Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(6): 05-10
Received: 05-07-2023
Accepted: 13-08-2023

Seema Dwivedi
Department of Botany, MMV, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Shachi Singh
Department of Botany, MMV, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Jasmeet Singh
Department of Dravyaguna, IMS, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Corresponding Author:
Shachi Singh
Department of Botany, MMV, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Effect of extraction solvent and plant part on the yield of phenolic compounds, plumbagin and biological activity of *Plumbago zeylanica*

Seema Dwivedi, Shachi Singh and Jasmeet Singh

DOI: https://doi.org/10.22271/phyto.2023.v12.i6a.14760

Abstract
Plants have always been important in traditional medicine, providing an extensive range of bioactive chemicals that are still of interest to science today. The present study evaluates the effect of several extraction solvents and plant parts (i.e., leaf, stem, and root) on the production of phenolic compounds, flavonoids, plumbagin and biological activity of *Plumbago zeylanica* L. Bioactive components were extracted using solvents ranging in polarity from non-polar hexane to polar water. The study found significant changes in extract yields depending on the solvent and plant part used, with aqueous extraction giving the most chemicals. Plumbagin, which is predominantly contained in the root, was extracted most effectively with hexane, chloroform, and acetone solvents. Water extracts were notably high in phenolics, whereas ethanolic extracts were high in flavonoids. The study also assessed the biological activities of the extracts, including their antioxidant, antibacterial, and antifungal effects. Results indicated that acetone extracts displayed the highest antioxidant activity, with leaves generally exhibiting superior properties compared to stems and roots. The antibacterial activity against Gram-positive bacteria was observed in hexane, chloroform, and acetone extracts, with plumbagin showing substantial antibacterial potential. In the case of antifungal activity, chloroform and acetone root extracts displayed significant inhibitory effects against *Candida* species, indicating potential use against fungal pathogens. This study focuses on the possible applications of *Plumbago zeylanica* L. and its constituents in medicine, pharmaceuticals, and other industries, highlighting the need of choosing the right solvent and plant part to produce the therapeutic benefits that are desired.

Keywords: Plumbago zeylanica, solvent, flavonoids, phenolic compounds, plumbagin, biological activity

Introduction
Plants have been extensively used for a wide range of pharmacological properties since prehistoric times, and this trend is continuing in the modern era [1]. Many of the medications that are currently available were derived from natural resources. For thousands of years, they have served as the foundation of many traditional medicinal systems, and they continue to supply with innovative treatments for every condition [2]. Pharmaceuticals of natural origin have lower toxicity and increased therapeutic effect, which leads to better patient compliance [3]. Due to fact that written data from historical times is lacking, there is a widespread need to perform a comprehensive study of naturally existing compounds to develop novel therapeutic moieties.

Plumbago is a genus of ten genera and 280 species in the Plumbaginaceae family. *Plumbago indica* L., *Plumbago auriculata* L., and *Plumbago zeylinica* L. are the three primary species of the genus *Plumbago*. *Plumbago zeylanica* L. is the most popular of these species due to its medicinal benefits [4]. *Plumbago zeylanica* L. is a well-known herbal plant that is also known as Ceylon leadwort, doctor bush, and wild leadwort [5]. Ayurvedic names for it include chitramula and Chitrak. Chitrak is a perennial plant grown in shaded areas of the garden for its beautiful inflorescence. It is readily available in India and Sri Lanka [6]. For a variety of therapies, ancient medical systems in various parts of the world used various amounts of *P. zeylanica.* The whole plant has medicinal properties, but the root has higher therapeutic applications [7]. *P. zeylanica* contains numerous secondary metabolites such as alkaloids, flavonoids, naphthaquinones, glycosides, steroids, saponins, triterpenoids, tannins, phenolic compounds, coumarins, carbohydrates, fixed oil and fats, and proteins. The most important active ingredient is 'plumbagin'. Plumbagin (5-hydroxy-2-methyl-1, 4- naphthoquinone-C11-H8-O3) is mostly stored in the root, with a smaller amount in the stem and leaves.

Plumbagin has antibacterial, antifungal, and antimicrobial properties [8]. The nature and amount of secondary metabolites recovered from medicinal plants have been observed to be affected by the solvents used during the extraction process [9]. Polar solvents, are used to extract polar compound for example phenolic compounds, glycosides, and saponins, whereas non-polar solvents are used to extract non polar compound like fatty acids and steroids. Many research have been conducted to determine the effect of various solvents on the content of secondary metabolites and/or their ability to inhibit free radicals in medicinal plant [10]. The majority of *P.zeylanica* research has been on the screening and identification of bioactive chemicals. However, no research has been conducted to investigate the effect of solvent on the extraction of bioactive components from different part of *P.zeylanica* and the biological activity of the extracts. In my research, I have studied the effects of various solvents on the extraction process. At the same time, I have also examined, how the solvent used effects the extraction of various plant parts. Overall, the results of this study provide some importance of solvent selection in the extraction process, as well as the differences in effects found when different parts of the plant are extracted using different solvents.

Materials and Methods

Collection of plant materials and preparation of plant extract

Plant was collected from the Ayurvedic garden of BHU. Leaf, stem and root of *Plumbago zeylanica* was air dried at room temperature. Different parts of the plant were crushed to make fine powder. This powder was subjected to successive extraction by maceration process in different solvents from non-polar to polar: hexane, followed by chloroform, acetone, ethanol and water. Each filtrate was concentrated and dried. These crude extracts were then weighed and kept in refrigerator for further investigation. Yield of each extract was calculated by the following formula:

Extractive yield value = Weight of concentrated extract/ Weight of plant dried powder × 100

Phytochemical analysis

The crude extract obtained with each solvent was diluted in its appropriate solvent to attain a concentration of 5 mg/mL. Following that, this solution was tested for phytochemical evaluation.

Estimation of total phenolic compound

The total phenolic content of the extracts was measured using the Folin-Ciocalteu reagent technique with minor modifications [11]. The total phenolics were expressed as mg/g gallic acid equivalent (GAE) using gallic acid as a standard. Plant extracts (0.5 ml) were diluted with 0.5 mL of methanol and placed in a test tube, along with 2.5mL of a 10fold dilute folin Ciocalteu reagent and 2mL of 7.5% sodium carbonate, and allowed to stand for 30 minutes at room temperature before being spectrophotometrically measured at 760 nm. Every experiment was carried out in triplicate. The folin-Ciocalteu reagent reacts with reducing substances such as polyphenols. It turns blue when exposed to light. This blue colour was spectrophotometrically measured.

Estimation of total flavonoid

The total flavonoid assay was carried out in accordance with Katasani Damodar [12], with minor modifications. The total flavonoid content was calculated using the aluminium chloride colorimetric technique. Each plant extract (0.5 mL) was mixed with 1.5 mL methanol, 0.1 mL 10% aluminium chloride, 0.1 mL 1M potassium acetate, and 2.8 mL distilled water. It was allowed to be kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured using a UV-Visible spectrophotometer at 510 nm. The calibration curve was created by preparing rutin solutions in ethanol at concentrations ranging from 2 to 8 µg/ml. The total flavonoid content was calculated as milligrams of rutin equivalents per gram of dried material.

Estimation of Plumbagin

In a succession of 5ml volumetric flasks, 0.2, 0.3, 0.4, 0.8, and 1 ml of standard stock solution of plumbagin were mixed with 1 ml of 10% alcoholic KOH and volume was built up to the mark with absolute alcohol [13]. The absorbance of the coloured solution measured at 520 nm in comparison to the reagent blank. The absorbance was plotted versus the concentration of plumbagin, and the concentration of the unknown solution was calculated using the calibration equation.

Antioxidant activity test (DPPH radical scavenging activity)

The DPPH radical scavenging assay was carried out using a modified version of the method described by Zhu, *et al.* [14]. Each sample was dissolved in ethanol to yield a concentration of 1 mg/ml, which was subsequently diluted to yield the series concentrations for antioxidant tests. The fractions' free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The stock solution was made by dissolving 25 mg DPPH in 100 mL of ethanol and storing it at 20 °C until needed. Each sample received 2ml of ethanol to generate a volume of 3ml, as well as 1ml of DPPH. The reaction mixture was thoroughly agitated before being incubated in the dark for 30 minutes at room temperature. The absorbance at 517 nm was then measured in comparison to a blank. Ascorbic acid standards have been prepared in a similar manner as positive controls. The sample's inhibition of the DPPH radical was estimated using the following formula:

% Inhibition of DPPH radical = [(absorbance of control − absorbance of sample) / (absorbance of control)] × 100

Antibacterial test

Preparation of culture medium and inoculation

The petriplates and nutritional agar media were sterilised at 1200C for 20 minutes. The second part of the treatment was completed under laminar air flow. Approximately 25ml of the media had been inserted into the sterile petriplates and allowed to solidify. After the medium had set, the bacterial organisms were distributed over it with a spreader.

Disc Diffusion Method

The disc diffusion method was used to investigate the antimicrobial activity of leaf, stem, and root extracts. Sterile nutrient agar plates were created for bacterial strains and inoculated under aseptic conditions using a spread plate approach. The 5 mm diameter filter paper disc (Whatman's No. 1 filter paper) was cut and sterilised. The leaf, stem, and root extracts were produced in specific concentrations (5

mg/ml) and added to each disc of holding capacity. With framing forceps, the sterile impregnated disc with plant extracts was put on the agar surface and gently pressed down to establish complete contact of the disc with the agar surface. Negative controls were filter paper discs saturated in solvent. All of the plates were incubated at 370 °C for 30 minutes. After the incubation period, the zone of inhibition was measured with a calliper [15].

Well diffusion method

Antimicrobial activity of the leaf, stem and root extracts was also tested using the disc diffusion method. Similar to the procedure used in disk-diffusion method, the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a tip, extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [16].

Antifungal test

Preparation of culture medium and inoculation

The Petri plates and the Mueller-Hilton agar medium were sterilized for 20 minutes at 1200C. The rest of the procedure was carried out in laminar air flow. Approximately 25ml of the media was poured into the sterile Petri plate and allowed to get solidified. The fungal colonies were suspended in 2 ml of sterile 0.9% Nacl, and the turbidity was adjusted to yield 1 × 10 5 -1 × 10 6 cells/ml (0.5 McFarland standard). After the media gets solidified the fungal organisms were spreaded on the medium using cotton swab.

Disc diffusion method

Antifungal activity of the leaf, stem and root extracts was tested using the disc diffusion method. Sterile agar plates were prepared for fungal strains and inoculated by a spread plate method under aseptic conditions. The filter paper disc of 5 mm diameter (Whatman's No. 1 filter paper) was prepared and sterilized. The leaf, stem and root extracts to be tested were prepared having particular concentration (5 mg/ml) and were added to each disc of holding capacity. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Filter paper discs soaked in solvent were used for negative controls and Ch-chkotrimazole (Cc 10) as antifugal disc was used as positive control. All the plates were incubated at 28 °C for 24 to 48hours. After incubation, the size (diameter) of the inhibition zones was measured [17].

Result and Discussion

Yield of extract

Extraction yield is affected by the solvent and extraction process [18]. The extraction process used must allow for full extraction of the chemicals of interest while avoiding chemical alteration [19]. In this study, crude extracts were made by macerating dry powder from each plant part with different solvents ranging from polar to non-polar. To acquire the true extraction yield, extracts were further concentrated on a water bath by completely evaporating the solvents. Determining the extraction percentage yield is an important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular solvent. Table 1 shows the yield of extracts obtained from samples using hexane, chloroform, acetone, ethanol, and water as solvents. The yield of plant extracts varies substantially depending on the solvent and plant parts used. Aqueous extraction frequently generated larger yields (leaf = 6.32%, stem = 6.05%, root = 11.22%) than organic solvents ranging from 0.25% to 1.75%. Among the organic solvents, ethanol yields the greatest extract (1.75%), especially in the stem. Hexane and chloroform extract yields are frequently lower (0.52% to 1.71%), with chloroform slightly better than hexane.

Table 1: Yield of plant extract obtained by various organic and inorganic solvent

Type of extract	Parts of plant	Amount of extract (mg/5 g)	Yield % (w/w)
Hexane	Leaf	76.25	1.52
	Stem	15.00	0.30
	Root	12.50	0.25
Chloroform	Leaf	85.00	1.71
	Stem	26.25	0.52
	Root	26.25	0.52
Acetone	Leaf	58.75	1.17
	Stem	35.00	0.70
	Root	43.75	0.87
Ethanolic	Leaf	71.25	1.42
	Stem	87.50	1.75
	Root	70.00	1.40
Aqueous	Leaf	316.25	6.32
	Stem	302.50	6.05
	Root	561.25	11.22

Quantitative estimation of Phenolic and flavonoid and their distribution within different plant parts

Phytochemicals, which are bioactive molecules found in plants, are gaining importance due to their potential health benefits and medical properties [20]. Quantitative phytochemical analysis is an important element of botanical research since it allows for the accurate detection and quantification of these compounds in plant extracts [21]. The concentration of bioactive components in *P.zeylanica* extracts varied significantly in the current study and was depended on the extraction solvent used (Table 2). Among the solvents tested, water appears to be the most effective in extracting phenolic and flavonoids from the leaves, stems, and roots of the plant. The highest levels of phenolic content were identified in water (30.81mg/g) and ethanolic extracts (2.6 mg/g), whereas flavonoid was maximum in ethanolic (0.88 mg/g), and water extract (0.47mg/g). Water is a highly polar solvent that is well-known for its ability to remove phenolic

chemicals [12]. The leaf contained the highest phenolic content (30.81 mg/g), followed by the root (8.56 mg/g) and the stem (6.42 mg/g). The leaf extract with the highest flavonoid content was obtained using water, ethanol, and acetone as the solvent, with concentrations of 0.47 mg/g, 2.6 mg/g, and 0.21mg/g, respectively. Flavonoid concentration in the water extracts of the stem (6.42 mg/g), root (8.56 mg/g), and in ethanol extracts of the stem (0.23 mg/g) and root (0.48 mg/g) was also remarkable. Flavonoids are also more polar chemicals, which is reflected in their extraction patterns [11]. Polar solvents such as ethanol and water were successful at extracting phenol and flavonoids, however non-polar solvents such as hexane and chloroform were not. Because phenol and flavonoids are known for their antioxidant characteristics and possible health advantages [19], the presence of these chemicals in these plant extracts may have implications for a variety of uses in the pharmaceutical, nutraceutical, and cosmetic industries. Phenolics and flavonoids are not evenly distributed throughout plant components. This distribution may be related to the varied biological roles of these compounds in different plant tissues. Plumbagin is a naturally occurring naphthoquinone molecule found in a variety of plant species and has been studied for its possible medical and biological characteristics [8]. Plumbagin was most commonly found in hexane, chloroform and acetone extracts, with acetone root extract having the greatest concentration (0.85mg/g). It was absent or found in minor amounts in other sections and solvent extracts of *P. zeylanica*. The solvent used for extraction has a considerable impact on the plumbagin content of the extracts. This distribution of plumbagin in different plant parts is consistent with earlier research [8], as certain plant secondary metabolites, particularly naphthoquinones like plumbagin, frequently accumulate in specific plant organs, such as the root.

Table 2: Quantitative estimations of phenolics, flavonoid and plumbagin in different parts of *P. zeylanica*

Extract	Part of plant	Total Phenolics (mg/g)	Total Flavonoid (mg/g)	Total Plumbagin (mg/g)
Hexane	Leaf	-	-	-
	Stem	-	-	-
	Root	-	-	0.28
Chloroform	Leaf	-	-	-
	Stem	-	-	-
	Root	-	-	0.39
Acetone	Leaf	-	0.21	0.08
	Stem	-	-	0.10
	Root	-	-	0.85
Ethanol	Leaf	2.6	0.88	-
	Stem	0.9	0.23	-
	Root	1.2	0.48	-
Water	Leaf	30.81	0.47	-
	Stem	6.42	0.30	-
	Root	8.56	0.09	-

Antioxidant activity

The antioxidant activity of plant extracts varies greatly depending on the extraction solvent used [22]. Table 3 shows the antioxidant activity (% inhibition) of *Plumbago zeylanica* extracts from various plant parts using different solvents, as well as the antioxidant activity of two reference compounds, Plumbagin and Ascorbic acid. Acetone appears to be the most effective solvent for extracting antioxidants from plant material, particularly leaves (inhibition 85.34%). Acetone's capacity to remove a wide spectrum of polar and non-polar molecules is consistent with our results. The antioxidant activity varies between different plant parts. Leaves exhibit higher antioxidant activity than stems and roots in all solvent extracts. The varied concentration of antioxidant molecules in different plant tissues could be responsible for this difference. Furthermore, the findings focus on the crucial role of Plumbagin, a component found in *Plumbago zeylanica*, in contributing to its antioxidant effects [23]. More research is needed to isolate and characterise specific antioxidant chemicals found in these extracts, as well as to investigate their potential applications in functional foods, medicines, and natural health products. Antioxidants are essential for protecting cells and tissues against oxidative damage, which has been linked to a variety of health problems, including ageing and chronic diseases [24, 25]. The variance in antioxidant activity reported here highlights the importance of considering both the plant part and the solvent utilised when studying or using plant extracts for antioxidant potential.

Table 3: Antioxidant activity of different parts of *P. zeylanica* in various extract

Extract	Part of plant	Antioxidant activity (%inhibition)
Hexane	leaf	53.45
	stem	30.22
	root	42.56
Chloroform	leaf	46.55
	stem	24.18
	root	37.09
Acetone	leaf	85.34
	stem	35.67
	root	65.78
Ethanol	leaf	29.03
	stem	03.41
	root	10.24
Water	leaf	20.62
	stem	02.56
	root	11.21
Plumbagin	-	89.59
Ascorbic acid	-	97.86

Antibacterial activity

The presence or absence of inhibitory zones was utilized to evaluate the antibacterial activity of *P.zeylanica* extracts against the tested microorganisms. According to Table 4, hexane, chloroform, and acetone extracts significantly inhibited the development of the gram-positive bacteria. In contrast, the water extract was shown to be ineffective against all of the tested bacterial strains. The root extracts of hexane, chloroform, acetone, and ethanol had a strong antibacterial

effect against Gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus. aureus*, with inhibition zones ranging from 8 to 27mm, whereas the leaf extracts with inhibition zone 8 to 10 mm and stem extracts with inhibition zone 6 to10, had a moderate antibacterial effect against these two gram-positive bacteria. The table also includes the results for streptomycin, an antibiotic used as a positive control, an extractive solvent used as a negative control, and the pure chemical plumbagin. According to the findings of the present study, the antibacterial activity of plant extracts differs depending on the extractive solvent and the indicator bacterium studied. *B. subtilis* shows greater sensitivity to plant extracts than *S. aureus.* The variation in susceptibility highlights the importance of include multiple indicators of bacteria in antibacterial research. Plumbagin, the pure chemical tested in this study, shows significant antibacterial activity against both indicator bacteria. This demonstrates that Plumbagin may be a beneficial natural molecule for future research and drug development. Surprisingly, the plant extracts that indicated antibacterial activity against specific bacteria were the same bacteria against which pure Plumbagin demonstrated antibiotic activity. The observed relationship between the antibacterial effects of our plant extracts and pure Plumbagin indicates that the plant's antibacterial activities may be due to the presence of Plumbagin in those part. This suggests that Plumbagin plays an important role in the plant's antibacterial action.

Table 4: Inhibition zone of *P. zeylanica* leaves, stem and root extracts using disc diffusion method and well diffusion method against bacteria species. (n=5)

Extracts	Study of Indicator Test Bacteria	Zone of inhibition of plant extracts (mm)					
		Leaf (5 mg/ml)	Stem (5 mg/ml)	Root (5 mg/ml)	PC (0.5 mg/ml)	NC	Plumbagin (0.5 mg/ml)
Hexane	*Staphyllococcus aureus*	10±1.3	6±0.0	7±0.2	17±1.4	-	30±2.4
	Bacillus subtilis	-	-	-	26±2.2	-	32±2.6
Chloroform	*Staphyllococcus aureus*	8±0.2	-	8±0.4	18±1.5	-	30±1.4
	Bacillus subtilis	10±0.8	-	18±1.5	30±2.6	-	29±1.7
Acetone	*Staphyllococcus aureus*	-	12±1.6	**27**±2.3	17±1.5	-	30±2.2
	Bacillus subtilis	9±0.2	12±1.6	**22**±2.4	26±2.8	-	26±1.4
Ethanol	*Staphyllococcus aureus*	-	-	-	21±2.2	-	20±0.8
	Bacillus subtilis	-	-	12±0.8	30±2.5	-	30±1.5
Aqueous	*Staphyllococcus aureus*	-	-	-	25±1.5	-	-
	Bacillus subtilis	-	-	-	30±2.1	-	-

PC = Streptomycin as positive control, NC = extractive solvent as negative control

Antifungal activity

The results demonstrate that chloroform and acetone root extracts exhibited significant antifungal efficacy against *Candida parasylopsis* (20 mm and 21mm respectively) and *Candida tropicali* (21mm and 21mm respectively) fungal pathogens. Ethanol root extracts also showed minor inhibition, with approximately 18mm zone of inhibition. Regardless of the solvent used, stem and leaf extracts have no inhibitory effect against any of the *Candida* species. Another tested fungal strain, *Candida utilis* was not inhibited by any of the extracts, irrespective of the solvent used for extraction. These results show that the root extracts contain antifungal compounds that are effective against certain *Candida* species. The inhibition zones in response to several solvents (chloroform, acetone and ethanol) for both *C. parasylopsis* and *C. tropicali* were rather similar, with values around 18-22 mm. This suggests that the antifungal compound in the root extracts is relatively stable across solvents. More research and analysis may be required to identify the active compounds responsible for these inhibitory effects and their potential antimicrobial use. Plumbagin, a naturally occurring molecule, on many Candida species. Plumbagin inhibited *C.parasylopsis* (10 mm) and *C.tropicali* (9mm) at the amounts tested. However *C. utilis* has no inhibition zone. This demonstrates that *C. utilis* possesses Plumbagin resistance mechanisms. The fact that both the tested plant extracts and pure Plumbagin exhibit antifungal activity against the same Candida species shows a relationship between the plant extracts' natural compounds and Plumbagin's antifungal capabilities. This relationship demonstrates that the presence of Plumbagin or compounds with similar antifungal activities may contribute to the active antifungal components in plant extracts.

Table 5: Inhibition zone of extracts of various parts of *P.zeylanica* plant and reference compound plumbagin against *Candida* species (n=5)

Extract	Candida species	Inhibition zone (mm)					
		Leaf (5 mg/ml)	Stem (5 mg/ml)	Root (5 mg/ml)	PC 10 µg	NC	Plumbagin (0.5 mg/ml)
Chloroform	*C.utilis*	-	-	-	18±1.2	-	-
	C.parasylopsis	-	-	22±1.2	15±0.5	-	10±0.2
	C.tropicali	-	-	21±0.8	12±0.2	-	9±0.0
Acetone	*C.utilis*	-	-	-	18±0.7		
	C.parasylopsis	-	-	21±1.5	15±0.5	-	
	C.tropicali	-	-	21±1.5	12±0.8	-	
Ethanol	*C.utilis*	-	-	-	18±1.2	-	
	C.parasylopsis	-	-	18±0.8	15±0.4	-	
	C.tropicali	-	-	18±1.2	12±0.0	-	

Positive control (Cc 10), NC= extractive solvent as a negative control

Conclusion

The findings of the study, which include plant extract yield, quantitative phytochemical analysis, antioxidant activity, antibacterial activity, and antifungal activity, provide important insights into the possible bioactive characteristics of *P. zeylanica*. The research shows that all parts of *P.*

zeylanica have the potential to be a source of bioactive compounds with antibacterial, antifungal, and antioxidant properties. The solvent and plant component used have a significant impact on the yield and bioactive chemical content of the extracts. As a result, selecting the appropriate extraction solvent is important for the desired medicinal effect of these extracts. We have also studied how the extractive solvent used affects the biological activity of the extracts. Examining the relationship between solvent choice and biological activity is an important component of our research and contributes to our understanding of the plant's probable uses. More research is needed to identify the active compounds causing these effects and to assess their potential applications in medicine and industry.

References

1. Khan MSA, Ahmad I. Herbal medicine: current trends and future prospects. In New look to phytomedicine Academic Press; c2019. p. 3-13.
2. Ji HF, Li XJ, Zhang HY. Natural products and drug discovery: can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia. EMBO reports. 2009;10(3):194-200.
3. Taylor WRJ, White NJ. Antimalarial drug toxicity: A review. Drug safety. 2004;27:25-61.
4. Shukla B, Saxena S, Usmani S, Kushwaha P. Phytochemistry and pharmacological studies of *Plumbago zeylanica* L.: A medicinal plant review. Clinical Phytoscience. 2021;7:1-11.
5. Akhilraj AR, Rukmini S, Amalraj AR. International Journal of Ayurveda and Pharma Research.
6. Reddy DM, Jain Vikas. An overview on medicinal plants for the treatment of acne. J Crit. Rev. 2019;6(6):7-14.
7. Chetty KM. Pharmaceutical studies and therapeutic uses of *Plumbago zeylanica* L. roots (Chitraka, Chitramulamu). Ethnobotanical Leaflets, 2006, 33(1).
8. Dohare B, Jain B, Khare S, Jain K. Comparative estimation of plumbagin in aerial and root part of *Plumbago zeylanica* using UV-visible spectrophotometric. Pharmaceutical and Biosciences Journal; c2015. p. 09-14.
9. Waris M, Kocak E, Gonulalan EM, Demirezer LO, Kır S, Nemutlu E. Metabolomics analysis insight into medicinal plant science. TrAC Trends in Analytical Chemistry. 2022;157:116795.
10. Dirar AI, Alsaadi DHM, Wada M, Mohamed MA, Watanabe T, Devkota HP. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. South African Journal of Botany. 2019;120:261-267.
11. Maurya S, Singh D. Quantitative analysis of total phenolic content in Adhatoda vasica Nees extracts. International Journal of PharmTech Research. 2010;2(4):2403-2406.
12. Damodar K, Bhogineni S, Ramanjaneyulu B. Phytochemical screening, quantitative estimation of total phenolic, flavanoids and antimicrobial evaluation of Trachyspermum ammi. Journal of Atoms and molecules. 2011;1(1):1.
13. Israni SA, Kapadia NS, Lahiri SK, Yadav G, Shah MB. An UV-visible spectrophotometric method for the estimation of plumbagin. Int J Chem Tech Res. 2010;2(2):856-859.
14. Zhu K, Zhou H, Qian H. Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. Process Biochemistry. 2006;41(6):1296-1302.
15. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. BMC complementary and alternative medicine. 2006;6(1):1-8.
16. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016;6(2):71-79.
17. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal susceptibility testing: current approaches. Clinical Microbiology Reviews. 2020;33(3):10-1128.
18. Jisieike CF, Betiku E. Rubber seed oil extraction: effects of solvent polarity, extraction time and solid-solvent ratio on its yield and quality. Biocatalysis and Agricultural Biotechnology. 2020;24:101522.
19. Zuo Y, Chen H, Deng Y. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta. 2002;57(2):307-316.
20. Chhikara N, Kaur A, Mann S, Garg MK, Sofi SA, Panghal A. Bioactive compounds, associated health benefits and safety considerations of Moringa oleifera L.: An updated review. Nutrition & Food Science. 2021;51(2):255-277.
21. Sultan P, Jan A, Pervaiz Q. Phytochemical studies for quantitative estimation of iridoid glycosides in Picrorhiza kurroa Royle. Botanical Studies. 2016;57(1):1-6.
22. Michiels JA, Kevers C, Pincemail J, Defraigne JO, Dommes J. Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. Food Chemistry. 2012;130(4):986-993.
23. Tilak JC, Adhikari S, Devasagayam TP. Antioxidant properties of *Plumbago zeylanica*, an Indian medicinal plant and its active ingredient, plumbagin. Redox report. 2004;9(4):219-227.
24. Singh S, Kumar M, Dwivedi S, Yadav A, Sharma S. Distribution profile of iridoid glycosides and phenolic compounds in two *Barleria* species and their correlation with antioxidant and antibacterial activity. Frontiers in Plant Science; c2022.
25. Singh S. Isolation and identification of pigment molecules from leaves of prosopis juliflora. International research journal of pharmacy. 2012;3(4):150-152.