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ORIGINAL ARTICLE **OPEN ACCESS**

Screening for Antimicrobial, Antioxidant and Antidiabetic activity of *Plumbago zeylanica* Plant Extracts

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ABSTRACT

Medicinal plants are sources of many potent & powerful drugs. The plant derived drugs are healthier and safer alternate to the synthetic drugs. In the present study P. zeylanica were collected from CCD farm, Kariapatti and processed. Aqueous & petroleum ether extracts of P. zeylanica were obtained using the soxhlet apparatus. Phytochemical analysis documents the presence of eight active components among aqueous & petroleum ether extracts of the plant. Antimicrobial activity of the extract was performed against the gram positive & gram negative organisms. Significant zone of inhibition was observed against the bacterial isolates. Significant antioxidant activity (p<0.0001)was observed in the plant extracts against DPPH scavenging assay & the study suggests that the antioxidant potential could be correlated by the presence of phenolic bioactive components in the plant extracts. The alpha glucosidase inhibition activity was found significant (p<0.0001) among the plant extracts of Plumbago zeylanica with increasing concentrations. HPLC results suggest the presence of photoactive components in the medicinal plant extracts Plumbago zeylanica. The study concludes that P. zeylanica could be a potential antimicrobial and antioxidant agent with rich profile of phytochemical components. Further research need to be expounded about the bioactive compounds of Plumbago species as successful therapeutic tool.

Keywords: *DPPH, α- glucosidase, Plumbago zyeylanica and Phenolic compounds.*

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INTRODUCTION

The genus Plumbago belonging to the family Plumbaginaceae, comprises 10 genera and 280 species [1, 2]. Three main species included in genus Plumbago namely, *Plumbago indica* L., *Plumbago auriculata* L. and *Plumbago zeylanica* L. (Plate 1). Among these species, *Plumbago zeylanica* L. is more popular due to its therapeutic properties. *Plumbago zeylanica* L. usually referred to as Ceylon leadwort, doctor bush and wild leadwort, is one of the well-known herbal plant. (3) It also named as chitramula and chitrak in Ayurveda. Chitrak is a perennial herb cultivated in shady places in the garden for its brilliant inflorescence [2,4,]. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [5]. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria [6]. The plants are the richest source for antimicrobial proteins and peptides and they may be used for industrial extraction and isolation of antimicrobial compounds which may find a place in medicine industry as constituents of antibiotics (7) Reactive oxygen species (ROS) is a collective term that includes both oxygen and non-radicals that are generated during normal metabolic processes [8]. A serious imbalance between the production and scavenging of ROS induces oxidative stress, leading to various diseases, such as allergies, cancer, cardiac and vessel injuries, and infectious and neurodegenerative diseases [9]. Many scientific studies have reported that antioxidants play an important role in reducing the pathological conditions caused by the effects of free radicals, because the antioxidant agents are stable enough to scavenge or deactivate the oxidants [10]. Plants are considered to be a good source of natural antioxidants because they contain a variety of secondary metabolites that have antioxidant capacities [11, 12]. Type 2 diabetes (T2D), characterized by hyperglycemia and abnormal carbohydrate metabolism, is a leading cause of morbidity and mortality worldwide and a major economic burden [13]. T2D which is developed due to insulin resistance and pancreatic β-cell dysfunction, leading to hyperglycemia [4]. The postprandial blood

glucose levels have been found to play an important role in the onset and developing complications of T2D [15]. One of the therapeutic strategies for managing postprandial hyperglycemia involves the inhibition of α-amylase and α-glucosidase [6]. The synthetic drugs such as acarbose and miglitol have strong inhibitory action against α-amylase and α-glucosidase; however, they may result in abdominal distention, flatulence, vomiting, and diarrhea [7]. *Plumbago zeylanica* L., is a pharmaceutically important plant. Present study is subjected to characterize the Phytochemical constituents and assess the *in-vitro* antibacterial, antioxidant and antidiabetic efficacy in the *Plumbago zyeylanica* plant extracts.

MATERIAL AND METHODS

Collection of plant sample and processing

Plumbago zeylanica was collected from CCD herbal nursery, Kariapatti in Madurai and brought to the laboratory in polythene bag and washed several times in clean water to remove dust. After cleaning, the plants were dried in shade at room temperature for one week. The dried plant material were homogenized to fine powder and further subjected to extraction.

Hot extraction method by soxhlet

10 g of plant powder was extracted in 250 ml of solvent (petroleum ether and water) by soxhlet extraction technique overnight at respective solvent temperatures (18). The obtained extracts were subjected to vacuum evaporator to evaporate excess solvents. After that, the dried crude extract yields were weighed and used for further experimental studies.

Preliminary characterization of Phytochemical constituents*:*

The phytochemical analysis of aqueous and petroleum ether extracts of the leaves of *P. zeylanica* were studied by slight modifications based on standard procedures described on different literatures. (19,20,21).

Antimicrobial Activity

Estimation of the antimicrobial potential is the first step towards finding the therapeutic value of the medicinal plant. Using the agar well diffusion method, the antimicrobial activity of Plumbago zyeylanica plant extracts against the common pathogens such as Escherichia sp., Salmonella sp., Shigella sp., Vibrio sp., and Pseudomonas sp., was estimated.(22)

In-vitro Antioxidant Activity:

DPPH Free Radical Scavenging Assay

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Free radicals scavenging activity of aqueous and pet-ether extracts of leaves of P. zeylanica plant were measured by DPPH. 0.1mm solution of DPPH in ethanol was prepared. This solution (1ml) was added to 1ml of different extracts in ethanol at different concentrations (50, 100, 150, 200, 250 µg/ml). Here, only those extracts are used which are solubilize in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, absorbance was measured at 510nm by using UV-spectrophotometer. (23)Reference standard compound being used was ascorbic acid and the experiment was done in triplicate. The percent DPPH scavenging effect was calculated by using the following equation

$$Inhibition\ (\%) = Ac - As\ /\ Ac \times 100$$

Where,

As – Absorbance of the sample

Ac- Absorbance of the control (ethanol &DPPH radical solution)

In-vitro Antidiabetic Activity:

α – Glucosidase Inhibiton Assay:

The a-glucosidase inhibitory assay was adapted from Li et al. (24) with modification. The yeast α-glucosidase was dissolved in 100mM of phosphate buffer of pH 6.8 and was used as the enzyme extract. 50µl of 5mM P-nitrophenyl-α-D-glucopyranoside (PNPG) in 100 mM PBS was used as the substrate. Plant extracts were used in the concentration ranging from 100-400 µg/ml. Different concentrations of plant extracts were mixed with 320 µl of 100mM PBS (pH 6.8) at 30°C for 5 minutes. 200 µl of 50mM sodium hydroxide was added to the mixture to stop the reaction and the absorbance was read at 405 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula

$$Inhibition\ (\%) = \frac{Abs_{405}\ (control) - Abs_{405}\ (extract) * 100}{Abs_{405}\ (control)}$$

The Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

HPLC Analysis:

For HPLC analysis the extract was centrifuged at 3000 rpm for 10 minutes and then filtered through whatmann No.1 filter paper using high pressure vaccum pump. The sample was diluted to 1:10 with the same solvent. To detect the UV-VIS spectrum profile of the crude extracts of *P. zeylanica*, the extracts were scanned in the wavelength ranging from 200-1100nm by using Shimadzu spectrophotometer and the characteristics peaks were detected. HPLC method was performed on Shimadzu LC-10 AT VP HPLC system, equipped with a model LC-10 AT pump.

Statistical Analysis

Statistical significance was analyzed by one-way analysis of variance (ANOVA) by Turkey HSD test using vassarstats tool p-(<0.0001). Values of <0.0001 were considered as statistically significant.

RESULTS AND DISCUSSION

Table 1 shows the identified phytoconstituents from Plumbago zeylanica extracts. Preliminary phytochemical analysis was carried out on solvents such petroleum ether and aqueous. Aqueous extracts shows positive results of phenolics, tannins, flavonoids, phytosterols, triterpeniods, carbohydrates, saponins and betacycine. On the other hand cabohydrates, phenolics, phytosterols and triterpeniods alone present in the Petroleum ether extracts. Phytochemical screening of *Plumbago zeylanica* shows that the plant have abundant amount of secondary metabolites [25]. Number of secondary metabolites such as naphthoquinones flavonoids, alkaloids, glycosides, saponins, steroids, tannins, triterpenoids, coumarins, carbohydrates, phenolic compounds, fixed oils, fats and proteins reported to be present in *Plumbago zeylanica L.* [26, 27]. The aqueous and petroleum ether extracts of P. zeylanica showed better antibacterial activity against clinical isolates *Escherichia sp., Salmonella sp., Shigella sp., Vibrio sp., and Pseudomonas sp.* (Table 2). The aqueous extract shows a significant zone of inhibition against the bacterial strains compared to the pet-ether extract. The antibacterial activities of the leaves were due to the presence of various secondary metabolites [28]. This study revealed that the antibacterial activity of the extracts enhanced with increasing concentration. The antioxidant activity of petroleum extracts was found between 10 and 50%, with the maximum of 45.5% in petroleum ether extract and a minimum of 11.59% in the aqueous extract of *P. zeylanica*. (Fig 1) Antioxidant activity was higher with an increase in the concentration of plant extracts and to found to be significant (*p<0.0001)* in *in-vitro* condition. A fair correlation between total phenolic content and antioxidant activity was also observed. These observations clearly indicated a cross linkage between phenolics and antioxidant activity [29]. Yohannes Weldemariam Getahun and his co-workers also examined the radical scavenging activity of chloroform and ethanolic crude extracts of *P. zeylanica*. The ethanolic extracts showed high percent of radical scavenging activity than chloroform crude extracts and comparable with that of standard ascorbic acid at higher concentration [30]. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes [31]. The antidiabetic activity of aqueous and petroleum ether extracts was found to be between 10-40% with the maximum of 36.06% in petroleum ether extract and with the minimum of 11.40% in the aqueous extract of *P. zeylanica.* (Fig 2) Antidiabetic activity was higher with an increase in the concentration of plant extracts. This study tends to show that extracts of *Plumbago zeylanica* show a significant ***(***p<0.0001) *in-vitro* inhibitory effect on *α*-glucosidase*.* It indicates that this plant extract could be useful in the management of postprandial hyperglycemia. The antidiabetic activity of plumbagin derived from the root of Plumbago zyeylanica L. and its implication on GLUT4 translocation in STZ-induced diabetic rats was performed by Christudas *et al*.(32). The HPLC profile of petroleum ether extract of *P. zeylanica* shows the maximum number of peaks, which indicates the presence of phytoactive components. The aqueous extracts of *P. zeylanica* were found to have a minimum of 3 peaks (Fig 3, 4). Thus, the results represent the presence of phenolic and other bioactive compounds in these plant extracts. *Plumbago zeylanica* L. exhibits a broad range of pharmacological activities, which include antibacterial, antifungal, anti-inflammatory, antidiabetic, anticancer, antioxidant, hepatoprotective, cytotoxic, and wound healing.

Table 1: Qualitative phytochemical composition of the *aqueous and petroleum ether of Plumbago zeylanica*

Secondary Metabolites	*Extracts*	
	Aqueous	*Petroleum Ether*
Carbohydrates	***+***	***+***
Flavonoids	***+***	***-***
Phenolics	***+***	***+***
Tannins	***+***	***+***
Triterpeniods	***+***	***+***
Saponins	***+***	***-***
Steroids	***+***	***+***
Alkaloids	***-***	***-***
Glycosides	***-***	***-***
Betacyanine.	***+***	***-***

Table 2: Antimicrobial activity of aqueous and petroleum ether extracts of *Plumbago zeylanica*

S.No	*Bacterial Isolates*	*Zone of Inhibition (in mm)*	
		Aqueous (10 mg/ml) 40µl	*Petroleum ether (5mg/ml) 40µl*
1.	***Streptococcus sp.***	***10***	***8***
2.	***Vibrio sp.***	***12***	***7***
3.	***Escherichia sp.***	***13***	***10***
4.	***Salmonella sp.***	***16***	***13***
5.	***Pseudomonas sp.***	***18***	***11***

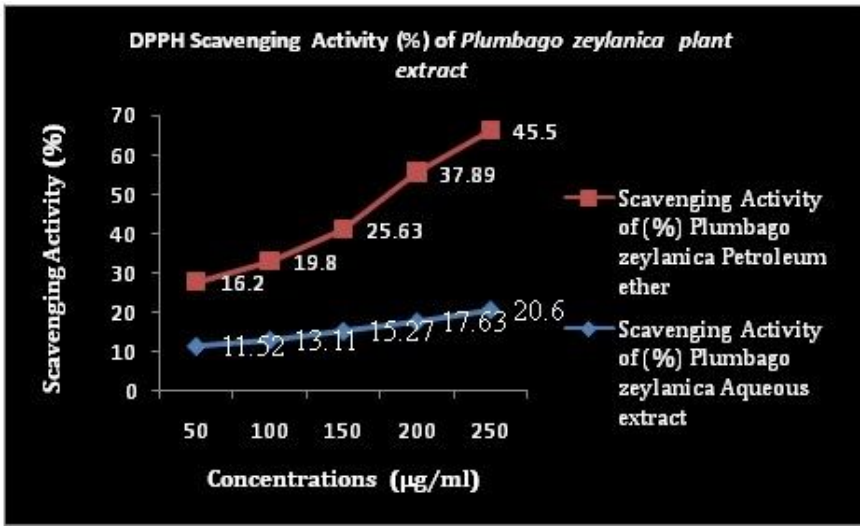

Fig 1. *In-vitro* Antioxidant Activity (%) of *Plumbago zeylanica* using DPPH scavenging assay

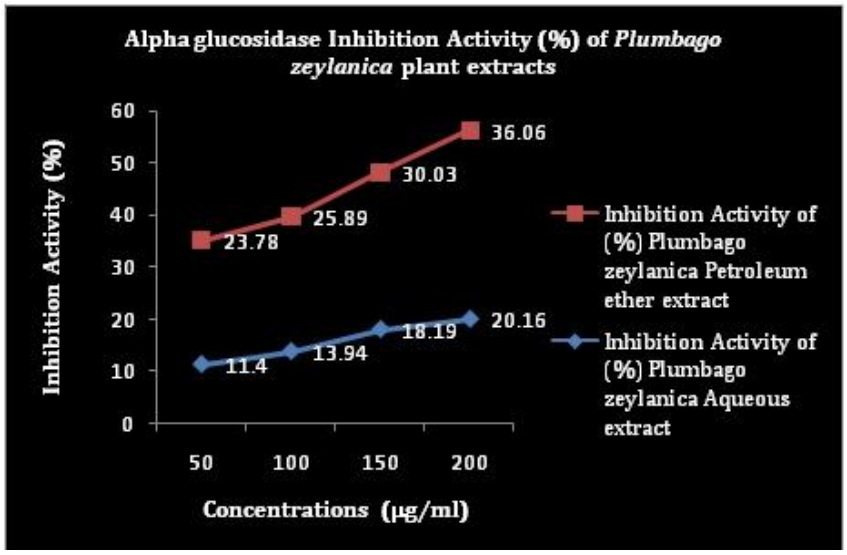

Fig 2. *In-vitro* Anti-diabetic Activity (%) of *Plumbago zeylanica* using alpha glucosidase inhibition assay

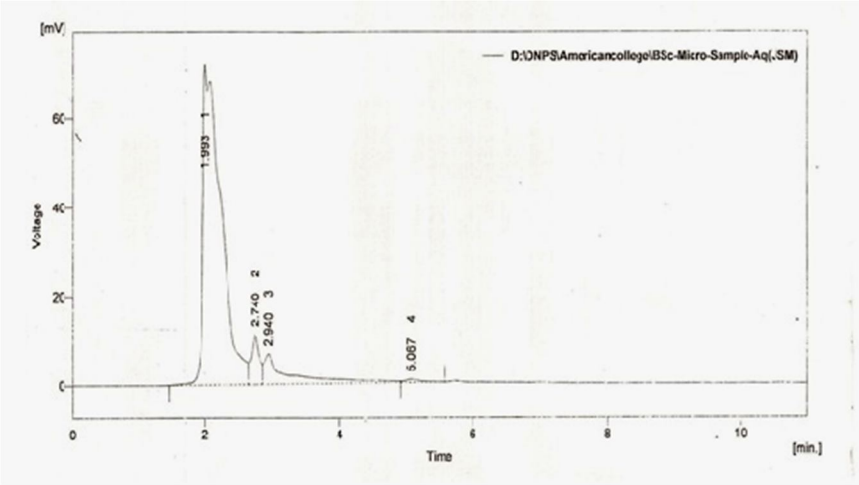

Fig 3. HPLC Profile for *P. zeylanica* (aqueous extract)

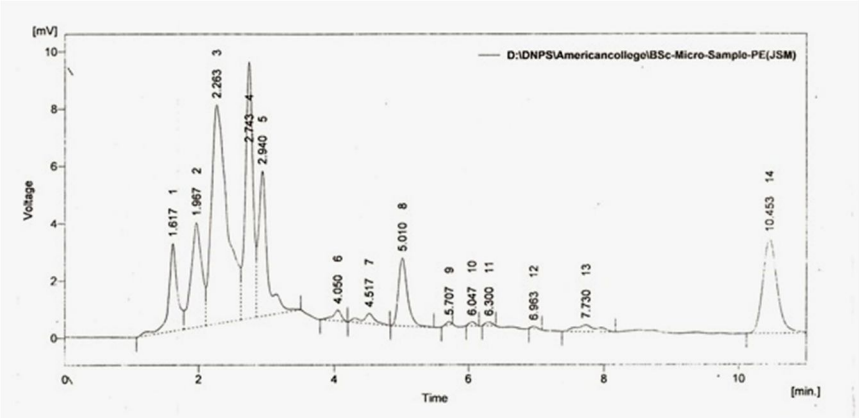

Fig 4. HPLC Profile for *P.zeylanica* (Petroleum ether)

Plate 1: *Plumbago zeylanica* plant

CONCLUSION

This study explored the traditional knowledge, phytochemistry, and pharmacological applications of the plant *Plumbago zeylanica.* It is chemically rich with its diverse content of active compounds, such as carbohydrates, tannins, phenols, flavonoids, triterpenoids, saponins, steroids and phytosterols constituents as multi-purpose medicinal agents. The results of the present study showed that *Plumbago zeylanica* L. has great potential to be integrated into conventional medical practice for the treatment of various health complications like oxidative stress, diabetes, and for the management of emerging multidrug-resistant organisms. Further research on the other bioactive components of *P.* zeylanica and *in-vivo* studies might explore more knowledge of its therapeutic value and would also have a noticeable socioeconomic impact.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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