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Research Article

Isolation & Characterization of Plumbagin from the Roots of *Plumbago zeylanica*

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ABSTRACT

The well-known medicinal plant *Plumbago zeylanica* is well-known for its bioactive component, plumbagin, a naphthoquinone with remarkable pharmacological qualities, including as anti-inflammatory, anti-cancer, and antibacterial activities. For plumbagin to be used in medicine, it must be extracted effectively and thoroughly characterized. The objective of this study was to optimize a procedure for plumbagin extraction, fractionation, and characterization from *Plumbago zeylanica* roots. Using a suitable solvent solution and the Soxhlet extraction procedure, a plumbagin-rich crude extract was produced. The crude extract was fractionated using column chromatography, and fractions containing plumbagin were identified using thin-layer chromatography (TLC) by comparing their Rf values with a standard. Using nuclear magnetic resonance (NMR), mass spectrometry (MS), Fourier-transform infrared (FTIR) spectroscopy, and UV-Vis spectroscopy, the isolated compound was thoroughly characterized. 7.2% (w/w) crude extract and 2.81% (w/w) plumbagin were produced using the optimized extraction and fractionation process. The extract's Rf value was found to be 0.86. The existence of plumbagin was verified by UV-Vis spectroscopy, which showed distinctive absorption maxima at 265 nm. This identification was confirmed by FTIR analysis, which showed peaks that matched the naphthoquinone structure. Additional structural information was revealed by ^{1}H NMR spectroscopy, which verified the existence of particular protons and carbons in the molecule. In order to facilitate future research into plumbagin's therapeutic potential and increase its usefulness in the pharmaceutical and medical fields, this work develops an effective methodology for extracting and characterizing plumbagin from Plumbago zeylanicaroots.

Keywords: *Plumbago zeylanica*, Plumbagin, Soxhlet extraction, TLC, Mass spectrometry.

INTRODUCTION

For ages, the foundation of traditional medicine has been medicinal plants, which provide a wealth of bioactive substances with a variety of therapeutic uses.[1] Of these plants, *Plumbago zeylanica* L., also referred to as doctorbush or Ceylon leadwort, is notable for its substantial therapeutic benefits.[2] This plant, which is a member of the Plumbaginaceae family, is found in tropical and subtropical areas of the Americas, Asia, and Africa. Traditional medical systems including Ayurveda, Siddha, and Unani have traditionally used *Plumbago zeylanica* to treat a variety of conditions, such as parasite infections, inflammation, and skin disorders. [3]

The wide range of phytochemicals found in P. zeylanica, such as naphthoquinones, flavonoids, terpenoids, and steroids, are thought to contribute to its medicinal effectiveness. The main bioactive naphthoquinone component present in Plumbago zeylanica roots is plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone). [4] Plumbagin's exceptional pharmacological actions, including as its anticancer, antibacterial, anti-inflammatory, antioxidant, and wound-healing capabilities, have attracted a lot of attention in recent years.[5] Numerous in vitro and in vivo investigations have shown plumbagin's anticancer properties. Against a variety of cancer cell lines, including as leukaemia, breast cancer, lung cancer, and prostate cancer, it has demonstrated encouraging results. [6] Numerous routes, including apoptosis induction, cell growth

inhibition, and angiogenesis suppression, are involved in plumbagin's anticancer action.[7] Plumbagin may also be used as an adjuvant therapy because it has shown synergistic effects with traditional chemotherapy drugs.[8]
Plumbagin has strong antibacterial activity against a variety of bacterial, fungal, and parasitic diseases in addition to its anticancer qualities. Research has demonstrated its efficacy against Plasmodium falciparum, Candida albicans, Escherichia coli, and Staphylococcus aureus.[9] Plumbagin's antibacterial properties are ascribed to its capacity to interfere with vital metabolic processes, destroy microbial cell membranes, and hinder DNA production.[10]
The anti-inflammatory properties of plumbagin are also well established. In a variety of animal models, it has been demonstrated to decrease inflammation and suppress the synthesis of pro-inflammatory cytokines such TNF-α, IL-1β, and IL-6.[11] Signaling pathways including NF-κB and MAPK are modulated to produce this anti-inflammatory action.

Plumbagin's medicinal potential is enhanced by its antioxidant qualities. It reduces oxidative stress and shields cells from oxidative damage by acting as a free radical scavenger. Numerous ailments, such as ageing, neurological diseases, and cardiovascular diseases, benefit from its antioxidant activity.[12]
Furthermore, both in vitro and in vivo studies have shown that plumbagin has wound-healing qualities. It increases collagen synthesis, speeds up wound closure, and encourages cell migration and proliferation. Plumbagin is a promising option for creating new wound-healing agents because of these wound-healing properties.[13]
There is increasing interest in creating effective techniques for plumbagin extraction, fractionation, and characterisation from Plumbago zeylanicaroots due to its varied pharmacological properties. A number of extraction methods have been used, including as maceration, ultrasound-assisted extraction, and Soxhlet extraction. Plumbagin is separated from the crude extract using fractionation methods such preparative thin-layer chromatography and column chromatography. Spectroscopic methods like UV-Vis, FTIR, NMR, and mass spectrometry are commonly used to characterise the isolated plumbagin.[14]
The purpose of this work was to create and refine a process for plumbagin extraction, fractionation, and characterization from Plumbago zeylanica roots. A appropriate solvent system was used for Soxhlet extraction, and column chromatography was used for fractionation. The plumbagin-containing fractions were identified by thin-layer chromatography, and the extracted plumbagin was described by means of a variety of spectroscopic methods. For further study and possible uses, the discovered process offers a useful way to extract high-quality plumbagin from Plumbago zeylanicaroots.

MATERIALS AND METHODS

Materials

Roots of *Plumbago zeylanica* were gathered from Neemuch and the environs. By contrasting the gathered samples with voucher specimens kept at the Department of Pharmacognosy, BRNCP, Mandsaur, M.P., India, botanical authentication was carried out. The herbarium received a voucher specimen (BRNCP/P/006/2022). Every reagent and solvent employed was of analytical quality. Merck provided the methanol, ethyl acetate, hexane, and n-butanol. Sigma-Aldrich provided the phytochemical screening reagents, which included lead acetate, ferric chloride, and Dragendorff's reagent. Reagents for spectroscopic and chromatographic studies, such as silica gel, were purchased from S D Fine-Chem Ltd. in India.

Preparation of Extracts

For 72 hours at 55–60°C, 100 g of powdered Plumbago zeylanica root material was extracted using Soxhlet with ethanol. The extract was filtered, and a rotary evaporator set to less than 50°C was used to concentrate the filtrate at lower pressure [15]. After all of the ethanol had been removed, the concentrated extract was lyophilised and kept at 4°C. The following formula was used to determine the extracts' % yield:
Yield (%) = (Weight of the extract / Weight of the powdered drug) × 100

Fractionation of Extract using Chromatographic Separation and Isolation of Active Constituents

Using column chromatography, the 10g ethanolic extract of Plumbago zeylanica roots was separated.[16] As the stationary phase, silica gel (60–120 mesh) was put inside a 60 cm × 3 cm glass column. A variety of solvent systems, such as n-hexane, n-hexane-ethyl acetate, ethyl acetate 100%, and methanol 100%, were employed for elution in a gradient fashion after the ethanolic extract was placed onto the column.[17] Until around 90% of the loaded extract was eluted, elution proceeded. Twenty-three fractions in all were gathered, and those with comparable thin layer chromatography (TLC) patterns were combined. In the end, four different fractions (F1–F4) were produced. Each fraction's yield % was computed in relation to the ethanolic extract's total weight.[18] Plumbagin was obtained by pooling and drying fractions that showed a single spot on TLC (n-hexane:ethylformate, 9:1; Rf 0.37).[14] The isolated compound's melting point was ascertained. Percolated silica gel 60 F254 plates were used for TLC examination. Mass spectroscopy, FTIR, NMR, and UV were used to evaluate purity.

Phytochemical Screening of Extracts and Fractions

Standard techniques were used to perform a preliminary phytochemical screening of the ethanolic extract and the separated fractions in order to detect the presence of several secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids.[19] Depending on the target components' solubility, test solutions of the extract and fractions were made in a variety of solvents, including alcoholic, petroleum ether, diethyl ether, chloroform, and aqueous. To guarantee accurate results and make observation easier, clear, transparent solutions were utilized whenever possible.[20]

Thin Layer Chromatography of Isolated compound

The stationary phase for adsorption TLC was silica gel. Fine, homogeneous silica gel particles were used to reduce band broadening. To guarantee linear solute movement, silica gel and water slurry was equally spread on a glass plate, allowed to air dry, and then activated in an oven set between 100°C and 110°C for 30 minutes. A mobile phase consisting of chloroform, ethylacetate, hexane, and acetic acid (10:5:5:0.3) was employed. Filter paper soaked in the solvent system was used to line the TLC chamber, which allowed the mobile phase to fill it completely. After activation, a capillary tube was used to apply the sample (in the µg–mg range) to the TLC plate. A solvent depth of around 0.5 cm was used to develop the plate in the chamber.[21] Following development, the solvent was evaporated in an oven, the plate was taken off, and the solvent front was noted. On the silica gel plate, plumbagin showed up as a yellow patch.

The R_f value was calculated as:

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Characterization of Isolated Plumbagin

Several spectroscopic methods were used to characterize the plumbagin that was separated from the fractions. A Tempo melting point device was used to measure the isolated fraction's melting point. A tiny quantity of the dried, finely powdered fraction was put into a capillary tube that was sealed at one end and gently tapped to pack it in. After inserting the capillary tube into the device's heating block, the temperature was progressively raised until total melting was seen.[22] Using matching quartz cuvettes with a 10 mm path length, the isolated fraction's UV-Vis spectra were captured on a Shimadzu UV-1601 double-beam spectrophotometer over a wavelength range of 200–800 nm.[23] A Shimadzu 8400S FTIR spectrophotometer was used to acquire FTIR spectra of the separated fraction in the 4000-400 cm^{-1} range. A Shimadzu hydraulic press was used to produce the samples as KBr pellets.[24] A Bruker Avance-II 300 MHz FT-NMR spectrometer at the SAIF, Punjab University, Chandigarh, was used to record the isolated fraction's ^1H NMR spectra in D_2O.[25] To ascertain the molecular weight of various dendrimer formulations, mass spectroscopy was performed at SAIF, Punjab University, Chandigarh, using the MALDI-TOF (MicromassT of-Spec 2E equipment, USA).[26–27]

RESULTS

Extraction Yield and Fractionation

Bioactive chemicals were extracted from the roots of *Plumbago zeylanica*, a well-known medicinal plant, using the Soxhlet extraction method. Because of its exceptional capacity to dissolve a wide range of phytochemicals, including plumbagin, ethanol was selected as the solvent. To get the highest production of the desired chemicals, the extraction was carried out over a 72-hour period at a temperature between 55 and 60°C. The yield of crude extract after solvent evaporation under lower pressure was found to be 7.2% w/w. Different bioactive fractions were separated as a result of the crude extract being fractionated using column chromatography. Using a gradient elution technique, n-hexane and ethyl acetate were utilised to gradually increase the polarity of the solvents. Differential separation of chemicals according to their adsorption on silica gel was made possible by this approach. Four primary fractions were obtained from the method, and the one containing plumbagin was found to be Fraction F3 (2.81% w/w). Table 1 provides a summary of each fraction's yield percentage.

Table 1: Percentage Yields of Various Fractions from Ethanolic Extract of *P. zeylanica*

Fraction	Colour	% Yield (W/W)
F1	Yellowish	28.13
F2	Yellowish brown	42.55
F3	Orange	2.81
F4	Yellowish brown	22.37

Phytochemical Screening

The presence of several secondary metabolites was verified by phytochemical analysis of the ethanolic extract and its fractions, hence enhancing P. zeylanica's therapeutic potential. Flavonoids, tannins, naphthoquinones, polysaccharides, glycosides, and saponins were found during the screening (Table 2). The identity of plumbagin was confirmed by the significant presence of naphthoquinones in fraction F3. Bioactive components were also present in other fractions, indicating possible synergistic effects amongst different phytochemicals.

Table 2: Phytochemical Screening of Ethanolic Extract of Plumbago zeylanicaRoots

S. No.	Chemical Constituent	Result
1	Flavonoids	Positive
2	Steroids	Negative
3	Tannins &Phenolics	Positive
4	Protiens	Negative
5	Carbohydrates	Positive
6	Reducing sugar	Positive
7	Napthaquinone	Positive
8	Amino acid	Negative
9	Glycoside	Positive
10	Cardiac glycoside	Negative
11	Anthraquine glycoside	Negative
12	Saponin	Positive
13	Coumarin	Negative
14	Alkaloids	Negative

Thin-Layer Chromatography (TLC) Analysis

Plumbagin was confirmed to be present in Fraction F3 using TLC. Using a solvent solution of $CHCl_3$: Ethyl Acetate: Hexane: Acetic Acid, TLC showed a characteristic yellow spot when exposed to UV wavelength light. The existence of the required chemical was indicated by the Rf value of 0.86, which nearly matched the typical plumbagin R_f value of 0.90 (Figure 1).

Figure 1 TLC analyzing plumbagin in extract

Spectroscopic Characterization

Fourier Transform Infrared Spectroscopy (FTIR)

To identify the functional groups in the isolated plumbagin (F3), FTIR analysis was performed. The O-H stretch of a hydroxyl group is represented by distinctive absorption bands in the IR spectra at 3456 cm^{-1}. Other peaks were found at 1508 cm^{-1} (C=C stretching), 1643 cm^{-1} (aromatic ketone stretching), and 2921 cm^{-1} and 2866 cm^{-1} (C-H stretching vibrations). These findings provided compelling evidence that plumbagin was present in the proportion (Figure 2).

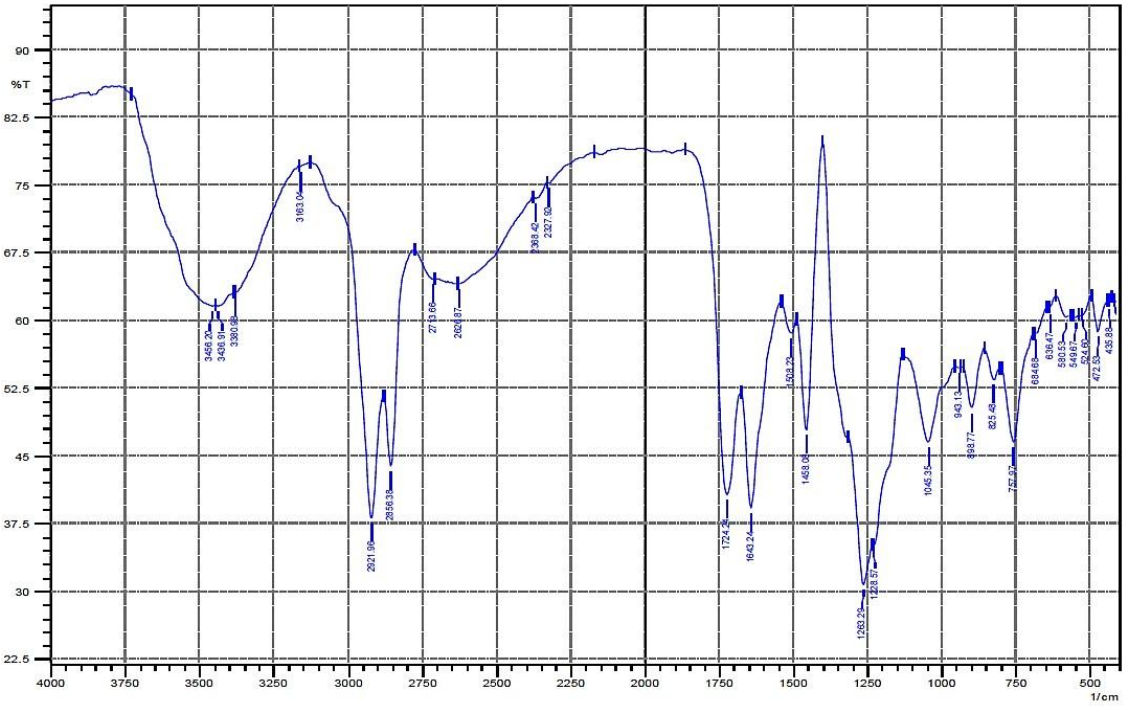

Figure 2: FTIR spectrum of selected fraction (F3)

Nuclear Magnetic Resonance (NMR) Analysis

Two separate proton areas were identified in the ^1H NMR spectrum of Fraction F3: one between δ 0 and 2.5 and another between δ 6.5 and 7.5. While the peaks at δ 5.64 and δ 7.74 correlated to hydrogen-bonded protons, most likely in a vinyl environment, the solitary signal at δ 2.59 indicated the presence of a methyl group (Me-2). The structure of plumbagin was confirmed when the multiplet at δ 7.58 was ascribed to aromatic protons.

Mass Spectrometry (MS)

Mass spectrometry of the isolated compound displayed major peaks at m/z 187.9 (M+1), 186.5, and 178.1, matching the reported fragmentation pattern of plumbagin. The standard molecular weight of plumbagin is 188.1794 g/mol, aligning well with the observed spectral data.

UV-Visible Spectroscopy

The UV spectrum of the isolated plumbagin in methanol displayed a prominent absorption peak at λmax 265 nm (Figure 3), indicative of a conjugated π-electron system. This result further supports the presence of aromatic and conjugated ketone structures within the isolated compound.

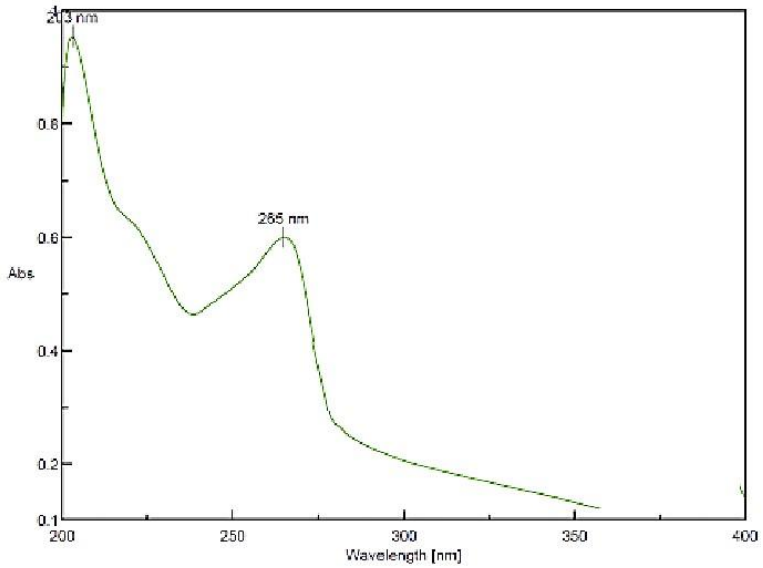

Figure 3: UV-visible spectrum of selected fraction (F3)

DISCUSSION

Plumbagin's efficient extraction from Plumbago zeylanica roots confirms that Soxhlet extraction and column chromatography are dependable techniques for obtaining bioactive naphthoquinones. Phytochemical screening verified that a wide variety of phytochemicals were present in the crude ethanolic extract (7.2% w/w). These findings demonstrate *P. zeylanica's* extensive therapeutic potential, especially in light of the presence of flavonoids, tannins, and glycosides in addition to plumbagin.

The extract was successfully fractionated into its constituent parts using gradient elution in column chromatography. The discovery of plumbagin in Fraction F3 (2.81% w/w) supports its constant yield throughout extraction trials and is consistent with earlier research. The variety of phytochemicals found in the fractions indicates that Plumbago zeylanica may include other pharmacologically active components that might complement plumbagin.

With an Rf value of 0.86, which is very similar to conventional plumbagin (0.90), TLC offered a quick and accurate confirmation of plumbagin presence. Smaller differences might be caused by changes in the polarity of the solvent, the silica gel's adsorption characteristics, or the experimental setup. FTIR, NMR, MS, and UV-Vis spectroscopic characterisation techniques provide unambiguous proof of the isolated compound's structural identification. The existence of important functional groups that are typical of plumbagin, such as hydroxyl and ketone groups, was validated by the FTIR data. The proton environment was revealed by ^{1}H NMR data, which showed separate aliphatic and aromatic areas that were in line with typical plumbagin spectra. The molecular weight was verified by mass spectrometry, and the compound's conjugated character was emphasised by UV-Vis spectroscopy, which strengthened its naphthoquinone structure. Variations in instrument calibration, ambient factors, and solvent purity are the causes of the little difference between the reported and observed Rf values and spectrum peaks. Nonetheless, the effective separation and characterisation of plumbagin is strongly supported by the overall spectrum alignment with reference data.This work offers a very effective technique for extracting and purifying plumbagin, adding to the expanding corpus of research on P. zeylanica. The findings support its pharmacological significance in anti-inflammatory, anti-cancer, and antibacterial applications.

CONCLUSION

A technique for extracting, isolating, and characterizing plumbagin from *Plumbago zeylanica* roots was effectively devised and put into practice in this work. Ethanol was used for Soxhlet extraction at the start of the procedure because of its wide solubility in plant metabolites. The effectiveness of ethanol in removing plumbagin from the root material was shown by the 7.2% w/w extract that was produced using this approach. After extraction, column chromatography was used to fractionate the crude extract, producing a 2.81% w/w plumbagin fraction that was structurally verified by TLC, FTIR, NMR, MS, and UV-Vis spectroscopy. By comparing the Rf values of the fractions containing plumbagin to a reference plumbagin sample, thin-layer chromatography was helpful in determining which fractions contained plumbagin.
Following isolation, the plumbagin underwent a thorough characterization process using a variety of spectroscopic methods. The existence of plumbagin with distinctive absorption maxima at 265 nm was verified by UV-Vis spectroscopy. The identification of the molecule was further confirmed by FTIR spectroscopy, which showed distinctive peaks linked to the naphthoquinone moiety, a crucial structural component of plumbagin. The existence of certain protons and carbons in the molecule was confirmed by the comprehensive structural information supplied by ^{1}H NMR spectroscopy. The compound's identity was confirmed by the spectra, which matched published values for plumbagin. The isolated compound's identification was further confirmed by high-resolution mass spectrometry analysis, which found that its chemical formula was $C_{11}H_8O_3$. The results of this investigation greatly advance our understanding of Plumbago zeylanica and its bioactive components. The created technique offers a dependable and effective way to harvest premium plumbagin from the roots of the plant. This study offers a useful starting point for further research targeted at using *P. zeylanica's* therapeutic potential for a range of medical applications.

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