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ORIGINAL ARTICLE

Phytochemical Profiling and Pharmacological Investigation of *Plumbago arabica*

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ABSTRACT
The current research work aimed to explore the pharmacological potential of the Plumbago arabica. To investigate the medicinally important substances present in the ethanol root extract of plants by using the analysis of phytochemicals, UV-Vis, FTIR and GC-MS. Phytochemical study of successive extracts showed a positive reaction for secondary metabolites. UV-Visible spectrum revealed the varying peaks in the range of 190-1100 nm recording the absorptions at its respective wavelengths. It showed a λ max peak at 245 nm. The FTIR spectroscopy revealed the existence of alcohol, alkanes, phenols, alkyl halides, aldehydes, carboxylic acid, aromatics and nitro compounds. The GC-MS analysis results show specific peaks that imply the existence of substances with diverse medicinal actions. The present investigation offers an organizing principle for screening several bioactive components for the treatment of various diseases.
***KEYWORDS**: Plumbago arabica, Phytochemicals, Characterization and Anti-microbial potential.*

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INTRODUCTION

Medicinally valuable plant research is currently focused on revealing novel secondary metabolites, which are substances derived from plants. Using natural drugs are more efficient than the chemical drugs and have none of side effects. There is an increasing need to search for new compounds with antimicrobial activity and microbial infection [1-2]. Plant includes several phytoconstituents such as phenol, carboxylic acid, glycosides, flavonoids, alkaloids, saponins, coumarins and terpenes. Plants are endowed with certain qualities and traits by these phytoconstituents. Thus, identifying the different biological activities of plants would be aided by the examination of their element [3]. Spectroscopy has developed into a powerful technique for the qualitative and quantitative examination of biological and pharmaceutical substances. *Plumbago indica* L., *Plumbago rosea* L., and *Plumbago zylenica* L. are blooming plants that may be found in several locations including South East Asia, the peninsula of Saudi Arabia, Europe, Malaysia, Indonesia, Africa, China, and India [4]. *Plumbago indica* L. exhibits notable pharmacological efficacy against certain viral illnesses. Conventional methods such as Siddha, Perso Arabic, and Homeopathy often recommend the use of Plumbago. The Plumbago roots extract contains plumbagin as its active ingredient, along with dihydro- flavanoids, chitonone, glucose, and steroids obtained from the flower, fruits and seeds respectively. Plumbagine exhibits a variety of health advantages, such as its antimicrobial properties, ability to prevent cancer, and capability to protect the cardiovascular system [5]. Plumbago arabica is a species of flowering plant in the genus plumbago. It is a subshrub native to western, central India, Oman, UAE in the southeastern Arabian Peninsula, where it grows in desert and dry shrublands. This Plumbago species is rare in India; no more research has been done in this medicinal plant. The current study investigates novel secondary metabolites of Plumbago arabica and characterization of compounds using GC-MS analysis to the presence of phytochemical constituents, with the goal of curing many disease and disorders. Plumbago, a genus belonging to plumbaginaceae family, consists of 280 species and 10 genera. The genus Plumbago is composed of *Plumbago zeylinica*, indica and auriculta. The

most well-known botanical plants among these species is *Plumbago zeylinica* L., which is locally referred to as Ceylon leadwort, doctor bush and wild leadwort. In Ayurveda it is also referred to as chitramula and chitrak. Chitrak is a perennial herb that is grown in shaded regions of the garden for its vibrant inflorescence. It is widely disseminated in India and Sri Lanka [6-10]. The research work indicates the root of *Plumbago zeylinica* L. a laxative, expectorant, tonic, abortifacient, and remarkable aperitif. It has also been reported to be beneficial in the treatment of splenic disease, dermatitis, laryngitis and rheumatism. Muscle discomfort is mitigated through the utilization of seed decoctions [11]. *Plumbago arabica* is a species of flowering plant that is a member of the Plumbago genus. It is a subshrub native to the south-eastern Arabian Peninsula, with a specific distribution in western central India, Oman, and the United Arab Emirates. This Plumbago species is not frequently encountered in India; there is a dearth of research on this medicinal plant. The aim of this investigation is to detect the presence of phytochemical constituents by employing GC-MS analysis to identify and characterize novel secondary metabolites of Plumbago arabica.

MATERIAL AND METHODS

Collection of plant materials

The plant material was procured from Chandanapuri Ghat, Sangamner (Located 19.49499^0N, 74.19651^0E) Ahmednagar district, Maharashtra in August 2023. Plant was authenticated at the Herbarium, Botanical Survey of India, Western regional center, Pune (M.H.) where voucher samples were deposited and signed certificate of identification was obtained scientist and plant taxonomists, also confirmed the plant.

Plant extracts preparation

The plant parts/materials were extensively disinfected with running tap water, dried naturally in a protected location and pulverized in a mixer. The substance was stored in plastic containers that were appropriately labelled [12, 37]. The soxhlet solvent extraction technique was employed to extract the plant powder. A thimble, a small container, was used to properly position approximately 15 grams of finely pulverized plant material. The thimble was then subjected to extraction using 250 ml of a variety of solvents, each of which was used independently. Water, ethanol, chloroform, and acetone are the solvents that are utilized for extraction. Subsequently, the extract was condensed using a rotary evaporator and preserved in a small container for successive use in phytochemical analysis [13-14].

Phytochemical group tests of Plumbago arabica

The extract was tested for the presence of bioactive compounds by using the following standard methods [15-18, 37].

Test for alkaloids:

Wagner's test

Dilute HCl was added to the crude extract; it was subsequently immersed in the water bath for five minutes before filter 1-2 ml reagent was added. The reddish-brown precipitate suggested a presence of alkaloids.

Dragendorff's test

The presence of an alkaloid was confirmed when an orange ppt was formed after mixing the filtrate with reagent.

Test for Phenolic:

Ferric chloride test

The plant extract was dissolved in D/W, and then 2 ml of a 5% $FeCl_3$ solution was added. Violet or blue-green color appears, it indicates the phenolic compounds.

Lead acetate test

The solution was made by dissolving the extract in deionized water, and then adding 1 to 2 drops of lead acetate solution. A white ppt developed indicates the presence of phenolic bioactive components.

Test for Glycosides:

Liebermann's test

A mixture was created by combining the crude extract with 2 ml of $CHCl_3$ and 2 ml of CH_3COOH. The solution was cooled in an ice bath and then carefully added conc.H2SO4. There is a noticeable change in color from violet to blue to green, that indicates the presence of glycosides.

Killer- Kiliani test

The extract was mixed with 2 ml of glacial acetic acid, which contained a few drops of a 2 % solution of ferric chloride. Afterward, the mixture was moved to a different test tube with 2 ml of conc. sulphuric acid. A brown ring in the interphase indicated the presence of glycosides.

Test for Saponins:
The plant extract was mixed vigorously with five to ten milliliters of distilled water in the test tube. Steady foam formed showed the potential existence of Saponins.
Test for Terpenoids-
2 ml of the extract was combined with 2 ml of chloroform and conc.H_2SO_4. An interface with a reddish brown tint suggests the positive test of terpenoids.
Test for Tannin:
2 and 3 ml of a solution containing 2 % $FeCl_3$ were added to the extract. Tannins may be identified by their appearance, which can be described as either blue-green or black in color as appropriate.
Test for flavonoids:
Shinoda test-
The raw extract was mixed with Mg ribbon pieces and concentrated HCl. After a few minutes, a pink color showed up, which meant flavonoids were present.
Lead acetate test-
The extract is subjected to treatment with 2 ml of lead acetate solution. The flavonoids are confirmed by the production of either a yellow color ppt or reddish color.
Test for Steroids
Plants extract mix with 2ml of Chloroform, followed by the addition of concentrated H_2SO_4. When observing the lower $CHCl_3$ layer, the appearance of a red color suggests the possible presence of steroids. To investigate further, a mixture of conc.H_2SO_4 and CH_3COOH was added to the mixture. The formation of dark green color indicates the presence of steroids.
Test for Anthraquinones
Two ml extract was mixed with a few drops of 10 % NH_3 solution. The development of a pink-colored precipitate indicated the presence of anthraquinones.
Taste for Phlobatanin-
A 2 % HCl solution was combined with the extract in a ratio of 2 ml to 1 ml. A red-colored substance is observed upon the detection of phlobatannins.
UV- VIS Spectrum Analysis
The extract was centrifuged at 5000 rpm for 20 min. and filtered through Whatmann filter paper no.41. The sample was diluted with appropriate solvent. The extract was scanned at a wavelength of 190 to 1100 nm using the Elico-SL-159 UV-Vis spectrophotometer, and the characteristic peaks were identified. Peak values were recorded [19].
FTIR Analysis
FTIR analysis was conducted using a dried powder of the test plant extract. One milligram amount of dehydrated powder was encapsulated within a 10 mg pellet of KBr, with the intention of creating transparent sample discs. The powdered sample of the pellet was placed into the FTIR spectroscopy (JASCO, Japan), which had scanning range of 400 to 4000 cm-1 and a resolution of 4 cm-1 [20].
Gas chromatography–Mass spectrometry (GC-MS) analysis:
The GCMS analysis was carried out using the ethanolic fraction of *Plumbago Arabica* root extract, analysed at the Department of Sophisticated Analytical Instrument Facility (SAIF), Panjab University, Sector, Chandigarh (India). Exactly 3 µl of previously concentrated extract was employed for GC-MS (Thermo Scientific TSQ 8000, Modes: Full scan, SIM, timed-SIM, combined full scan/ SIM, combined full scan/ timed-SIM, timed-SRM, combined SRM/ full scan, Model: TRACE 1300 GC, Specification: Ion source type: EI source programmable to 350^0c, Mass range : 2.11 amu, Split/ Split less injectors and multi-mode including on-column, PTV, column temperature 400^0c, polar as well as nonpolar columns are available, Detectors: Flame Ionization Detector and Electron Capture Detector) analysis. The analysis was carried out using Agilent 7880 with 30 mm column length 0.25mm ID, 0.32 µ film thickness. The He gas was used as carrier gas at a constant rate of 1 ml/min. Injector temperature set at 100^0c. The oven temp. programmed from 50to 280^0c. The sample injected in split mode as 50:1.The MS taken at 70 eV. GC-MS is equipped with the NIST Library [38].
Antimicrobial Assay (Discs diffusion method)
The bacterial strains were obtained from the laboratory unit of Department of Microbiology, Pravara Medical Trust (PMT) Loni, and confirmed using standard biochemical tests as described by Cheesbrough [22]. The isolates were kept on freshly prepared NA-nutrient agar slants. The NA plates were carefully dried in a drier for approximately 15 min. in order to eliminate surface moisture. The petri dish inoculated with the test micro-organism using aseptic techniques to ensure uniform distribution. Discs contain standard antibiotics Gentamicin for bacteria and discs containing DMSO extract used as controls.

The glass petri plates incubated at a temperature of 37^{0}c for a period of 24 to 36 hours in the incubator. The measurement of the zone of inhibition was recorded in millimetre (mm) [23-26].

RESULT AND DISCUSSION

Qualitative phytochemical analysis

The qualitative phytochemical study of Plumbago arabica extracts is summarized in table 1. The aqueous extract was subjected to phytochemical examination, which verified the presence of secondary metabolites such as phenols, saponins, terpenoids, steroids, and phalobatanins. The ethanol extract was shown to include bioactive metabolites such as alkaloids, phenols, glycosides, Terpenoids, tannins, flavonoids, steroids and anthraquinones. The presence of alkaloids, glycosides, tannin, steroids and anthraquinones was detected in the chloroform extract, while the presence of alkaloids, saponins, terpenoids, flavonoids, steroids and anthraquinones was verified in the acetone extract.

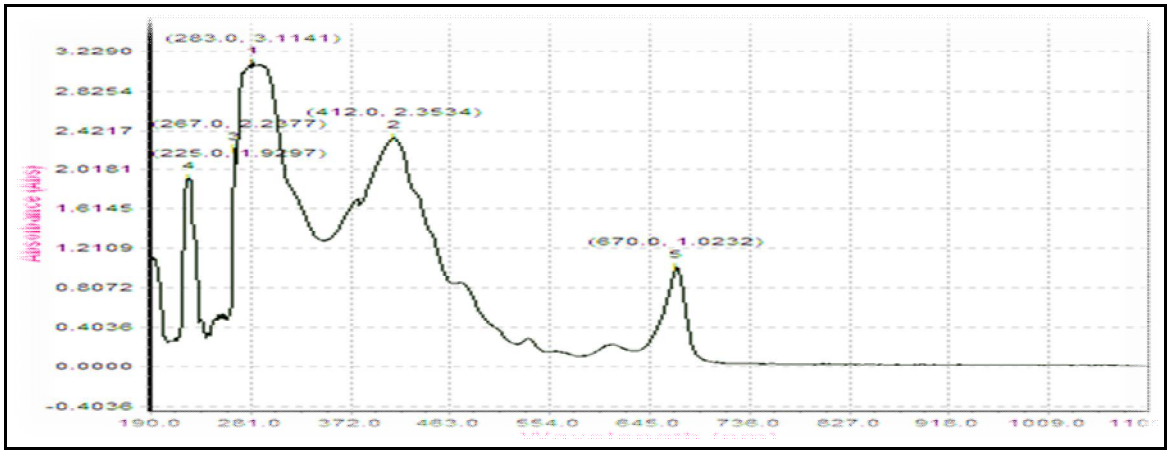
Table 1: Qualitative phytochemical screening of *Plumbago arabica* root extract

Obs.No.	Test	WE	EX	CE	AE
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. WE-Water Extract, EX-Ethanol Extract, CE-Chloroform Extract, AE-Acetone Extract

Ultraviolet- visible spectrophotometry (UV-Vis) analysis of Ethanol Extract

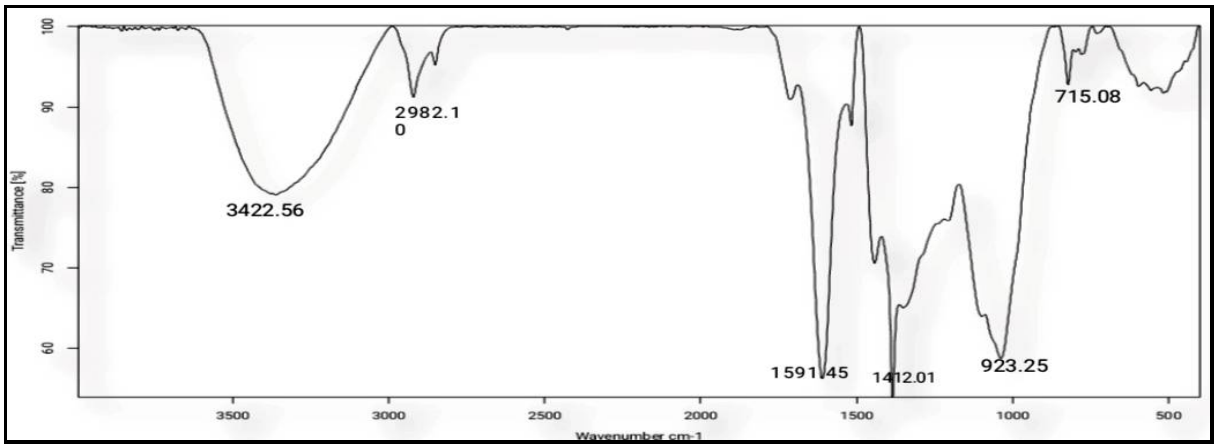
The UV-Vis spectrum profile of the Plumbago Arabica root extract, a wavelength range of 190 to 1100nm was selected. This range was chosen because it exhibited a clear peak and a well-defined baseline. The ethanol extract peaks at 225,267,283, 412 and 670 nm, accompanied by absorption values of 1.92, 2.23, 3.11, 2.35 and 1.02 as illustrated in graph 1. An analysis was conducted on plant extract using UV-Vis spectroscopy to detect the presence of tannins, flavonoids, alkaloids, and terpenoids. Based on the spectroscopy profile, it was observed that certain compounds were detected in specific wavelength ranges. Specifically, there was evidence of the presence of tannins and flavonoids in the 230-290nm range, while alkaloids and terpenoids exhibited a peak at 400 to 650 nm. Through UV-Vis spectra analysis, it was discovered that there are a multitude of phytoconstituents present, each possessing valuable medicinal properties. These compounds are categorized as secondary plant metabolites.



Graph-1.UV-Vis absorption spectra of ethanolic root extract of *Plumbago arabica*

Fourier transforms infrared spectrophotometry (FT-IR) analysis of Ethanol Extract

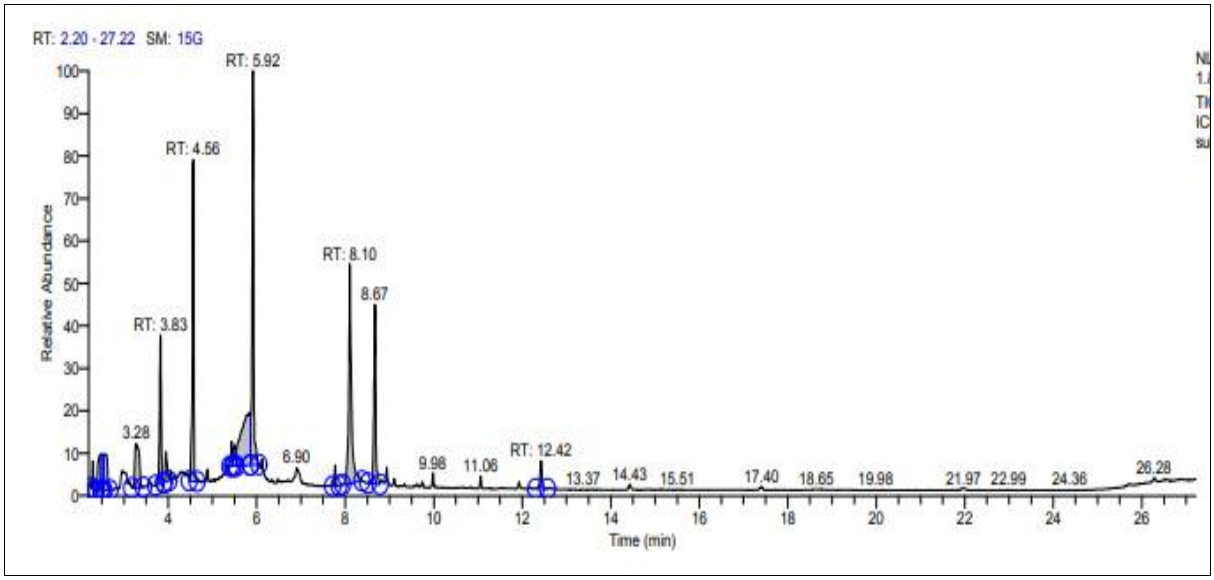
The FT-IR spectrum was used to analyse the functional groups of effective components presence in extract based on the peaks values in the region of FTIR radiation. Based on their peak ratios, the functional groupings of the components were separated. The results of FT-IR peak values and functional group were represented in graph 2. The FTIR gave a broad peak at 3422.56 cm^{-1} which indicated the presence of N-H stretching. A strong peak at 2982.10 cm^{-1} which indicated the presence of C-H stretching, 1591.45 cm^{-1} attributed to C-C stretching in ring, the peak around 1422.01 cm^{-1} are due to aromatic group, 923.25 cm^{-1} O-H bend indicated the presence of carboxylic acid and 715 cm^{-1} C-H rock. These observations imply that the plant extracts are rich in secondary metabolites.



Graph: 2. FTIR spectrum analysis of ethanolic extract of *Plumbago arabica*

Gas chromatography–Mass spectrometry (GC-MS) analysis of Ethanol Extract

The study consisted of determining unique bioactive components from the root extract of P.arabica using GC-MS analysis. The compounds that were found, displayed in the total ion chromatogram of the ethanolic extract, revealed the GC-MS profile. (Table 2, Graph 3).Various compounds were identified in the ethanol fraction of Plumbago arabica by GC-MS analysis. The prevailing compounds were 2-fluoropropene, allyl fluride, acetic acid, ethoxy -2-prpanol, 3 hydroxy 3-methyl 2 butanone2-butanal, furan 2,3,-dihydro-2-butenal, 1,3-butadien-1-ol, Propane, 2,2-diethoxy- Urea, (2-hydroxyethyl)-Thiocyanic acid, ethyl ester, di-isopropyl ether, 2- butanone, 3 ethaoxy-3-methyl, 4-ethaoxy-2-butanone, 3-Hexanol, 3-methyl, 2,2,4-Trimethyl-3-pentanol, 3-Pentanol, 2,3-dimethyl,4-Isobutoxy-2-butanone, Pentanol, 2,3-dimethyl-3-heptanol, 4-methyl-ethanol, 2-furancarboxylic acid, tetrahydro-3-methyl-5-oxo hexanoic acid, isoxazole, Trimethylsilyl methyl sulfide, 1,3-Propanediol, tert-butyl dimethyl silyl ether, 1,3-Propanediol, trimethylsilyl ether, 1,1-Difluoro-2,2,3-trimethyl-cyclopropane, 1,2,4-Triazine-3,5(2H,4H)-dione, 6-benzoylthio, Acetophenone, 1-Pentanone, 1-phenyl, ç-Chlorobutyrophenone,7-Benzoylheptanoic acid, a,à-Dichloroacetophenone, 1-Hexanone, 5-methyl-1-phenyl, Phenacyl thiocyanate,1-Pentanone, 1-phenyl, 5-Benzoylpentanoic acid, 1-phenyl-chlorobutyrophenone, 7-benzoyl heptanoic acid, dichloro acetophenone, Cyclo hexasiloxane,heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradeca methyl octa-siloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, 3,3,5-Triethoxy1,1,1,7,7,7 hexamethyl-5 (trimethyl silyloxy) tetrasiloxane silane, Phenol, 2-methoxy-3-(2-propenyl)- 3-Allyl-6-methoxyphenol, Eugenol, Phenol, 2-methoxy-4-(1-propenyl)-,(Z)-trans-Isoeugenol, Caryophyllene, Bicyclo [7.2.0] undec-4-ene, Isocaryophillene, Bicyclo [5.2.0] nonane, 2-Methylene-4,8,8-trimethyl-4-vinyl, Dibutyl phthalate, Phthalic acid, butyl hept-4-yl ester, 1,2-Benzene dicarboxylic acid [27-36].



Graph: 3. Chromatogram obtained from ethanol extract of *Plumbago arabica* root by GCMS

Table: 2. GCMS Analysis of ethanolic root extract of *Plumbago arabica*

Peak	R.Time	Area	Area %	Height	Compound Name
1.	2.30	21359692.39	0.89	12576227.65	2-fluoropropene, allyl fluride, acetic acid, ethoxy -2-prpanol, 3 hydroxy 3-methyl 2 butanone
2.	2.46	78070647.74	3.26	16180300.86	2-butanal, furan 2,3,-dihydro-2-butenal, 1,3-butadien-1-ol
3.	3.28	132069213.80	5.51	19423065.32	Propane, 2,2-diethoxy- Urea, (2-hydroxyethyl)- Thiocyanic acid, ethyl ester, di-isopropyl ether, 2-butanone, 3 ethaoxy-3-methyl
4.	3.83	159098235.31	6.63	65495019.30	4-ethaoxy-2-butanone, 3-Hexanol, 3-methyl, 2,2,4-Trimethyl-3-pentanol, 3-Pentanol, 2,3-dimethyl, 4-Isobutoxy-2-butanone
5.	3.95	40470442.33	1.69	13417186.74	Pentanol, 2,3-dimethyl-3-heptanol, 4-methyl-ethanol
6.	4.56	313611612.61	13.08	141287080.30	2-furancarboxylic acid, tetrahydro-3-methyl-5-oxo hexanoic acid, isoxazole
7.	5.43	22384882.58	0.93	10936267.88	Trimethylsilyl methyl sulfide, 1,3-Propanediol, tert-butyl dimethyl silyl ether, 1,3-Propanediol, trimethylsilyl ether, 1,1-Difluoro-2,2,3-trimethyl-cyclopropane, 1,2,4-Triazine-3,5(2H,4H)-dione, 6-benzoylthio.
8.	5.50	28752133.86	1.20	9236857.83	Acetophenone, 1-Pentanone, 1-phenyl, ç-Chlorobutyrophenone, 7-Benzoylheptanoic acid, a,à-Dichloroacetophenone
9.	5.83	333113731.11	13.89	23139481.40	1-Hexanone, 5-methyl-1-phenyl, Phenacyl thiocyanate, 1-Pentanone, 1-phenyl, 5-Benzoylpentanoic acid
10.	5.92	434993116.69	18.14	173350741.25	1-phenyl-chlorobutyrophenone, 7-benzoyl heptanoic acid, dichloroacetophenone
11.	7.77	18002871.79	0.75	9083361.60	Cyclo hexasiloxane, hepta siloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, 3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5 (trimethyl silyloxy) tetrasiloxane silane

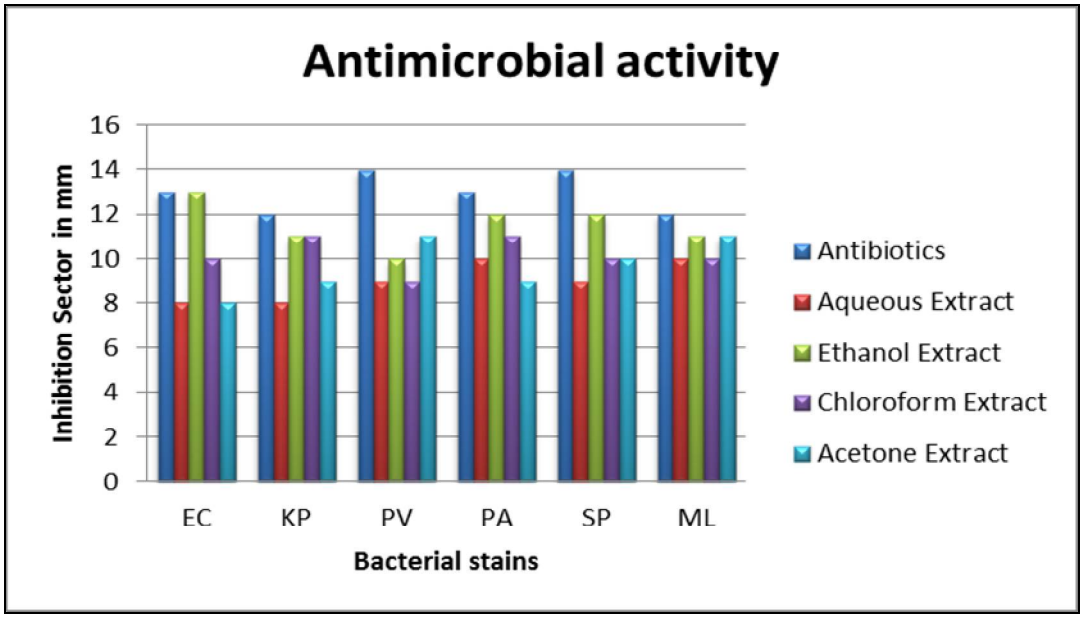
12.	8.10	401535145.13	16.74	96527801.80	Phenol, 2-methoxy-3-(2-propenyl)- 3-Allyl-6-methoxyphenol, Eugenol, Phenol, 2-methoxy-4-(1-propenyl)-, (Z)- trans-Isoeugenol.
13.	8.67	229555454.69	9.57	78850337.74	Caryophyllene,Bicyclo[7.2.0]undec-4-ene, Isocaryophillene, Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl.
14.	12.42	42942231.35	1.79	12398370.93	Dibutyl phthalate, Phthalic acid, butyl hept-4-yl ester, 1,2-Benzene dicarboxylic acid.

Antimicrobial properties of *Plumbago arabica* root extract

Table 3 and graph 4 shows the effects of plant extract on antibacterial activity, applying the disc diffusion method the ethanol extract of PA was examined for its ability to inhibit both Gram positive (G^{+ve}) and Gram negative (G^{-ve}) bacteria. Antimicrobial action is shown by Plumbago arabica root extract demonstrating a significant antibacterial effect against microorganisms. The current research it appears that the sample extracts have antibacterial properties that could be utilized as antimicrobial agents in novel therapeutic medications. Important pharmacological research that results in the creation of more potent medication is the antibacterial property of biochemical substances. Pharmacological analysis and therapeutic antimicrobial activity are used to these medicinally useful plants.

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

Sr.No.	Bacterial isolates	Zone of Inhibition (in mm diameter)				
		Gentamicin (Gram negative and positive)				
		Antibiotics	Aqueous Extract	Ethanol Extract	Chloroform Extract	Acetone Extract
1.	*Escherichia coli*	13	8	13	10	8
2.	***Klebsiella pneumonia***	12	8	11	11	9
3.	*Proteus vulgaris*	14	9	10	9	11
4.	*Pseudomonas aeroginosa*	13	10	12	11	9
5.	*Staphylococcus* ***pneumonia***	14	9	12	10	10
6.	*Micrococcus luteus*	12	10	11	10	11



Graph: 4. Graphical representation for antimicrobial activity of *Plumbago arabica*

CONCLUSIONS

This study was to identify a range of bioactive chemicals extracted from the root of Plumbago arabica using FT-IR and GC-MS analysis, indicating the initial discovery and characterization of these compounds. The bioactive compounds are an extensive spectrum of therapeutic and pharmacological properties. The

study serves as strong evidence of the antimicrobial properties of P. arabica extracts. The bioactive compounds eugenol, trans-isoeugenol, caryophyllene, biocyclo nonane, isoxazole, acetophenone, cyclo hexasiloxane, hepta siloxane, octasiloxane, tetrasiloxane silane, dibutyl phthalate, benzene dicarboxylic acid showed antimicrobial potential. Based on the research findings, it has been discovered that the plant has the ability to create reliable and effective medications for a wide range of illnesses. To identify new medication formulations, further research is necessary to explore their bioactivity and conduct clinical studies.

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