergistic manner. Hence, it suggest that extensive work on more number of tumor cell line, selective toxicity studies and *in vivo* experiments could help to bring out the application of *P.zeylanica* tissue extracts for anticancer therapies.

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CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

ABBREVIATION USED

DPPH: 2,2-diphenyl-1-picrylhydrazyl; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **IC_{50}:** half maximal inhibitory concentration; **HPTLC:** High Performance Thin Layer Chromatography; **DMSO:** Dimethyl sulfoxide; **COG:** Clusters of Orthologous Groups; **KAAS:** KEGG Automatic Annotation Server.

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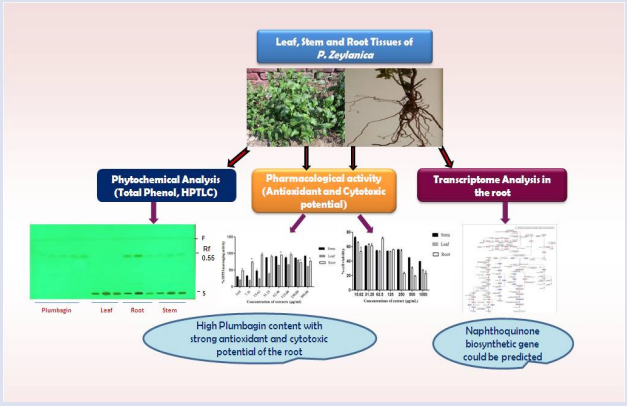
GRAPHICAL ABSTRACT



HIGHLIGHTS OF PAPER

- The ethanolic root extract of *P. zeylanica* with high plumbagin content demonstrated maximum antioxidant and cytotoxic potential
- The strong antioxidant potential shown by the tissue extracts of *P. zeylanica* demonstrates its ability to protect the non-target (normal) cells against oxidative stress, while the cytotoxic effects suggest that it could inhibit the growth of cancerous cells.
- Bio-efficacy of leaf tissue plotted as function of Plumbagin content revealed maximum pharmacological activity suggesting synergistic action of other bio-active molecules
- Preliminary analysis on the quinone pathway derived from the root transcriptome predicted gene transcripts for biosynthesis of naphthoquinone.

AUTHOR PROFILE

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Prof. S.P. Thyagarajan: Prof. Thyagarajan, was the Vice-Chancellor of the University of Madras during 2003-2006, and is currently Professor of Eminence &Dean (Research) at Sri Ramachandra University, Chennai. He has been conferred with several Fellowship titles including those by National Academy of Sciences and National Academy of Medical Sciences. He has 46 years of teaching and research experience and successfully completed 60 research projects funded by national and inter-national funding agencies. He has published 340 research papers and 16 books in the area of infectious diseases and Drug development from Natural products; He serves as member of several expert committees of UGC, ICMR, DST.,DBT, DOD, Ministry of Health, WHO etc., He has guided 32 candidates for their Ph.D. He is the inventor of a patented drug for the treatment of Chronic jaundice, called Hepatitis-B from the Indian medicinal plant, *Phyllanthus amarus*, [Kizhanelli in Tamil], which has already been marketed by an University-Industry agreement under the brand name "VIROHEP" by Rallis India Pharmaceuticals/ Shreya Life Sciences, Mumbai.

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