

***In-vitro* antimicrobial screening with phytochemical study of *Plumbago zeylanica* L. collected from two regions of Eastern Himalayas- A comparative study**

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Abstract

Geographical variation plays an important role in the phytoconstituents of the plant species. The present study was an investigation of phytochemical and antimicrobial properties of a same species of plant *Plumbago zeylanica* L. collected from two different Eastern Himalayan regions (Sikkim and Assam). The solvent used for extraction of plants were chloroform, acetone and ethanol. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, triterpenoids, gum, mucilage, fatty acids, protein and saponine in both region plants. The qualitative phytochemical study revealed important information about the different phytoconstituents content present in the various extracts of same plant collected from two different regions. The *in-vitro* antimicrobial evaluation was carried out by agar disc diffusion method and it was performed on microbial strains like gram negative species *Salmonella typhi* and *Pseudomonas aeruginosa* and gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Standard antibiotic Amoxicillin was used as a reference drug to screen the antimicrobial properties of extracts. Ethanolic extract exerted the maximum activity in both regions plants. The ethanol extracts of both region plants showed significant antibacterial activity against all the organisms, while chloroform and acetone fractions showed moderate activity. As this was a comparative study so, the interesting finding was that Assam plant extract was having more potency especially ethanol extract in comparison to Sikkim plant extracts. Therefore, the detail result and analysis of this study helped to distinguish the differences in characters with special attention towards antimicrobial properties of *P. zeylanica* L. species collected from these two Himalayan regions.

Keywords: *Plumbago zeylanica* L., Comparative, Phytochemical, *In-vitro*, Antimicrobial, Amoxicillin

1. Introduction

The irrational and increasing use of antibiotics and poor patient compliance has led to the development of bacterial resistance to antibiotics. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost.¹ *Plumbago zeylanica* L. belongs to the family Plumbaginaceae commonly known as Chitraka having pungent, astringent, diuretic, germicidal, antiviral, antiseptic ethno-medicinal properties.² The leaves and roots of *P. zeylanica* contains an alkaloid called plumbagin, which possesses most of the therapeutic effects.³ In India it is usually used to treat fever or malaria. Pharmacological studies have indicated that *P. zeylanica* extract has antiplasmodial, antimicrobial, antifungal, anti-inflammatory, antihyperglycemic, hypolipidaemic and antiatherosclerotic activities.⁴ Geographical variation plays an important role in the phytoconstituents of the plant species, thus it leads to differentiation in their therapeutic potency. This present investigation was done to reveal the variation in phytoconstituents and pharmacological activity of the plant *Plumbago zeylanica* collected from the different regions of Eastern Himalayas. A detail Pharmacognostical, phytochemical comparison has not been carried out on *P. zeylanica* so far, hence the present study is carried out. In this study the antimicrobial activity of *P. zeylanica* ethanolic leaf extracts were assessed *in-vitro*.

2. Materials and Method

2.1 Collection of plant materials and Authentication

The plant material was collected in the month of August 2013 from Gangtok, Sikkim and in the month of July 2013 from Ambagan, Nagaon, Assam, then taxonomically authenticated with the help of standard literature in Botanical Survey of India (BSI), Gangtok, Sikkim, India and the authentication no was SHRC-5/02/2012-Tech.54.

2.2 Preparation of plant extract:

The dried plant material was pulverized into fine powder using mixer grinder. About 70g of powdered material was extracted in Soxhlet extraction apparatus with 200ml of each of the following solvents; chloroform, acetone and ethanol.⁵ The extracts were further processed by evaporating the solvent (at 40°C) with the help of rotary evaporation (Buchi Rotavapor 011; Brinkmann Instruments, Inc., Westbury, USA). The sticky greenish-brown substances were obtained and stored in refrigerator for prior to use.⁶ The extracts of each solvent were used for the qualitative phytochemical screening for the detection of the various classes of phyto-constituents, using standard methods.^{7,8} All the dried extracts were exposed to UV rays (Camag Universal UV lamp TL-600) (200-400 nm) for 24 h and sterility was checked frequently by streaking on nutrient agar plates.

2.3 Preparation of microorganism

Isolation of bacterial species of Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Salmonella typhi* and *Pseudomonas aeruginosa*) were taken place. The gram staining and biochemical test of cultures were performed to check the purity and they were grown in nutrient broth at 37°C and maintained in nutrient agar slants at 2-8°C. In this antibacterial assay nutrient agar media was used as bacterial culture medium.

2.4 Preparation of microorganism

To ensure the activity of standard antibiotic against the test organisms, Amoxicillin (30µg/disc) discs were used as positive control and as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. The purity of the antibiotic was found to be 99.8%.

2.5 Dilutions and Inoculum preparations

The dried plant extracts of *P. zeylanica* and antibiotic Amoxicillin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 50, 100mg/ml. The inoculums of *S. aureus*, *B. subtilis*, *S. typhi* and *P. aeruginosa* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hours. The cultures are stored in the refrigerator at 2-8°C for analysis after growth was observed.

2.6 Antimicrobial Screening

Agar diffusion method using a paper disc was performed for anti microbial screening.^{9,10} The required amount of Petri plates was prepared and autoclaved at 121°C for 15 minutes and these were allowed to cool under laminar air flow. About 20 ml of media into each sterile Petri dish was aseptically transferred and allowed to solidify. 1ml inoculums suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different concentrations of about 50, 100mg/ml of plant extracts of *P. zeylanica* of both the region separately and antibiotic Amoxicillin into each separate disc of about 100µl. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm) and the data were compared for both region plant extract.

3. Results and Discussion

3.1 Percentage yield of extract and Phytochemical screening

The % yield of various extract of Sikkim region plant were 2.077% w/w, 0.948% w/w, 6.3976% w/w for chloroform, acetone and ethanol respectively. On the contrary, for Assam region plant these were 0.577%w/w, 0.848%w/w, 6.8106%w/w for chloroform, acetone and ethanol respectively. The results of qualitative screening of phytochemical constituents in the leaves of *P. zeylanica* revealed the presence of alkaloids, glycoside, flavonoids, saponins, simple phenolics, fatty acids, tannins and mucilage (Table 1).

Table 1- Phyto-chemical screening of extracts of Sikkim and Assam region plant

Test	For Sikkim Plant			For Assam Plant		
	C	A	E	C	A	E
Alkaloids	-	-	+	-	-	+
Carbohydrates	-	-	+	-	-	+
Glycosides	+	-	+	+	-	+
Flavonoids	-	-	+	-	-	+
Fixed oil and fats	-	-	-	-	-	-
Phenolic compound and Tannins	-	-	+	-	-	+
Saponins	-	-	+	-	-	+
Proteins and Aminoacids	-	+	+	-	+	+
Phytosterols	+	+	-	+	+	-
Gums and Mucilage	-	-	-	-	-	-

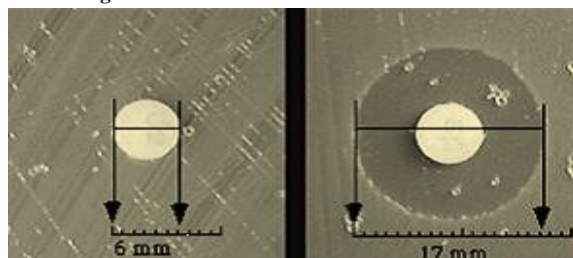
(+) - Present, (-) – Absent, C- Chloroform, A- Acetone, E- Ethanol

3.2 Antimicrobial Activity

Antimicrobial action of Sikkim and Assam plant extracts was expressed through graph. The ethanol extract of Sikkim plant showed best zone of inhibition of 17mm diameter against *P. aeruginosa* and the least activity of 5mm diameter was recorded by acetone extract against *S. typhi* at 100mg/ml concentration. Similarly, in case of 50mg/ml concentration of extracts the best response showed by ethanol extract, where the diameter of zone of inhibition was found to be 10mm against *P. aeruginosa* and least activity was 3.5mm against *S. typhi* by acetone extract.

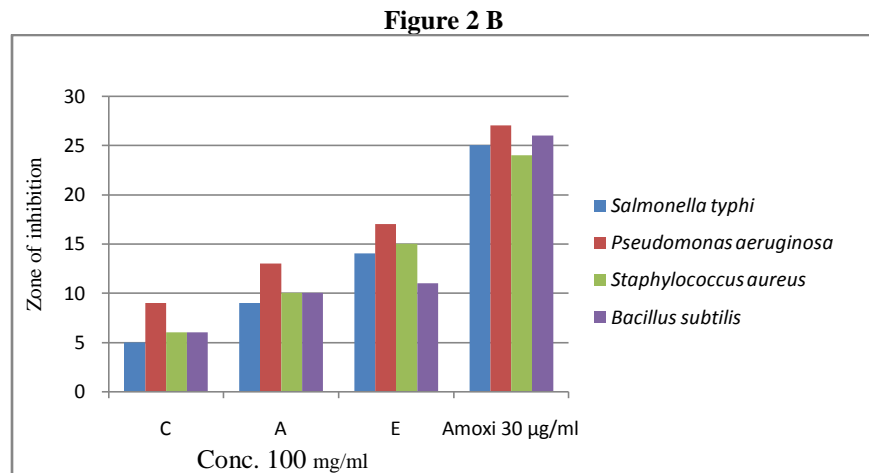
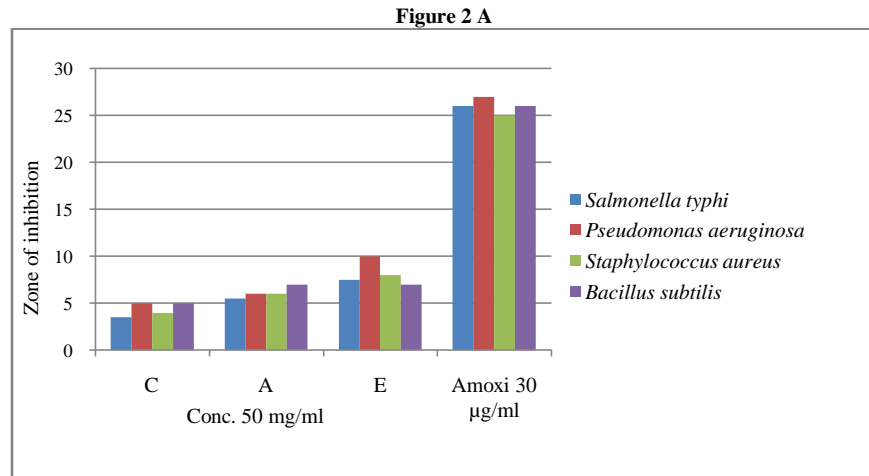
Similarly, the best zone of inhibition of 17mm diameter against *P. aeruginosa* (Figure 1) was recorded by ethanol extract and least activity of 5mm diameter against *S. typhi* and *S. aureus* at 100mg/ml concentration was recorded in case of acetone extract. Likewise, ethanol extract of 50mg/ml concentration showed the best activity as the diameter of zone of inhibition was found to be 10mm against *P. aeruginosa* and acetone extract exhibited least activity of 3.5mm zone of inhibition against *S. typhi*.

Fig 1: Determination of clear zone of inhibition



3.2.1 Antibacterial efficacy of different solvent extracts of *Plumbago zeylanica* leaves of Sikkim region

Figure 2 (A&B): Antibacterial efficacy of different solvent extracts at 50mg/ml (A) and 100mg/ml (B) conc. of *P. zeylanica* leaves of Sikkim region plant.



3.2.2 Antibacterial efficacy of different solvent extracts of *Plumbago zeylanica* leaves of Assam region

Figure 3 (A&B): Antibacterial efficacy of different solvent extracts at 50mg/ml (A) and 100mg/ml (B) conc. of *P. zeylanica* leaves of Assam region plant.

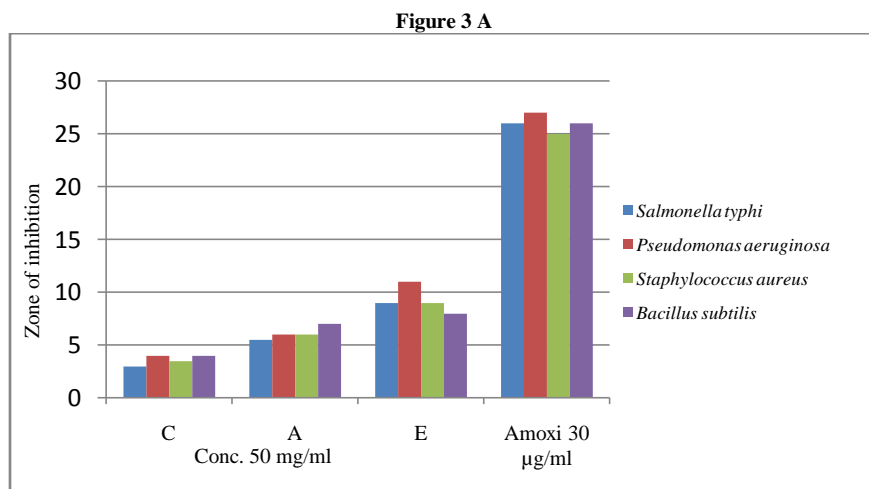
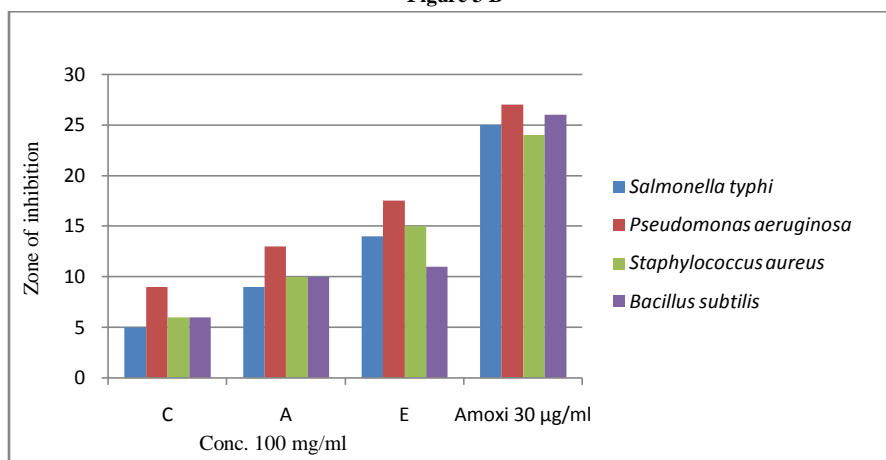


Figure 3 B



4. Discussion

An attempt had been initiated to evaluate the comparative phytochemical parameters of *P. zeylanica* plant of Sikkim and Assam regions. Alkaloid, carbohydrate, protein and amino acids and flavonoids were found positive in acetone and ethanol extract of both region plants, while glycosides were present in chloroform and ethanol extracts only. Oils and fats, gum and mucilage contents were absent in all the studied extract. Phenolic contents and Saponins were positive in ethanol and acetone fractions. From this experimental data it was found that ethanol extracts of both region plants contained most of the phytoconstituents. These metabolites have been reported to show antimicrobial activity.¹¹

The qualitative phytochemical investigation gave valuable information about the different phytoconstituents present in the various extracts of same plant collected from different regions which may help regarding the selection of the particular extract for further investigation of isolating the active principle.

In this experiment all the extracts of *P. zeylanica* Linn. were tested against selected Gram-negative and Gram-positive bacteria. Activities of the various extracts were comparable to those of standard antibacterial agent Amoxicillin. In the present antimicrobial activity of both plant extracts towards drug resistant or clinically significant microbes were reported and it was noted that active constituent of plant material seep out in organic solvent to display biological activity.

The ethanol extracts of both region plants showed significant anti bacterial activity while chloroform and acetone fractions showed moderate activity against all the organisms. Ethanol extract of both region plants showed highest zone of inhibition against *P. aeruginosa* and least zone of inhibition was noted against *S. typhi* by acetone extracts.

Interestingly, this comparative study revealed an important fact that Assam plant extracts were more potent especially ethanol extract in comparison to Sikkim plant. The geographical variations might be the reason of these differences which lead to differences in active metabolites in plants.

4. Conclusion

This comparative study does stand a link between the phytoconstituents and antimicrobial activity of *P. zeylanica* L. collected from different regions of Eastern Himalayas. The study showed that the chloroform, acetone and ethanol extract of *P. zeylanica* L. have anti-microbial action. A conclusion can be made on behalf of this comparative study that according to the geographical variations of plant species their pharmacological action also varies.

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