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Research Article

PHYSICOCHEMICAL, PHYTOCHEMICAL, SPECTROSCOPIC (LCMS, AND H[1]-NMR) ANALYSIS OF EXTRACTS OF *PLUMBAGO ZEYLANICA*

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ABSTRACT

Objective: This study aimed to find the physicochemical, phytochemical analysis, and spectroscopic analysis of solvent extracts of the roots of *Plumbago zeylanica.*

Methods: The Soxhlet apparatus was employed to extract individual solvent extracts from the roots of *P. zeylanica*. In this study, solvent extracts made from the roots of *P. zeylanica* are tested for their physicochemical properties, phytochemical make-up, and spectroscopic properties. Spectroscopic investigations were conducted with the Bruker 400 MHz nuclear magnetic resonance (NMR) system, manufactured in Switzerland, as well as liquid chromatography mass spectroscopy (LC-MS), a mass spectrometer.

Results: The physicochemical study of *P. zeylanica* roots revealed a moisture content of 10.51%, a total ash content of 2.06%, and an alcohol-soluble extract of 1.72%. In addition, many physical parameters such as color, taste, aroma, and nature were examined. The phytochemical analysis of *P. zeylanica* revealed the detection of significant phytonutrients, including tannins, carbohydrates, proteins, flavonoids, alkaloids, and sterols, in the root sample. The presence of tannins, carbohydrates, proteins, flavonoids, alkaloids, and sterols in extracts of *P. zeylanica* was established through spectroscopic analysis using H1-NMR and LCMS.

Conclusion: The examination of solvent extracts obtained from the roots of *P. zeylanica* involved physicochemical, phytochemical, and spectroscopic techniques. This research revealed the presence of many biologically active metabolites, including alkaloids, amino acids, flavonoids, phenols, tannins, and terpenoids. The identification of these metabolites presents a promising prospect for substituting conventional chemical methods in the management of clinically pathogenic and phytopathogenic microorganisms.

Keywords: *Plumbago zeylanica*, Phytochemical, Physicochemical, LCMS, [1]H-NMR.

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INTRODUCTION

Plants, bestowed on humanity by the divine, hold a prominent position in the human experience. In previous times, several components of plants, including barks, roots, flowers, and leaves, have been employed for their analgesic, antibacterial, antioxidant, and other medicinal applications [1]. Plants have a diverse array of bioactive substances, which are essentially metabolites. These bioactive molecules have played a crucial role in the preservation of human health [2]. Natural goods serve as the primary sources from which contemporary medications, readily available on the market, are derived. Metabolites derived from natural sources, specifically secondary metabolites, are poised to assume a pivotal position within the pharmaceutical industry's drug development program, primarily in the creation of medicinal compounds.

A phytochemical refers to a bioactive compound that is naturally synthesized by plants as a result of their regular metabolic activities. The phrase "secondary metabolites" is employed to denote these chemical compounds. This category encompasses alkaloids, flavonoids, coumarins, gums, tannins, terpenes, phenols, and other chemicals. Phytochemicals, which are present in plant-based foods, play a crucial role in safeguarding the body against diseases through their interaction with nutrients and dietary fiber. Phytochemicals have been recognized as a significant constituent of the diet, which collaborates with nutrients present in fruits, vegetables, and nuts to effectively mitigate the progression of aging and lower the susceptibility to various ailments such as cancer, heart disease, stroke, hypertension, cataracts, osteoporosis, and urinary tract infections.

Phenolic substances, such as flavonoids, tannins, lignans, coumarins, and phenolic acids, are found in medicinal plants and have many biological effects, including antibacterial and antioxidant properties [3]. There is a current search for antioxidants that are generated from plant-based sources. Researchers are currently exploring the substitution of synthetic antioxidants with plant-derived antioxidants due to their elevated costs and associated detrimental effects, such as carcinogenic properties. The application of plants containing antioxidants in the field of medicine is attributed to the presence of free radicals generated by these antioxidants [4-6]. *Plumbago zeylanica*, a plant indigenous to several regions of India, such as the southern, eastern, and northern areas, possesses extracts that exhibit many medicinal uses, including antibacterial, anti-inflammatory, and antifungal activities [7-9]. Plumbagin, 5-beta-sitosterol, and bakuchiol are metabolites derived from *P. zeylanica* that exhibit potential suitability for the aforementioned applications.

The current investigation focused on the examination of the physicochemical, phytochemical, and spectroscopic properties of solvents such as chloroform, pet ether, methanol, and hexane extracts derived from the roots of *P. zeylanica*. To ascertain the presence of derivatives in the extracts, a preliminary investigation involving physicochemical and phytochemical screening was conducted. The findings of these investigations were further corroborated through the

use of liquid chromatography-mass spectroscopy (LC-MS) and ^{1}H-NMR spectroscopic analyses.

MATERIALS AND METHODS

Materials

Chemicals

Chloroform, methanol, pet ether, hexane, and D_6-DMSO (For nuclear magnetic resonance [NMR]) solvents were purchased from Sigma-Aldrich Co., USA.

Equipment

Soxhlet flask for the extraction by different solvents. Spectroscopic studies were carried out using Bruker 400 MHz NMR system [10] (Switzerland) and LC-MS (mass spectrometer, mass accuracy ± 0.2 u; USA).

Methods

Chloroform, hexane, methanol, and petroleum ether were utilized in the extractions. For extraction, Soxhlet and flask extraction processes were adopted. 10 g of the powered samples of the roots of the *P. zeylanica* were placed in muslin cloth and used in a Soxhlet apparatus to extract each solvent at a temperature below its boiling point. A part of the powdered plant samples was soaked in solvent in a conical flask, wrapped in aluminum foil, and shaken for 48 h at 120–130 rpm in a shaker. The extracts were filtered using What-man filter paper No: 1 after 48 h. The solvent was extinguished.

Physicochemical analysis of roots of *P. zeylanica*

Moisture content

The moisture content of *P. zeylanica* was determined by properly weighing 5 g of the sample in a dry and level petri dish. The sample was then dried in an oven at a temperature of 110ºC. The drying process continues until two successive measurements exhibit a maximum difference of 5 mg. The weight loss was quantified in terms of percentage.

pH (1% aqueous)

The material was dissolved in 100 mL of distilled water, with frequent agitation, and subsequently left undisturbed for 18 h. The pH of the solution was determined by employing a pH meter, which underwent filtration and subsequent verification.

Total ash

Accurately weighed and dried, 2–5 g of plant material (specifically, the root) were added to a crucible that had been previously fired and tared. The substance should be ignited through a slow increase in temperature, reaching a range of 500–600° Celsius, until it achieves a white coloration. The sample was subjected to cooling within a desiccator and subsequently measured for weight. The proportion of total ash content was determined through calculation.

Acid insoluble ash

In the crucible containing the entirety of the ash, 25 mL of hydrochloric acid (about 70 g/l) were introduced. The crucible was then covered with a watch glass and subjected to gentle boiling for 5 min. The watch glass was rinsed with a volume of 5 cc of hot water, and then this liquid was introduced into the crucible. Collected the insoluble debris on an ash-less filter paper and washed it with hot water until the filtrate was neutral. The filter paper containing insoluble matter was ignited in a crucible until a consistent weight was achieved. The sample was subjected to cooling within a desiccator and subsequently measured for weight. The percentage of acid-insoluble ash concentration was determined through calculations.

Alcohol soluble extract

Accurately measure approximately 4 g of air-dried root material from the plant species *P. zeylanica* and place it in a conical flask with a glass stopper. The sample was subjected to a treatment involving the addition of 100 mL of 100% alcohol, followed by a 6-h period of agitation at regular intervals. Subsequently, the mixture was left undisturbed for 18 h. The filtrate was swiftly filtered, ensuring the preservation of all solvents. Subsequently, 25 mL of the filtrate was carefully transferred to a petri dish with a flat bottom that had been previously weighed. The solution was then evaporated completely over a water bath. The sample was subjected to a drying process at 105°C for 6 h. Following this, the sample was allowed to cool in a desiccator for 30 min, after which its weight was measured. The percentage of alcohol-soluble materials was determined through calculation.

Phytochemical analysis of *P. zeylanica*

The dried powder of the roots of *P. zeylanica* was treated to Soxhlet extraction with 50% ethanol for 2 h. The solution was subjected to evaporation until complete dryness using a rotary evaporator and thereafter stored in a refrigerated environment. The extracted substances were used for the purpose of screening phytochemical compounds.

The plant extract from the root was subjected to phytochemical qualitative analysis using the established standard technique. The plant extract underwent a first phytochemical screening to determine the presence of several compounds, including phenols and tannins, terpenoids, carbohydrates, proteins, amino acids, flavonoids, alkaloids, and glycosides. In accordance, with established protocols [11-13].

Test for tannins

The gelatin test involves subjecting the test solution to a gelatin solution, resulting in the formation of a white precipitate. This precipitate serves as an indication of the presence of tannins.

Test for carbohydrates

The Benedict's test involved the addition of a small amount of Benedict's reagent, which is an alkaline solution containing a complex of cupric citrate, to the test solution. The mixture was then heated in a water bath, and the formation of a reddish-brown precipitate was observed. This precipitate indicated the presence of a carbohydrate in the test solution.

Test for proteins

The biuret test was conducted by treating the test solution with a 10% solution of sodium hydroxide and adding two drops of a 0.1% solution of copper sulfate (CuSO4). The resulting mixture was then studied for the appearance of a violet or pink hue, which serves as an indication of the presence of proteins.

Test for free amino acids

The ninhydrin test involves subjecting the test solution to boiling in the presence of a 0.2% solution of ninhydrin. This process leads to the development of a purple hue, indicating the presence of unbound amino acids.

Test for flavonoids

The ferric chloride test was conducted by adding a small amount of ferric chloride solution to the test solution. This resulted in the creation of a blackish-red color, indicating the presence of flavonoids.

Test for alkaloids

The Hager's Test involved the addition of a small amount of saturated picric acid solution, also known as Hager's reagent, to the test solution. The observation of a yellow colored precipitate indicates the existence of alkaloids.

Test for glycosides

For the Keller-Kiliani test, a small amount of glacial acetic acid and a few drops of a solution of ferric chloride ($FeCl_3$) were added to the test solution and then mixed well. The addition of concentrated sulfuric acid was performed gradually, and the resulting mixture was examined

for the occurrence of phase separation into two distinct layers. The presence of glycosides can be indicated by the lower reddish-brown layer and the higher acetic acid layer, which undergoes a color change to bluish-green.

Test for saponins
The foam test involved the combination of the test solution with water, followed by agitation and a subsequent 15-min incubation period. The observed foam exhibits stability for 15 min, which suggests the existence of saponins.

Analysis using NMR and MS
The analysis of plant extracts derived from *P. zeylanica* was conducted using NMR and MS techniques to validate the existence of flavonoids, alkaloids, tannins, and other relevant compounds. The plant extracts are dissolved in deuterated DMSO-d6 [14] for the purpose of conducting an H^1-NMR study [15]. On the other hand, the root extracts are dissolved in ethyl acetate to facilitate mass spectroscopic analysis.

RESULTS

Physicochemical analysis of roots of *P. zeylanica*
The physicochemical analyses reveal that the moisture content of the sample is 10.51%, while the ash content is measured at 2.06%. The percentage of alcohol-soluble extractives is 12.72%, whereas the acid-insoluble ash content is 0.148%.

The procedures recommended in the WHO guidelines [16], Indian pharmacopeia [17], and AOAC [18].

Table 1 : Physicochemical Analysis of roots of *Plumbago zeylanica*

S. No	Parameters observed	Result (%)
1	Moisture content	10.51
2	Ash value	
	Acid insoluble ash	0.148
	Water soluble ash	0.71
	Total ash	2.06
3	Alcohol-soluble extractive	12.72
4	Other matter	0.4
5	Physical parameters	
	Color	Brown
	Taste	Bitter
	Odor	Unpleasant
	Nature	Coarse powder

Phytochemical analysis of roots of *P. zeylanica*
The findings derived from the phytochemical analysis of solvent extracts obtained from *P. zeylanica* demonstrated the existence of beneficial phytonutrients. The results indicated that the extracts of chloroform, hexane, and methanol contained reducing sugar. The presence of flavonoids has been verified in the extracts obtained from chloroform and methanol. β-Sitosterol, a steroid compound, has been identified in all of the extracts. Tables 2 and 3 present the results of the phytochemical screening conducted on extracts of *P. zeylanica*.

Analysis using NMR and MS
The identification of secondary metabolites, such as β-Sitosterol, bakuchiol, biplumbagin, and β-Psoralen, in the roots of *P. zeylanica* has been established by the utilization of NMR and mass spectroscopic methodologies. The provided data include mass (LC-MS) and NMR spectra of plant extracts.

DISCUSSION

The physicochemical examination provides evidence of the existence of 10.51% moisture and 2.06% ash content. The analysis determined that the alcohol-soluble extractive content of the plant materials was 12.72%, indicating the existence of polar chemicals such as anthraquinones, alkaloids, glycosides of flavonoids, and triterpenoids. Finally, the detection of acid-insoluble ash at a concentration of 0.148% serves as an indication of the existence of siliceous material within the pharmaceutical substance. The results of the phytochemical study have provided confirmation of the existence of reducing sugar in the extracts obtained from chloroform, hexane, and methanol. In addition, the presence of flavonoids has been confirmed in the chloroform and methanol extracts. The physico-chemical investigation of the roots of *P. zeylanica* revealed the existence of inorganic materials and siliceous substances. In addition, phytochemical analysis of the roots of *P. zeylanica* identified the presence of alkaloids, phenols, flavonoids, tannins, and terpenoids. The identification of secondary metabolites such as β-Sitosterol, Bakuchiol, Biplumbagin, β-Psoralen in the root extracts of *P. zeylanica* was verified using spectroscopic analysis, namely mass and H^1-NMR spectroscopy.

The existence of beta-sitosterol and bakuchiol can be inferred from the peaks observed at 414 and 279 in the LC-MS analysis of the chloroform extract, as depicted in Fig. 1. The LC-MS analysis of the hexane extract (Fig. 2) reveals the presence of bakuchiol, biplumbagin, and beta-Sitosterol, as indicated by the peaks seen at

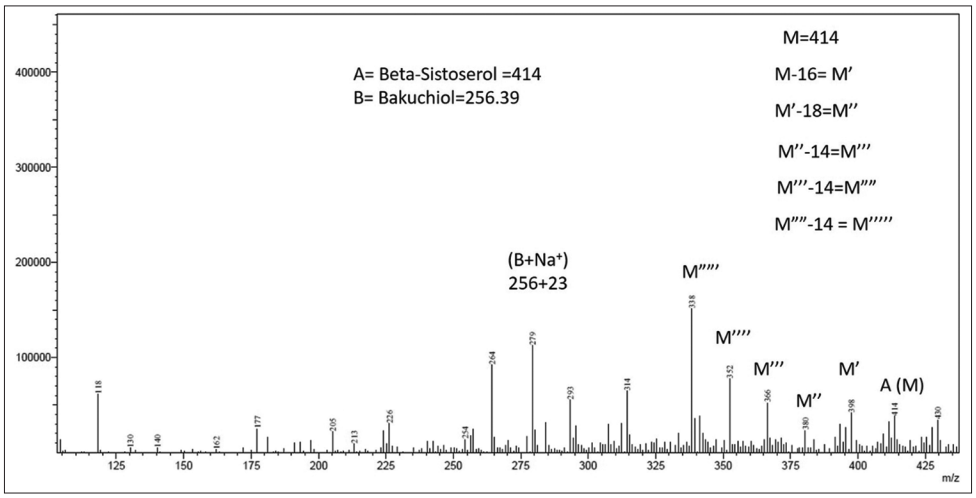

Fig. 1: LC-MS of Chloroform extract of roots of *Plumbago zeylanica*

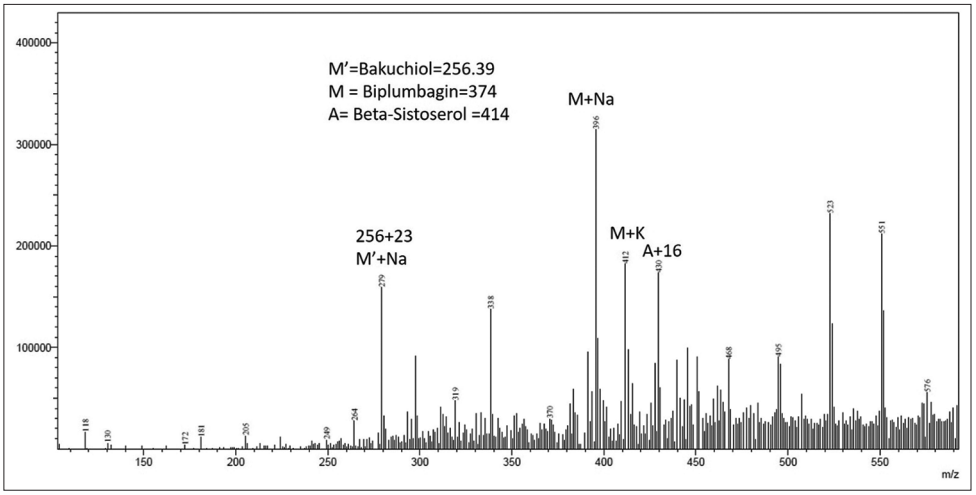

Fig. 2: LC-MS of hexane extract of roots of *Plumbago zeylanica*

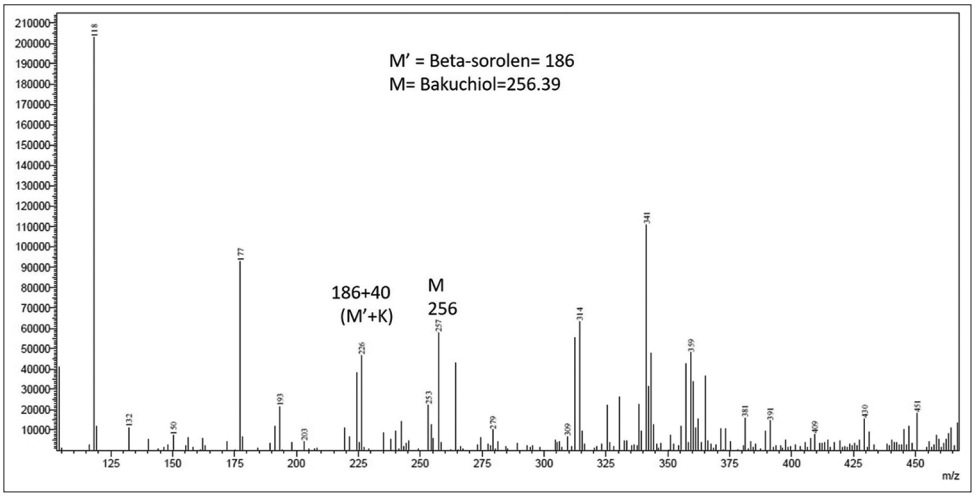

Fig. 3: LC-MS of methanol extract of roots of *Plumbago zeylanica*

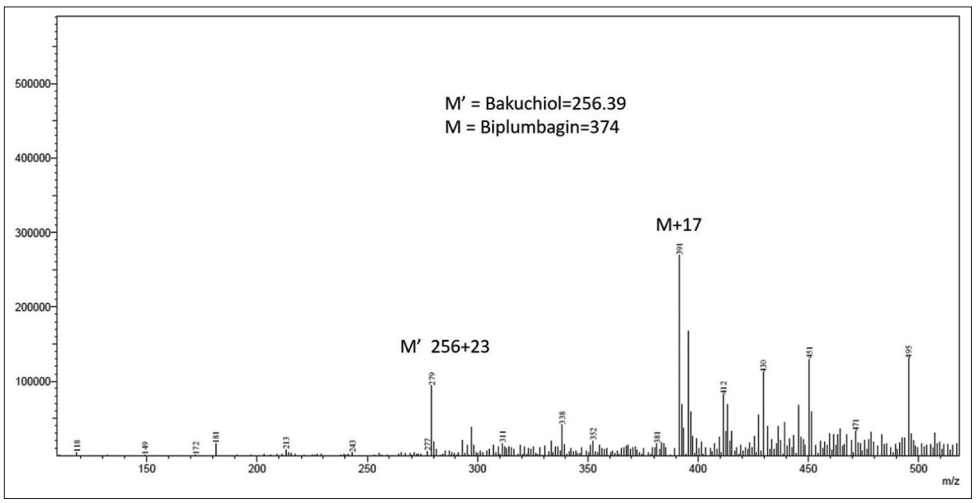

Fig. 4: LC-MS of pet ether extract of roots of *Plumbago zeylanica*

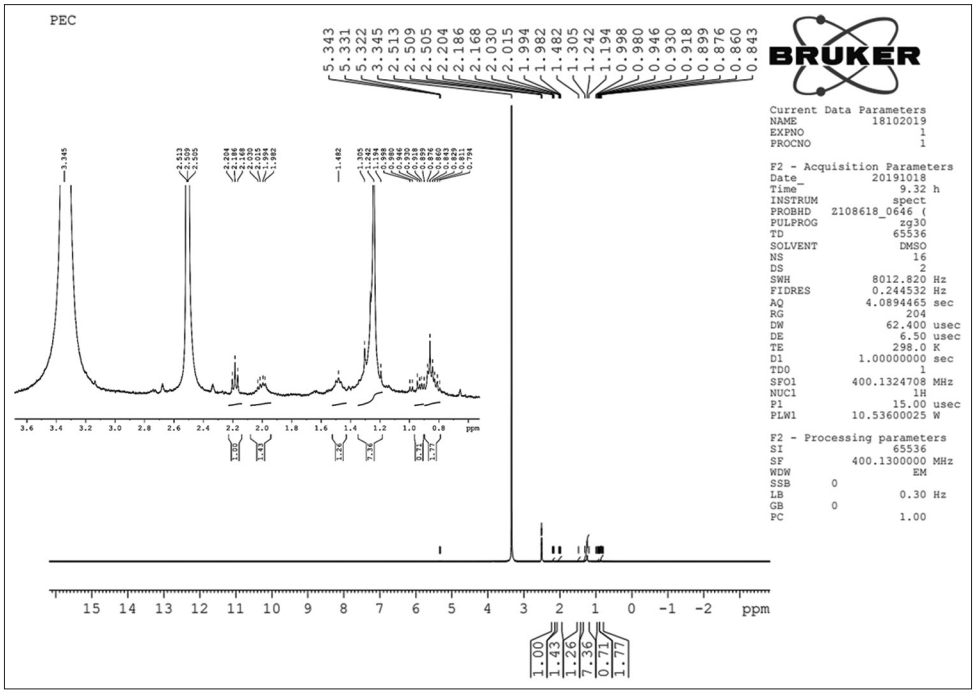

Fig. 5: [1]HNMR spectrum of chloroform extract of roots of *Plumbago zeylanica*

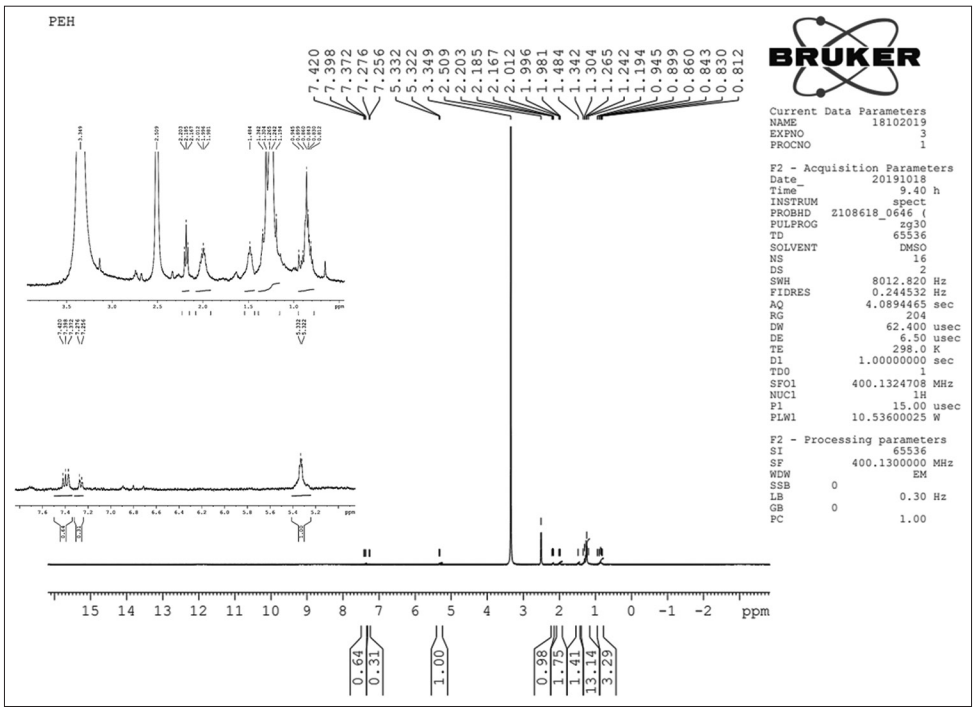

Fig. 6: [1]HNMR spectrum of Hexane extract of roots of *Plumbago zeylanica*

Table 2: Phytochemical analysis of *Plumbago zeylanica* [19]

S. No	Chemicals	Present (+) or Absent (-)
1	Phenolic and tannins	+
2	Terpenoid	-
3	Carbohydrates	+
4	Proteins	+
5	Amino acids	-
6	Flavonoids	+
7	Alkaloids	+
8	Glycosides	+
9	Steroid	-
10	Sterol	+

279, 396, and 430, respectively. The LC-MS analysis of the methanol extract (Fig. 3) reveals two prominent peaks at 226 and 257, which can be attributed to the presence of beta-Sitosterol and bakuchiol, respectively. The LC-MS analysis of the pet ether extract (Fig. 4) reveals the presence of Bakuchiol and biplumbagin, as evidenced by the peaks seen at 279 and 391, respectively. The verification of the aforementioned secondary metabolites was additionally conducted using H1-NMR spectroscopy (Figs. 5-8).

Based on the aforementioned findings, it can be concluded that the root extracts of *P. zeylanica* have broad-ranging uses in the realm of antibacterial activity and may hold potential in the field of oncology for the treatment of select cancer types.

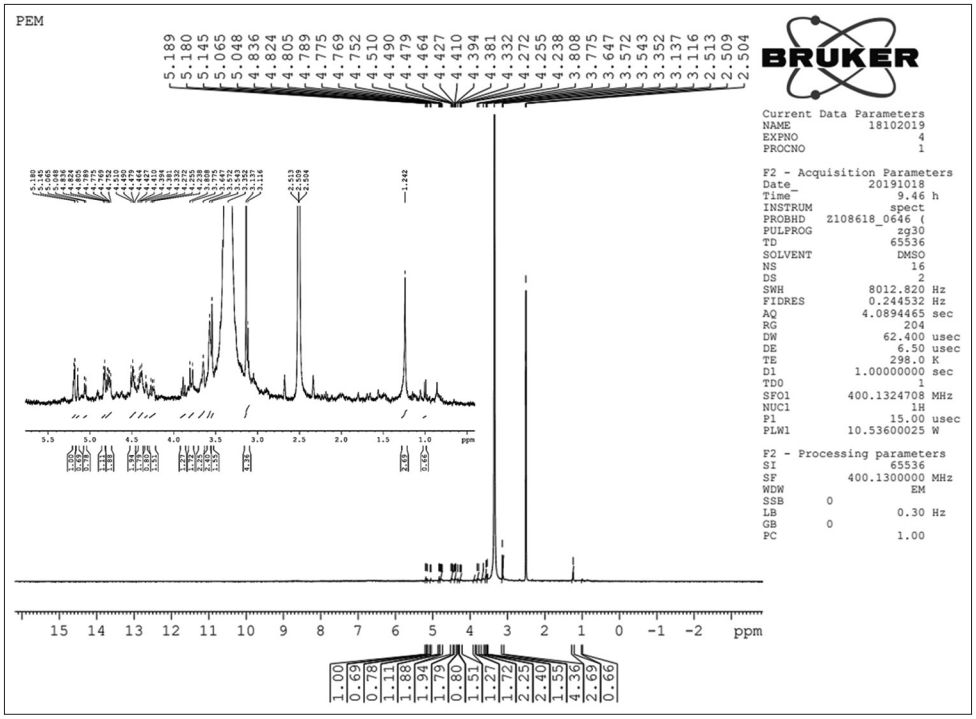

Fig. 7: [1]HNMR spectrum of methanol extract of roots of *Plumbago zeylanica*

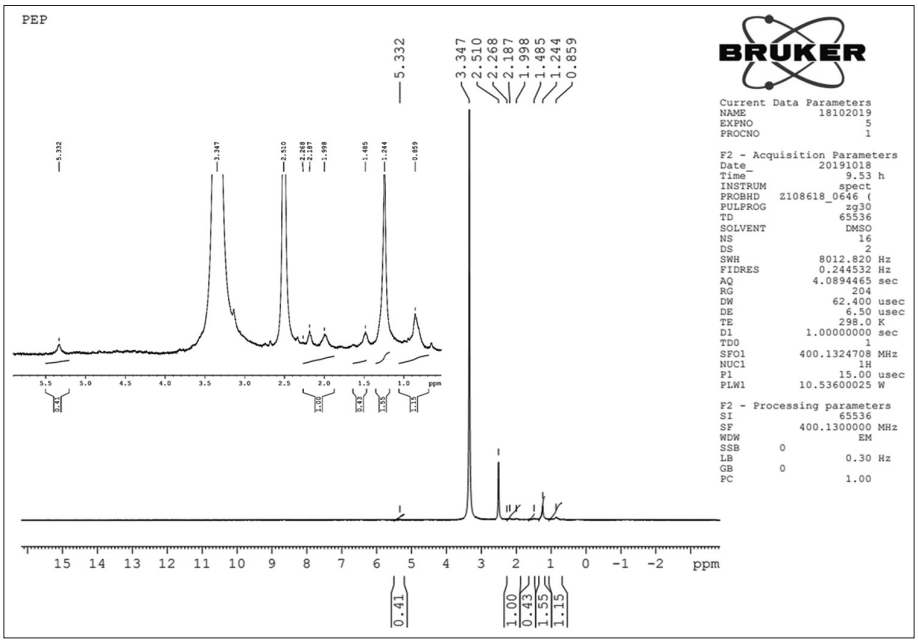

Fig. 8: [1]HNMR spectrum of Pet Ether extract of roots of *Plumbago zeylanica*

Table 3: Phytochemical screening of roots of *Plumbago zeylanica* extracted with different solvents [(+: present) (-: absent)] [20,21]

S. No	Chemical constituents	Pet ether extract	Chloroform extract	Hexane extracts	Methanol
1	Carbohydrates	–	+	+	+
2	Flavonoids	–	+	–	+
3	Tannin	–	+	+	+
4	Terpenoid	–	+	–	–
5	Phenol	–	–	+	–
6	Alkaloid	–	–	+	+
7	Quinone	–	+	–	–
8	Steroid	+	+	+	+

CONCLUSION

The roots of *P. zeylanica* were subjected to physicochemical, phytochemical, and spectroscopic investigation, which revealed the presence of many biologically active metabolites, including alkaloids, amino acids, flavonoids, phenols, tannins, and terpenoids. The identification of these metabolites presents a promising prospect for substituting conventional chemical methods in the management of clinical pathogenic and phytopathogenic microorganisms. Furthermore, the progression of natural antimicrobials will contribute to the reduction of adverse effects caused by synthetic medications. Hence, the utilization of *P. zeylanica* extracts has broad potential in the field of antimicrobial and antifungal applications, as substantiated by previous research [22].

AUTHORS CONTRIBUTION

All authors have made an equal contribution.

CONFLICTS OF INTERESTS

Nil.

AUTHORS FUNDING

Nil.

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