

Spectroscopic profile as pharmacognostic criteria to distinguish the medicinally important *Plumbago* Species

Renisheya Joy Jeba Malar Tharmaraj¹, Johnson Marimuthu Antonysamy^{1*}

¹Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India

* Author for Correspondance: ptjohnson@gmail.com, Tel: +919786924334, Fax: +914622561765

Abstract

The root of the *Plumbago* species were examined phytochemically and their potentials are well studied. The spectrometric and chromatographic screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. Objective: The present study was aimed to reveal the inter-specific similarity and variation among the selected *Plumbago* species viz., *Plumbago zeylanica* Linn, *Plumbago auriculata* Lam, *Plumbago rosea* Linn. using UV-VIS and FTIR profiles. Methods: To know the various phytoconstituents and functional groups and similarity and variation present in the *Plumbago* species aerial parts, the UV-VIS spectroscopic analysis and FTIR analysis was carried out using Shimadzu and Perkin Elmer spectrophotometer respectively. Result: The UV-VIS spectroscopic analysis revealed the presence of various functional compounds and groups and similarity and variation in the aerial parts of studied *Plumbago* species. The FTIR analysis identified various functional groups like alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines presence in the crude powder of *Plumbago* species aerial parts. Conclusion: The results of the present study paved a way to predict and compare the phytoconstituents presence in all the three selected *Plumbago* species which can be used for further characterization. In addition it provides the pharmacognostical marker to distinguish the medicinally important *Plumbago* species using relatively simple, cost-effective spectroscopic profile.

Keywords

Plumbago zeylanica; *Plumbago auriculata*; *Plumbago rosea*; UV-Vis; FTIR.

Introduction

Plants fabricate a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compound, have sustained to play a prevailing role in the maintenance of human health since ancient times [1]. In pharmacognosy, the phytochemical assessment is one of the important and vital tools for quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques such as fluorescence, UV-VIS, FT-IR, HPLC, HPTLC and GC-MS.

The spectrometric and chromatographic screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [2]. However, the determinations of phytoconstituents performed by relatively simple, cost-effective and rapid tests for detecting phytocomponents are necessary. Spectroscopic (UV-Vis, FTIR) methods together or separate can be used in this sense as well as predictable methods [3]. Fourier Transform Infrared Spectroscopy (FTIR) is a rapid, non invasive, high-resolution analytical tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular

fingerprint [4]. It has been shown to be a valuable tool for differentiating and discriminating closely related microbial strains, plants and other organism [5-8].

Spectroscopic methods have become firmly established as a key technological podium for secondary metabolite profiling in both plant and other species [9,10]. *Plumbago zeylanica* is usually known as white chitraka, belongs to family Plumbaginaceae. *P. zeylanica* is a perennial sub-scandent shrub, grows all over India, especially in moist places [11]. *P. zeylanica* has been used by rustic and tribal people since hundreds of years as a traditional system of medicine [12]. The word comes from two Latin words *plumbum* (lead) and *agere* (to resemble), a 'leadlike ore', alluding to historical use as a cure for lead poisoning [13]. The whole plant and its roots have been used as a folk medicine for the treatment of rheumatic pain, dysmenorrheal, carbuncles, and contusion of the ulcers, extremities and elimination of intestinal parasites [14]. Chemical examination of *P. zeylanica* exposed that the root contains plumbagin, 3-chloroplumbagin, 2,3-biplumbagin, 6,6-biplumbagin, isozeylinone, zeylinone, chitranone, droserone, plumbagic acid, plumbazeylanone, glucose, fructose, enzymes as invertase and protease. The leaves and stem contains little or no plumbagin. The aerial parts

contain naphthoquinones, lupeol, lupenylacetate, sitosterol and amino acids [15]. *Plumbago capensis* Thumb. Syn. *P. auriculata* Lam. (Plumbaginaceae) is a small sub-scandent shrub with quadrilateral spatulate leaves and pale blue flowers, native to South Africa and grown in gardens in India as a decorative plant [16]. A decoction of the plant is taken as an antidote for black water fever. The powdered root is taken as a snuff to relieve headache [17]. The root of the *Plumbago rosea* contains plumbagin which stimulates the central nervous system. The plant is able to develop the digestive power and endorse appetite. It also has antiseptic property. The juice of the *P. rosea* leaves oil was used to cure rheumatism and paralysis. It contains plumbagin (2-Methoxy-5-hydroxy-1-4-Napthoquinone), which is a natural naphthoquinone possessing a mixture of pharmacological activities such as antimalarial and antimicrobial [18-19]. However, there is no report on the spectroscopic study on the aerial parts of *Plumbago* species (*Plumbago zeylanica* L., *Plumbago auriculata* Lam, *Plumbago rosea* Linn.). With this background the present study was aimed to study the inter-specific similarity and variation between the selected three *Plumbago* species using spectroscopic profile.

Materials and Methods

Sample collection

The aerial parts of *Plumbago* species (*P. zeylanica*, *P. auriculata* and *P. rosea*) were collected from natural habitats at Tenkasi (Tamil Nadu), Kerala and Mysore, India respectively. The collected samples were washed in the running tap water for 5 min and the samples were dried and powdered leaves (50 g) were extracted successively with 300 mL of petroleum ether, chloroform, acetone, ethyl acetate, ethanolic and aqueous by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuum at 40°C using rotary evaporator.

UV-Vis analysis

To know the functional groups and metabolites presence and similarity and variation present in the crude extract of selected *Plumbago* species the UV-Vis analysis was carried out using Shimadzu spectrophotometer [20]. The extracts were examined under visible and UV light for proximate analysis. For UV-VIS spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 (1 mg/10 ml) with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm and the characteristic peaks were detected. The UV-Vis analysis was repeated twice and confirmed the spectrum. The peak values of UV-Vis were used to distinguish the selected *Plumbago* species. To reveal the *inter-specific* similarities among the selected *Plumbago* species, the UV-VIS spectral profile was converted in to "1" and "0" matrix,

to indicate the presence or absence of peak values respectively. UV-VIS Spectral similarities (GS) were estimated according to Nei and Li [21]. To demonstrate the inter-specific relationship, a cladogram was constructed by UPGMA using NTSYSpc- 2.0 software.

FTIR analysis

To know the functional groups presence, the FTIR analysis was performed using IR spectrophotometer (Shimadzu 8400S) system. Infrared spectra of the test plants were measured using the methods cited in Harborne [22]. About 1.0 mg of the three plant powders were separately made into thin discs with 10-100mg of potassium bromide using a mould and press under anhydrous conditions. It was then measured in an automatic recording IR spectrophotometer (Shimadzu 8400S) in the range of 667 to 4000 cm^{-1} . The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum. To reveal the interspecific similarities among the studied *Plumbago* species, the FTIR profile was converted in to "1" and "0" matrix, to indicate the presence or absence of peak values, respectively. FTIR similarities (GS) were estimated according to Nei and Li [21]. To demonstrate the *inter-specific* relationship among the selected *Plumbago* species, a cladogram was constructed by UPGMA using NTSYSpc- 2.0 software.

Results

The qualitative UV-VIS fingerprint profile of extracts of *Plumbago* species were selected at wavelength from 200 to 1100 nm due to sharpness of the peaks and proper baseline (Fig. 1-3).

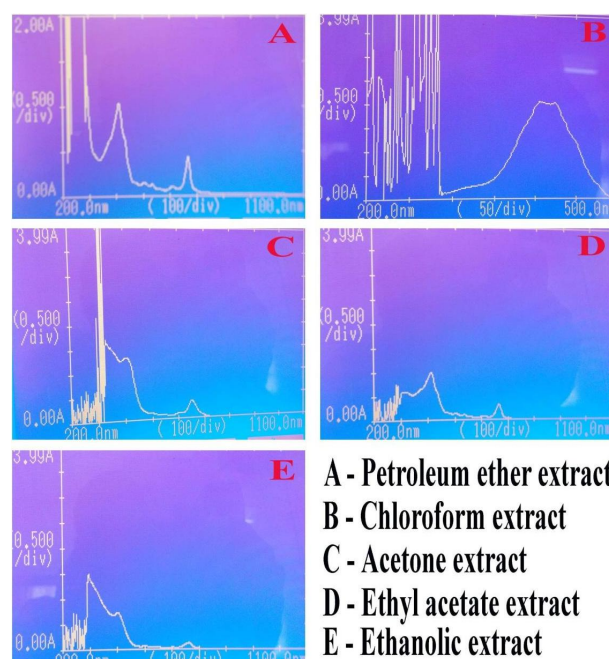


Fig 1 UV- Vis Spectrum of *Plumbago zeylanica*

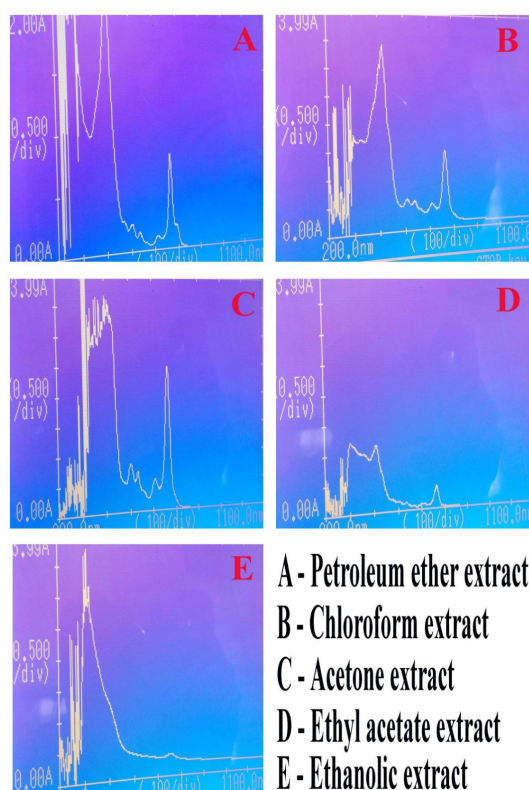


Fig 2 UV- Vis Spectrum of *Plumbago auriculata*

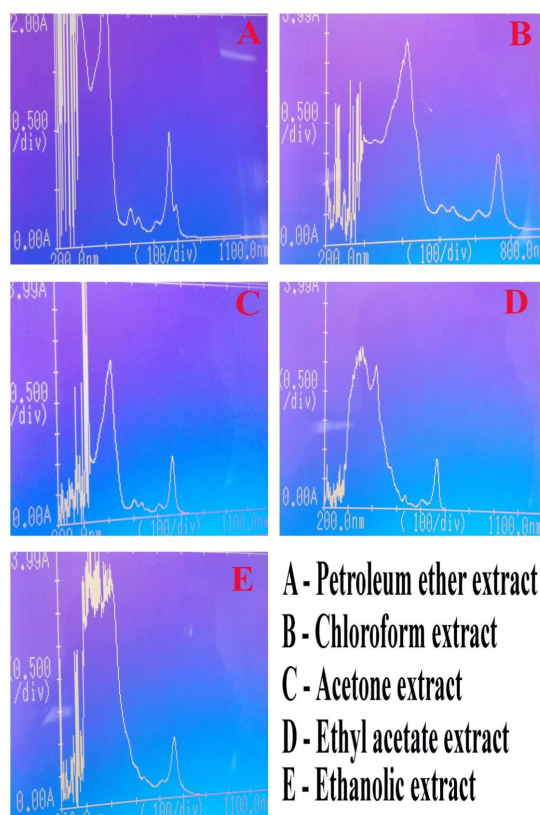


Fig 3 UV- Vis Spectrum of *Plumbago rosea*

The profile showed the peaks at the range of 300 - 1100 nm with the absorption range of 0.015 - 3.678 respectively. The results of UV-vis analysis revealed the similarity and variation between the studied species. The petroleum ether extract profile

of *P. zeylanica* showed the metabolites and functional group presences in the nm of 668, 608, 533, 505 and 409 with the absorption of 0.437, 0.109, 0.138, 0.155 and 1.037 (Fig. 1 A). The chloroform extract profile of *P. zeylanica* showed the metabolites and functional groups presence in the nm of 1091, 1021, 952, 667, 608, 538, 505 and 416 with the absorption of 0.059, 0.062, 0.065, 1.022, 0.29, 0.354, 0.392 and 2.659 respectively (Fig. 1 B). The acetone extract profile of *P. zeylanica* showed the metabolites and functional group occurrence in the nm of 651, 604, 532 and 403 with the absorption of 0.381, 0.124, 0.107 and 1.336 respectively (Fig. 1 C).

The ethyl acetate extract profile of *P. zeylanica* showed the metabolites and functional group presence in the nm of 665, 607, 534, 504, 410 and 319 with the absorption of 0.334, 0.088, 0.118, 0.139, 1.019 and 0.611 respectively (Fig. 1 D). The ethanolic extract profile of *P. zeylanica* showed the metabolites and functional group presence in the nm of 976, 662, 605, 532, 502, 401 and 295 with the absorption of 0.011, 0.136, 0.046, 0.56, 0.073, 0.741 and 1.497 respectively (Fig. 1 E). The aqueous extract profile of *P. zeylanica* does not show any peak detection.

The petroleum ether extract profile of *P. auriculata* showed the metabolites and functional group presence in the nm of 669, 611, 533, 502 and 407 with the absorption of 0.818, 0.121, 0.163, 0.238 and 2.237 (Fig. 2 A). The chloroform extract profile of *P. auriculata* showed the metabolites and functional group presence in the nm of 1052, 929, 667, 609, 538, 507 and 415 with the absorption of 0.119, 0.126, 1.455, 0.482, 0.556, 0.59 and 3.436 respectively (Fig. 2 B). The acetone extract profile of *P. auriculata* showed the metabolites and functional group presence in the nm of 1021, 665, 606, 534, 503 and 400 with the absorption of 0.012, 2.48, 0.550, 0.690, 0.829 and 3.675, respectively (Fig. 2 C). The ethyl acetate extract profile of *P. auriculata* showed the metabolites and functional group occurrence in the nm of 665, 606, 534, 410 and 303 with the absorption of 0.014, 0.390, 0.110, 0.144, 1.155 and 1.202 respectively (Fig. 2 D). The ethanolic extract profile of *P. auriculata* showed the metabolites and functional group presence in the nm of 1086, 1009, 872 and 656 with the absorption of 0.21, 0.222, 0.241 and 0.38 respectively (Fig. 2 E). The aqueous extract profile of *P. auriculata* does not show any peak detection in aqueous extract.

The petroleum ether extract profile of *P. rosea* showed the metabolites and functional group presence in the nm of 1022, 695, 669, 610, 533, 503, and 407 with the absorption of 0.022, 0.329, 0.971, 0.193, 0.231, 0.311 and 2.466 respectively (Fig. 3 A). The chloroform extract profile of *P. rosea* showed

the metabolites and functional group presence in the nm of 1022, 992, 666, 605, 503 and 415 with the absorption of 0.117, 0.118, 0.384, 0.259, 0.334 and 1.110 respectively (Fig. 3 B). The acetone extract profile of *P. rosea* showed the metabolites and functional group presence in the nm of 1020, 1005, 665, 606, 533, 504 and 411 with the absorption of 0.014, 0.013, 1.020, 0.220, 0.271, 0.321 and 2.683

respectively (Fig. 3 C). The ethyl acetate extract profile of *P. rosea* showed the metabolites and functional group occurrence in the nm of 666, 607, 558, 411 and 354 with the absorption of 0.879, 0.178, 0.099 and 2.451 respectively (Fig. 3 D). The ethanolic extract profile of *P. rosea* showed the metabolites and functional group presence in the nm of 1062, 916, 664, 610, 532 and 320 with the absorption of 0.012, 0.014, 0.982, 0.299, 0.385 and 4.000 respectively (Fig. 3 E). The aqueous extract profile of *P. rosea* does not show any peak detection.

The plumbagin dissolved in petroleum ether, chloroform, acetone, ethylacetate and ethanolic extracts showed only one peak in the nm ranged 406, 408, 409, 411 and 416 with the absorption of 2.111, 1.668, 2.352, 1.928 and 1.384 respectively (Fig. 4 A-E). The cladogram based on UV- Vis spectroscopic profile showed two clusters, of which cluster 2(C₂) includes only one species viz., *P. rosea* showed 100% of divergence from other two studied species. Cluster 1 (C₁) showed two nodal (N) branches, (C₁N¹ and C₁N²). C₁N¹ was *P. zeylanica* and C₁N² was *P. auriculata* (Fig. 5).

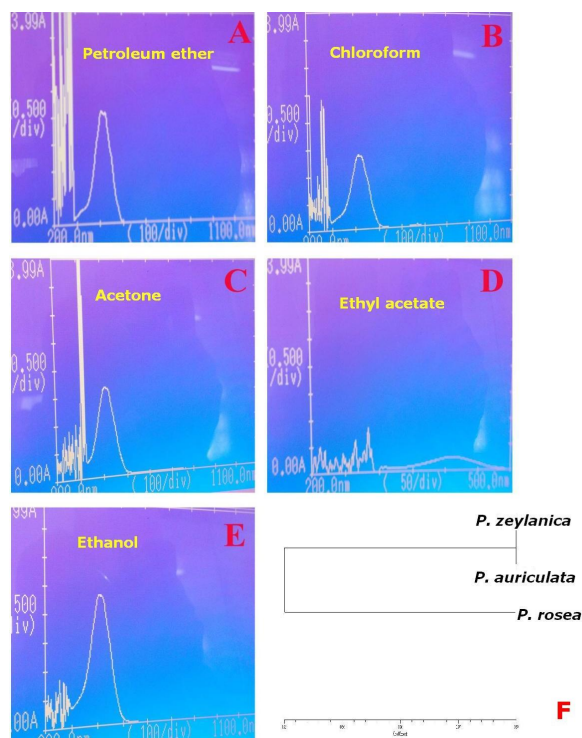


Fig 4 UV- Vis Spectrum of Plumbagin

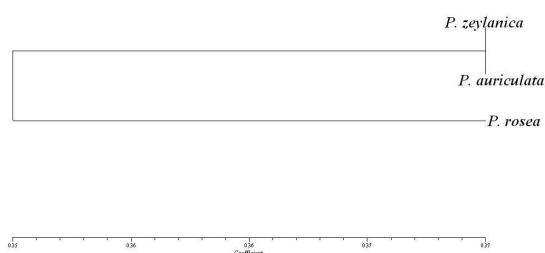


Fig 5 Cladogram of the selected *Plumbago* species based on UV- Vis spectrum profile

The FTIR spectrum and the peak values are used to identify the functional group of the active components. The results of the present study confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines ranging the peak value of 3778.29 – 462.88 in the crude powder of *Plumbago* species aerial parts. The results of FTIR peak values and functional groups were represented in Table 1 (Fig. 6 A - C).

Functional Group	Ranges	Pz	Pa	Pr
Unknown compound	-	+	+	-
Amides(bonded NH)N-H str	3180-3220 nm	+	+	+
Phosphorous compounds (P-OH)P-H str	2350-2440 nm	-	+	+
α-β-γ-δ- Unsaturated aliphatic (carbonyl compound)	1660-1680 nm	+	+	+
Alkanes (-CH2-) C-H def (1445-1485nm)	1445-1485nm	+	+	+
Phenols, tert carboxyl	1310-1410 nm	+	+	+
Sulfur compounds (syllfonamides). S=O str.sym	1300-1400 nm	+	+	+
Sulfonic acid	1150-1210 nm	+	+	+
Secondary alcohols	1100-1120 nm	+	+	+
Halides	790 - 840 nm	+	+	+
Halogen compounds (C-Cl equatorial)C-Cl str.	750-790 nm	+	+	+
Halogen compounds (C-Br axial)C-Br.str.	600-800 nm	+	+	+
Sulfur compounds (C-S str)	570-700 nm	+	+	+
unknown	-	+	+	+
Total		13	14	13

Table 1 FTIR peak values for crude powder of *Plumbago* species Pz – *Plumbago zeylanica*, Pa- *Plumbago auriculata*, Pr- *Plumbago rosea*, + sign indicates the functional group presence; - sign indicates the functional groups absence in the crude powder

The cladogram based on FTIR spectroscopic profile showed two clusters, of which cluster 2 includes only one species viz., *P. rosea* showed 100% of divergence from other two studied species. Cluster 1 (C₁) showed two nodal (N) branches, (C₁N¹ and C₁N²). C₁N¹ was *P. zeylanica* and C₁N² was *P. auriculata* (Fig. 6 D). The amalgamated cladogram was constructed based on the UV- Vis and FTIR analysis (Fig. 6 E). The amalgamated cladogram revealed the *inter*-species relationship between the studied *Plumbago* species. Cluster 1 (C₁) showed two nodal (N) branches, (C₁N¹ and C₁N²). C₁N¹ was *P. zeylanica* and C₁N² was *P. auriculata*, of which cluster 2 (C₂) includes only one species viz., *P. rosea* showed 100% of divergence from other studied two species (Fig. 6 E).

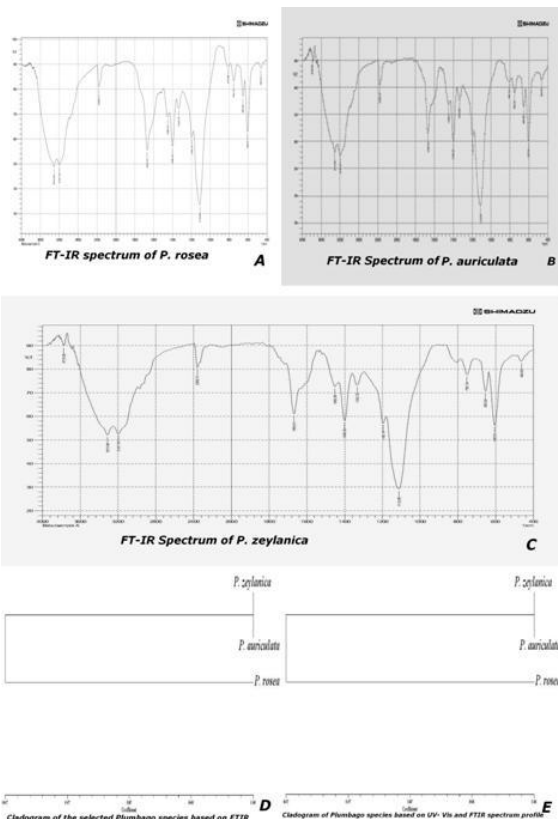


Fig 6: A-C UV- Vis Spectrum of *Plumbago* species D Cladogram of the selected *Plumbago* species based on FT-IR spectrum profile E Amalgamated Cladogram of the selected *Plumbago* species based on UV- Vis and FT- IR spectrum profile

Discussion

Spectral differences are the objective reflection of componential differences. By using the peak values of FTIR spectrum, the origin of different functional groups can be tested accurately and effectively, various constituents in the plant can be traced, medicinal materials can be identified true or false and even evaluate the qualities of medicinal materials. Therefore, FTIR spectrum is a most probable method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine [5]. Many workers applied the FTIR spectrum as a tool for differentiating and discriminating closely related plants and other organisms [23-24]. In the present study also, the FTIR analysis results revealed the inter-relationship between the studied *Plumbago* species. The results of the present study were directly coincided with previous studies and supported their observations.

UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition [25]. Kishore *et al.* [26] characterized difuranonaphthoquinones from *P. zeylanica* roots by spectral analysis (UV, IR, 1D and 2D NMR and MS) and identified naphthoquinones, lapachol, plumbagin, 2-isopropenyl-9-methoxy-1,8-di-oxa-dicyclopenta(b,g) naphthalene-4,10-dione, 9-hydroxy-2-isopropenyl-1,8-dioxo-dicyclopenta(b,g) naphthalene-4,10-dione, 2-(1-hydroxy-1-methyl-ethyl)-9-methoxy-1,8-dioxo-dicyclopenta(b,g)

naphthalene-4,10-dione and 5,7 -dihydroxy-8-methoxy-2-methyl-1,4-naphthoquinone. Similar to Kishore *et al.* [22] observation, in the present study also the FTIR analysis was carried out in the crude powder of *Plumbago* species aerial parts. Hazhar *et al.* [27] studied the spectroscopic data (IR, ¹H NMR, and ¹³C NMR) of *P. europaea* and confirmed the chemical structure of the isolated constituent (A) to contain the important functional groups of plumbagin. Ariyanathan *et al.* [28] isolated caposione, isoshinalane, diomuscione, plumbagin, sitosterol from the methanolic extract of *P. auriculata* using IR and NMR spectrum studies which reported the presence of carbonyl, phenol and hydroxyl groups in the nm of 3300, 1730, 1700. The results of *Plumbago* species FTIR analysis is directly coincided with Ariyanathan *et al.* [24] observations.

Shalini *et al.* [29] estimated the plumbagin content in the root of different *Plumbaginales* namely *P. zeylanica*, *P. auriculata* and *P. rosea* collected from Ahmedabad using UV-Vis spectrophotometric method at the wavelength of 520 nm and determined that *P. rosea* showed maximum of (1.68%), followed by *P. zeylanica* (0.413%) and *P. auriculata* (0.13%). Contrary to Shalini *et al.*, observations, in the present study, the chloroform extract of *P. zeylanica* showed higher concentration of plumbagin at the nm of 401 – 420 with the absorbance of 2.659, 3.436 and 1.11 elucidated the presence of various compounds. Similarly, the chloroform extract of *P. auriculata* and ethyl acetate extract of *P. rosea* showed higher concentration of Plumbagin of 3.436 and 2.451 Abs. Our results were directly coincided with the standard plumbagin. In addition to Shalini *et al.* [25] observation, we revealed the UV-Vis qualitative spectroscopic profile of the *P. zeylanica*, *P. auriculata* and *P. rosea*. Kumar and Murugan [7] have used the FT-IR analysis in taxonomic identification of various accessions of *Solanum capicoides*. Hora and Malik [8] studied the genetic relationships among three genera (*Trigonella*, *Melilotus* and *Medicago*) through the chemical fingerprinting using FT-IR. Similarly in the present study also the FT-IR spectroscopic peak values are employed to distinguish the medicinally important *Plumbago* species.

Conclusion

These spectroscopic profiles will act as pharmacognostic marker to distinguish the medicinally important *Plumbago* species using relatively simple, cost-effective spectroscopic profile from its adulterants in the pharmaceutical industries. The FTIR spectrum recognised more or less comparable peaks unless viewing a trivial variation among the three *Plumbago* species, where as UV-Vis spectrum showed a wide range of variation among the different extracts of *Plumbago* species, and also the UV spectrum of plumbagin also exposed distinct absorbance range in different extracts. The results of the present study paved a way to predict and compare the phytoconstituents presence in all the three selected *Plumbago* species which can be

used for further characterization. In addition the result of the FT-IR analysis helped to distinguish the medicinally important plants. The results may be used in the pharmaceutical industries to distinguish the medicinal sources from its adulterants.

Conflict of Interest

We declare that we have no conflict of interest.

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