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GC-MS ANALYSIS, PHYTOCHEMICAL PROFILE, AND ANTIBACTERIAL EFFICACY OF THE MEDICINAL PLANT *PLUMBAGO ARABICA*

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ABSTRACT

The present study focused on determining the phytochemical composition of the ethanol root extract of *Plumbago arabica* through GC-MS analysis and characterization. The desiccated root were pulverized and immersed in various solvents of ascending polarity: chloroform, methanol, ethanol, and water. The phytochemical constituents in the methanolic extracts were identified using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The spectra of unknown compounds were analysed in comparison to known substances using the National Institutes of Standards and Technology database. The substances were considered to possess significant pharmacological and biological relevance. The extracts shown significant antibacterial activity when evaluated against the development of specific bacteria and fungi. The substances were considered to have significant pharmacological and physiological relevance. This study highlights the plant's significance as a phytopharmaceutical resource by demonstrating that it includes a number of bioactive chemicals.

KEYWORDS: *Plumbago arabica*, Phytochemicals, GC-MS, Antimicrobial.

1. INTRODUCTION

Natural products, such as plant extracts in their pure form or as standardized extracts, show immense potential for innovative therapeutic discoveries because of their unique chemical

diversity.[1] The World Health Organization (WHO) indicates that more than 80% of the global population relies on traditional medicine as their primary source of healthcare. The use of herbal remedies in Asia reflects a long-standing relationship between humans and their surroundings. Plants used in traditional medicine include a wide variety of compounds that are effective in addressing both chronic and infectious diseases.[2] In light of the rise of adverse effects and microbial resistance to synthetic drugs, people have increasingly sought alternatives through traditional medicinal practices. They identified numerous plant-derived compounds that act as safe and widely effective options with reduced adverse effects. A wide array of beneficial biological activities has been recorded, such as anticancer, antibacterial, antioxidant, antidiarrheal, analgesic, and wound healing properties. Numerous people claim the notable benefits of certain natural or herbal products. The active pharmacological components contribute to the creation of new drug entities through both in vitro and in vivo strategies.

The importance of the genus Plumbago in terms of its traditional medicinal uses and classification motivated us to explore the chemical signals present in its species. The genus Plumbago, belonging to the family Plumbaginaceae, comprises 10 genera and 280 species.[3,4] The genus Plumbago includes three main species: *Plumbago indica* L., *Plumbago auriculata* L., and *Plumbago zeylanica* L. Among these species, *Plumbago zeylanica* L. is recognized for its therapeutic properties. *Plumbago zeylanica* L., often referred to as Ceylon leadwort, doctor bush, and wild leadwort, stands out as a significant herbal plant.[5] In Ayurveda, it is also known as chitramula and chitrak. Chitrak is a perennial herb widely found throughout India and Sri Lanka. Plumbago species have been utilized for centuries because of their various medicinal properties. This herb demonstrates significant therapeutic efficacy. In Ayurveda, it is considered a rejuvenating practice. The root, bark, flower, and leaves of this herb are utilized in creating various Ayurveda treatments. In conventional practices, it is acknowledged for its significant protective benefits on an enlarged liver and spleen, along with its properties that combat cancer, viruses, and bacteria. This plant is acknowledged for its properties as a laxative, expectorant, tonic, abortifacient, and a stimulating appetizer. The *Plumbago arabica*[6] species is rarely found in India, therefore there is a lack of research on this medicinal plant.

Gas Chromatography-Mass Spectrometry (GCMS), an analytical method, is becoming more important in the assessment of phytochemicals. GC-MS has become a widely recognized

advanced method for identifying and quantifying secondary metabolites, even in minimal concentrations.[7] This study seeks to quantify secondary metabolites, perform an antibiotic assay, and identify the chemical contents of the methanolic extract of P. arabica, given the lack of a report on the GC-MS analysis of Plumbago arabica from India.

2. MATERIALS AND METHODS

2.1 Procurement and analysis of plant specimens

The plant materials (roots) were collected from the natural environments of Chandanapuri, Maharashtra, India. The plant the specimen was verified by the BSI-Botanical Survey of India, Western Regional Centre, Pune (M.H.). The roots were cleansed with water and distilled water three times prior to being dried in the shade. A mechanical grinder was utilized to pulverize the roots. The powdered P. arabica plant material was utilized for further investigation.

2.2 Sample extraction and phytochemical screening

Twenty-five grams of air-dried root powder undergo a comprehensive extraction process using 250 ml of methanol, maintained at a temperature below its boiling point, facilitated by a Soxhlet extractor. The methanol extract acquired from the Soxhlet extractor went through filtration before being subjected to evaporation with a rotary evaporator. The resultant dry extracts went through phytochemical analysis. The methanol extract's chemical phyto-constituents were identified through GC-MS analysis. The extracts were subjected to initial phytochemical screening using the standard method to determine the presence of various chemical groups or components, such as alkaloids, phenols, glycosides, terpenoids, tannins, flavonoids, steroids, and anthraquinones.[8-10]

2.3 Gas Chromatography-Mass Spectrometry analysis-Identification of compounds

The root extracts were analysed using GC-MS with a Thermo Scientific TSQ 8000 system. This setup included an AOC-20i auto-sampler and a Gas Chromatograph interfaced with a Mass Spectrometer (GC-MS), equipped with an Elite 5MS fused capillary column (30.0 m, 0.25 mm ID, 250 µm df). The analysis took place at the Department of Sophisticated Analytical Instrument Facility (SAIF), Panjab University, Sector, Chandigarh, India. An electron ionization device was employed in electron impact mode, utilizing ionization energy of 70 eV for GC-MS detection. Helium gas (99.99%) was utilized as the carrier gas, maintained at a consistent flow rate of 1 ml/min, with an injection volume of 1 µl and a split ratio of 10:1. The injector temperature was established at 250 °C, while the ion source

temperature was maintained at 240 °C. The oven temperature was programmed to initiate at 60 °C, remaining isothermal for 2 minutes, then increasing at a rate of 10 °C/min until reaching 300 °C, and finishing with a 6-minute isothermal phase at 280 °C. Mass spectra were obtained at 70 eV, utilizing a scanning period of 0.5 seconds, encompassing fragments ranging from 45 to 650 Da. The solvent delay varied between 0 and 2 minutes, while the total duration for GC/MS was 30 minutes. The average peak area of each component was compared to the total area to determine its relative proportion. This investigation employed the Flame Ionization Detector/ Electron Capture Detector, along with the software used for processing mass spectra and chromatograms. The mass spectrogram obtained from GC-MS was examined using databases that encompass around few lacks chemicals.[11,12] The spectra of the unknown compounds were compared with those of the known compounds stored in the NIST-2015 libraries.[13] The names, molecular formulas, and structures of the examined materials were determined.

2.4 Antimicrobial efficacy of P. arabica plant extarcts

In consideration of their potential clinical and pharmacological relevance, six bacterial strains Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus pneumoniae, and Micrococcus luteus were selected for screening, along with two fungal strains, Candida albicans and Aspergillus niger. The microbial cultures were obtained from the Department of Microbiology at Pravara Medical Trust (PMT) in Loni (M.H.). The cultivation of bacteria and fungi was conducted in nutrient broth and potato dextrose agar at a temperature of 37˚c. Subsequently, slants were prepared on nutrient agar and PDA, and those were stored at 4˚c.

2.4.1 Antibacterial assays by disc diffusion method

Investigations into the antibacterial properties of water, chloroform, methanol, and ethanol extracts were carried out using the disc diffusion method.[14,15] Nutrient agar (pH 7.4) was prepared and subjected to sterilization for duration of 15 minutes. The sterilized media was carefully dispensed onto sterile petri dishes, followed by the inoculation of the isolated bacterial pathogens into the plates. Different concentrations of the plant solvent extracts, diluted in DMSO (Dimethyl Sulfoxide), were administered to each disk. Gentamicin was employed as the positive control, whereas DMSO functioned as the negative control. The experiment was carried out in two separate instances. The bacterial plates were then

incubated at 37°C for duration of 24 hours, after which the antibacterial activity was evaluated by measuring the inhibitory zone in millimetres.[16]

2.4.2 Antifungal screening

The antifungal efficacy of the four extracts was evaluated by examining their effects on the zone of inhibition/growth inhibition of specific fungi. Two distinct strains of human pathogenic fungi, Candida albicans and Aspergillus niger, were assessed. The biological activity of the extracts was assessed in vitro by measuring the zone of inhibition both in the presence and absence of the extract. Fluconazole was employed as the positive control, whereas DMSO was used as the negative control. The experiment was carried out in two separate instances. The fungus plates were then incubated at 37°C for duration of 24 to 48 hours, followed by an assessment of antifungal activity through the measurement of the inhibitory zone in millimetres.[17]

3. RESULT AND DISCUSSION

3.1 Qualitative phytochemical analysis

Secondary metabolites display a diverse array of biological activities. The findings of this study indicate positive results for alkaloids, phenols, glycosides, saponins, terpenoids, tannins, flavonoids, steroids, anthraquinones, and phlobatannins (Table 1). The identified chemical exhibits antifungal and antibacterial properties.

Table 1: Qualitative phytochemical analysis-Inference of secondary phyto-compounds in Plumbago arabica.

Obs.No.	Phytochemicals	Aqueous extract	Chloroform extract	Methanol extract	Ethanol extract
1.	Alkaloids	-	+	+	+
2.	Phenols	+	-	+	+
3.	Glycosides	-	-	+	-
4.	Saponins	+	-	-	+
5.	Terpenoids	-	+	+	+
6.	Tannin	+	-	+	+
7.	Flavonoids	+	+	+	+
8.	Steroids	+	+	+	-
9.	Anthraquinone	-	-	+	+
10.	Phlobatanins	-	-	-	-

+ indicates presence and – indicates absence of activity

3.2 Bioactive chemical compounds identified in the extracts (GC-MS analysis)

The methanolic root samples of P. arabica underwent analysis via GC-MS to identify phyto-components and various bioactive chemicals. The GC-MS technology facilitates the identification and quantification of chemical compounds through their unique fragmentation patterns observed at specific retention times. The GC-MS analysis identified a wide variety of chemicals, as detailed in table 2 and figure 1. Previous investigations into diverse medicinal flora have revealed 25 bioactive phytochemicals in the present study, encompassing eugenol, trans-isoeugenol, phenol, acetophenone, chlorobutyrophenone, heptasiloxane, along with additional phytoconstituents. The compounds demonstrate a diverse array of bioactive properties, including antioxidant, anti-inflammatory, anticancer, antimicrobial, hypoglycemic, hepatoprotective, anticoronary, antiandrogenic, and antiarthritic effects.[18-21]

Table 2: Bioactive constituents from methanolic extract of *Plumbago arabica* roots.

Peak	R.Time	Area	%Area	Height	Compound Name
1.	2.40	438540991.58	23.25	131145451.79	Propane, 2,2-dimethoxy- 2-Hydroxy-2-methylbutyric acid 2-methoxy-2-methyl
2.	3.50	49659457.99	2.63	22433712.90	3-Penten-2-one, 4-methyl, 2 Pentanone, 3-methylene, 2,4-Azetidinedione,Cyclopropane, 1,1,2,3-tetramethyl
3.	3.89	133278729.65	7.06	59221887.68	2-Pentanone, 4-hydroxy-4-methyl-2-Hexanol, 2-methyl- 2-Pentanol, 2,4-dimethyl, 2,3-dimethyl-pentamethyl
4.	5.84	378164408.62	20.05	40393366.73	Acetophenone,1-Hexanone, 5-methyl-1-phenyl,1-Pentanone, 1-phenyl,Phenylglyoxal, ç-Chlorobutyrophenone
5.	5.92	202750660.94	10.75	72614801.63	Acetophenone,1-Pentanone, 1-phenyl,Hexanone, 5-methyl-1-phenyl, Benzoylpentanoic acid, Phenylglyoxal
6.	7.78	25116401.48	1.33	12933000.05	Cyclohexasiloxane, dodecamethyl, Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl, 3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5-(trimethylsilyloxy) tetrasiloxane ,Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, Silane, dimethyl (dimethyl(dimethyl (2 isopropylphenoxy) silyloxy) silyloxy) (2-isopropylphenoxy)
7.	8.08	274130502.45	14.53	77120146.29	Phenol, 2-methoxy-3-(2-propenyl),

					Eugenol, 3-Allyl-6-methoxyphenol, Phenol, 2-methoxy-4-(1-propenyl)-, (Z), trans-Isoeugenol
8.	8.14	134864054.15	7.15	33453853.73	Eugenol, trans-Isoeugenol, Phenol
9.	8.68	226293116.62	12.00	72518834.28	Caryophyllene, Bicyclo[7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene, Isocaryophillene ,Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene, Bicyclo [5.2.0] nonane, 2-methylene-4,8,8-trimethyl-4-vinyl
10.	8.94	23715173.98	1.26	10492944.05	Cyclohexasiloxane, tetradecamethyl, Heptasiloxane, 3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5-(trimethylsilyloxy) tetrasiloxane ,Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, Hexasilane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl

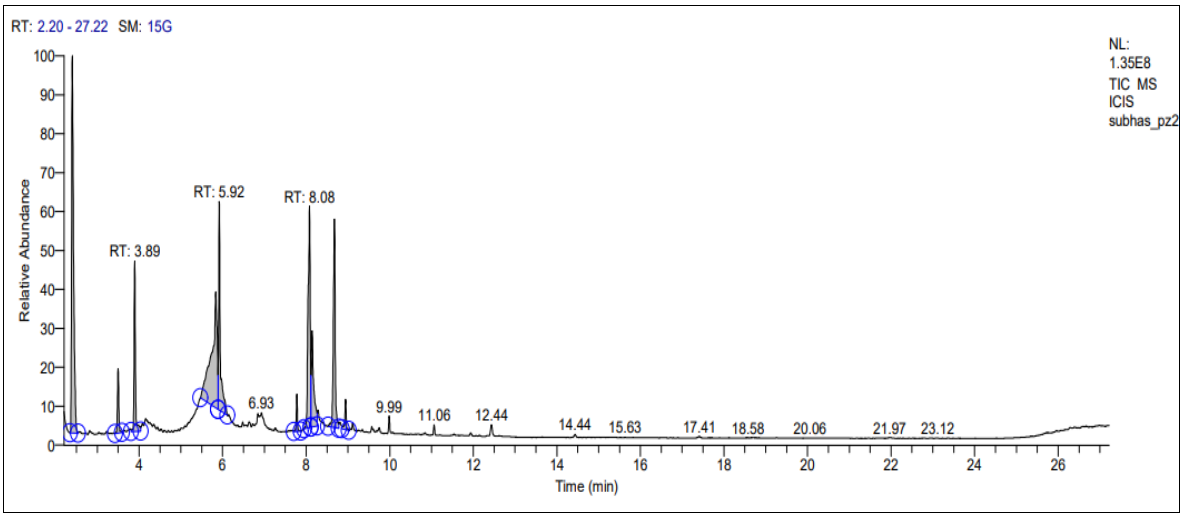

Figure 1: GC-MS Chromatogram of methanol extract of *Plumbago Arabica.*

3.3 Antimicrobial activities

3.3.1 Antibacterial efficacy

The antibacterial efficacy of various root extracts from Plumbago arabica was evaluated through the disc diffusion method, utilizing gentamycin as the positive control. All four extracts exhibited antibacterial activity; however, methanol and ethanol showed greater antimicrobial potential, as indicated by the zones of inhibition presented in table 3 and figure 2.

Table 3: Antibacterial activities of *Plumbago arabica* root extract.

Sr. No.	Bacterial Isolates	Zone of Inhibition in mm (ZOI)				
		Gentamicin				
		Antibiotics	Aqueous Extract	Chloroform Extract	Ethanol Extract	Methanol Extract
1.	*Escherichia coli*	15	9	8	12	13
2.	*Klebsiella pneumonia*	14	7	9	10	12
3.	*Proteus vulgaris*	14	8	9	12	12
4.	*Pseudomonas aeroginosa*	12	8	8	11	13
5.	*Staphylococcus pneumonia*	15	11	8	12	12
6.	*Micrococcus luteus*	16	8	10	11	13

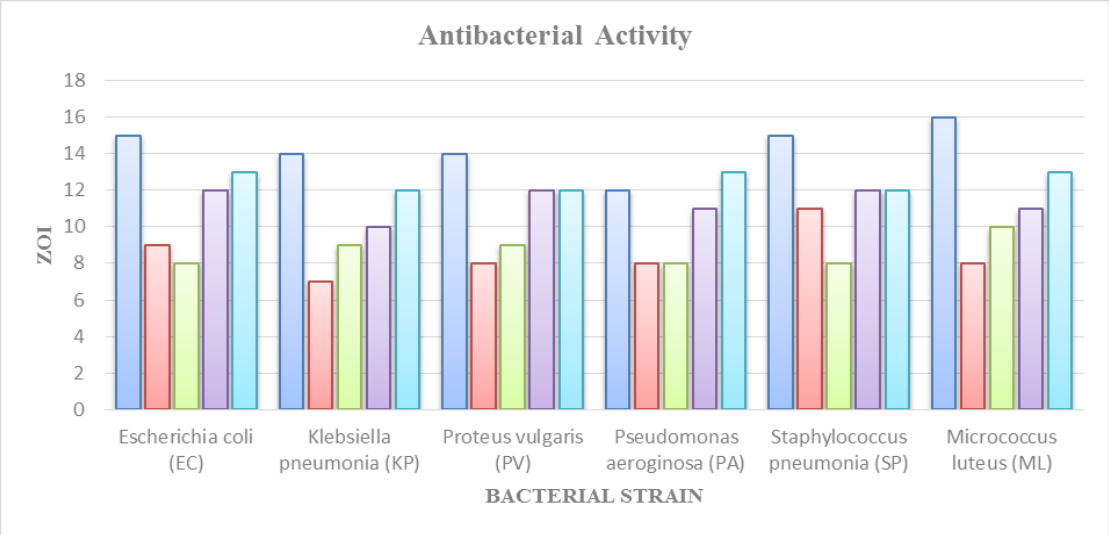

Figure 2: Graphical representation for antifungal activity of *Plumbago Arabica.*

3.3.2 Antifungal activity

The antifungal efficacy of various extracts from the plant P. arabica was investigated utilizing the zone of inhibition technique. All four extracts exhibited notable antifungal activity, as indicated by the zones of inhibition detailed in table 4 and figure 3. The results suggest that Plumbago root extract play a role in mitigating human disorders associated with the previously mentioned bacterial and fungal infections.

Table 4: Antifungal activities of *Plumbago arabica* root extract.

Sr. No.	Fungal Strain	Zone of Inhibition in mm (ZOI)				
		Fluconazole				
		Antibiotics	Aqueous Extract	Chloroform Extract	Ethanol Extract	Methanol Extract
1.	*Candida albicans* (CA)	18	8	9	12	13
2.	*Aspergillus niger (AN)*	16	7	8	11	12

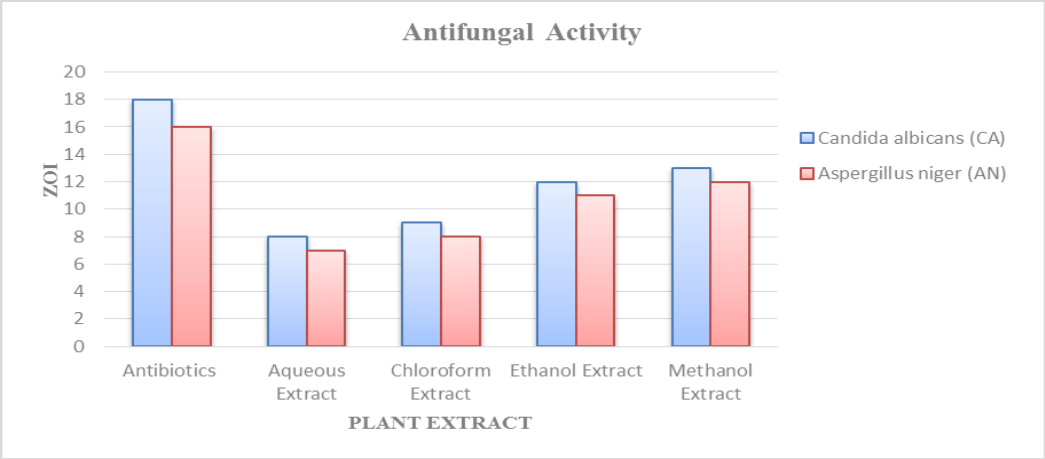

Figure 4: Graphical representation for antifungal activity of *Plumbago Arabica.*

4. CONCLUSION

This study offers valuable findings with the initial report on the GC-MS analysis and biological activity of Plumbago arabica. A variety of phyto-bioactive compounds present in this therapeutically important plant exhibited effective inhibition against a wide range of infections. The Plumbago plant exhibits characteristics that suggest its potential as a source of various bioactive compounds. Therefore, it is recommended that additional in-vitro and in-vivo studies be conducted to evaluate the pharmacological activities, especially the antioxidant, anticancer, and antiviral properties of compounds present in the methanol extract of P. arabica.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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