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INVESTIGATION ON PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF THE LEAF AND STEM EXTRACTS OF *IRESINE HERBSTII*

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ABSTRACT

Many plants have been known to synthesize active secondary metabolites to protect themselves from microbial attack which have potential therapeutic applications. The soxhlet extracts of leaf and stem of *Iresine herbstii* using ethanol, acetone, dichloromethane and petroleum ether were investigated for their phytochemical and antibacterial activity against pathogenic bacteria and compared with the standard antibiotics. Preliminary qualitative phytochemical screening of the extracts was carried out to reveal the phytochemicals constituents of the plant. Antibacterial activity was performed by using agar well and disc diffusion methods. Phytochemical screening exposed that leaf and stem contained alkaloids, carbohydrates, flavonoids, glycosides, phenols, phytosterols, proteins, resins, saponins, tannins and thiols. The results indicated that the leaf ethanolic extract was found to be most effective against pathogenic bacteria at varying zone of inhibition (10.20±0.50 to 15.13±0.33 mm). The results revealed that leaf extracts were found to be more effective than stem extracts.

KEYWORDS: ***Iresine herbstii,*** **phytochemicals, pathogenic bacteria**

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INTRODUCTION

Plants have been the earliest companion of mankind, providing food, shelter and serving humanity in curing different ailments[1]. Several medicinal plants in India are used in the form of crude extracts, infusions or plaster to treat common infections without scientific evidence[2]. Researchers have revealed that phytochemicals present in the plant not only protect themselves but also save humans from harmful diseases[3]. Medicinal plants are used as food, flavors, cosmetic, ornamental, fumigants, insect deterrents and medicine and they are the best resources from which novel bioactive substances are discovered[4]. The most important bioactive compounds of these plants are alkaloids, flavanoids, tannins and phenolic compounds[5]. Nowadays, scientists are forced to search for newer anti microbial substance from various sources including medicinal plants because of the less availability and high cost of new generation antibiotics as well as increase in number of infectious diseases due to multiple drug resistance bacterial strains[6].

Iresine herbstii belongs to *Amaranthaceae* family and commonly known as blood leaf, chicken gizzard, beefsteak plant and herbst's blood leaf (English), Naayurivi (Tamil). Bloodleaf, probably first collected in Brazil, is native to tropical South America. But it is also available in the tropical forest in several parts of India and tropical Asia and has multiple applications in different folk medicine. *I. herbstii* is traditionally used in the Northern Peruvian Andes for black magic with the ritual aim to expel bad spirits from the body[7], and also to diagnose various illnesses. *I. herbstii* was reported as an additive of ayahuasca[8] and ingredient of san pedro decoction, with possible hallucinogenic properties[9]. *I. herbstii* leaves are used in wound healing, anticancer agent, post-labor tonic[10], and externally against skin depurative such as eczemas, sores and pimples[7]. Moreover, the plant is also used in astringent, diuretic, spasmolytic, whooping cough, antimicrobial agent and roots in hemicranias[11]. Leaves and flowers are used in decoction, for fever and kidney problems [12] and also as a relaxant and antipyretic[7]. Literature survey revealed that there is no previous report on the antibacterial activity and phytochemical analysis of *I. herbstii*. Therefore, the present study was undertaken to report the phytochemicals present in the plant and its antibacterial activity.

MATERIALS AND METHODS

I. Chemicals

All solvents- ethanol, acetone, dichloromethane and petroleum ether were purchased from Merck, India. Nutrient agar, Mueller-Hinton broth, Mueller-Hinton agar was purchased from Hi-Media, India. All chemicals used in the study were of analytical grade.

II. Collection and processing of plant samples

Healthy, disease free leaf and stem of *I. herbstii* were collected during the month of May 2010 in and around the villages of Bankura district of West Bengal, India. Plant with complete herbarium was identified and authenticated from Post Graduate and Research Department of Botany, PSGR Krishnammal College for Women, Coimbatore, South India. The collected leaf and stem were washed properly in the tap water followed by detergent water and finally rinsed with distilled water until no foreign material remained (damaged leaves were removed). The fresh plant materials were left to dry in a closed room (25-28ºC) for approximately five days. The dried plant parts were pulverized to obtain a powder by using sterile electrical blender. The powdered samples were stored in air tight container, protected from sunlight for further use.

III. Extract Preparation

Twenty five grams of powdered plant materials were continuously extracted with different solvents like ethanol, acetone, dichloromethane and petroleum ether for successive solvent extraction based on polarity using soxhlet extraction apparatus at the boiling point of the respective solvents for 12-16 h or until the colour of the extracted solvent become clear. Different extracts were concentrated under reduced pressure using rotary evaporator and they were poured into a pre-weighed vial, further dried in a desiccating chamber until a constant dry weight was obtained. The extract vials were stored at 4ºC for further studies. The yield from 100 gm of dried powdered material was calculated as follows:

$$\text{Product yield} = \frac{\text{Amount of product}}{\text{Amount of sample added}} \times 100$$

IV. Phytochemical Analysis

Phytochemical analysis of the leaf and stem extracts of *I. herbstii* were analyzed using standard qualitative methods as described by Harborne and Baxter[13], 1995 and Kokate et al[14], 2003. The components were analyzed for phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, phytosterols, proteins, resins, saponins, tannins and thiols.

V. Antibacterial Screening

a. Bacterial pathogens and their growth conditions

The pathogens *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were obtained from Microbiology laboratory, Kovai Medical Center and Hospital (KMCH), a 500 bed multispecialty hospital, Coimbatore, South India. The strains were maintained on nutrient agar slants at 4°C.

b. Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) that were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh Mueller-Hinton broth to achieve optical densities corresponding to 0.5 i.e. 10^5 to 10^6 CFU/ ml using McFarland's standard

c. Agar well diffusion method

A Mueller-Hinton agar plates were swabbed on three axis with sterile cotton tipped swab, which were dipped in the freshly prepared diluted culture. A 6 mm hole was bored aseptically using sterile cork borer. The agar plugs were taken out carefully, so as not to disturb the surrounding medium. The holes were filled with different concentrations of leaf and stem extracts (50, 100, 150, 200 and 250 mg/ml) dissolved in 10% dimethyl sulfoxide (DMSO) and allowed to stand for one hour for perfusion of the extracts and kept for incubation at 30°C for 24 h. After incubation, the petriplates were observed for the antibacterial activity and measured in terms of diameter in millimetre of the inhibition zone. DMSO (10%) were used as negative control and the antibiotics like kanamycin (30 µg/disc), norfloxacin (10 µg/disc), ciprofloxacin (5 µg/disc) were used as positive control.

VI. Statistical Analysis

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD.

RESULTS AND DISCUSSION

a) Yield of plant extracts in different solvents

The obtained yield of the plant extracts varied from each plant parts as well as with the

different solvents used for the extraction (Table 1). It was found that among the leaf and stem extracts of *I. herbstii*, ethanolic extract of leaf exhibited highest yield (3%), whereas the lowest yield was obtained against petroleum ether extract of stem (0.5%). The differences in the extract yields from the tested plant materials in the present analysis might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants[15]. Among other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds might also be very important[16].

Table 1
Yield of leaf and stem extracts of I. herbstii in different solvents

Solvents used	% Yield from plant powder in gm	
	Leaf	Stem
Ethanol	3.6	2.3
Acetone	3.2	1.8
Dichloromethane	1.2	0.7
Petroleum Ether	0.85	0.5

b) Phytochemical investigation of leaf and stem extracts of I. herbstii by biochemical methods

Phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, phytosterols, proteins, resins, saponins, tannins and thiols (Table 2). All the phytochemicals were present in aqueous extracts of leaf but in case of stem aqueous extracts, saponins and resins were absent. Similarly, in the case of ethanolic extract of leaf all the phytochemicals were present but in the petroleum ether extract only alkaloids and glycosides were present. Saponins, phenols, resins and thiols were absent in ethanolic extract of stem but petroleum ether extracted only alkaloids and flavonoids. As far as *I. herbstii* is concerned, the presence of carbohydrates in all extracts except petroleum ether extract of leaf and stem needs a special attention. Sugar not only aids in providing energy and building plant body but also they play a vital role in ecological balance like plant-environment interaction[17]. Flavonoids have been reported to have useful properties including anti-inflammatory, enzyme inhibition and antimicrobial activity[18]. Phenolic compounds possessed anti inflammation as well as antimicrobial, antiviral and anticancer property[19]. Steroids and saponins are the subgroup of tri-terpenoids, which indicates the presence of tri-terpenoids in *I. herbstii.* Tannins present in ethanol and aqueous extract have antimicrobial effect with increasing concentration and because of their astringent property; they serve as barrier to herbivores[20]. In plants, these secondary metabolites function to attract beneficial and repel harmful organisms, serve as phytoprotectants and respond to environmental changes. However, in humans, the compounds have beneficial effects including antioxidant, anti-inflammatory effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of steroid metabolism and antibacterial and antiviral effects[21]. From table 2, it is clear that the extraction of phyto-constituents gradually reduced with the polarity of solvents used for extraction.

Table 2
Qualitative analysis of phyto-constituents various extracts of leaf and stem of I. herbstii

Compounds	Leaves extracts				Stem extracts			
	Ethanol	Acetone	DCM	Ether	Ethanol	Acetone	DCM	Ether
Alkaloid	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	-	+	+	+	-
Flavonoid	+	+	+	-	+	+	+	+
Glycoside	+	+	-	+	+	+	+	-
Phenol	+	+	-	-	+	-	-	-
Phytosterol	+	+	+	-	+	+	-	-
Protein	+	+	+	-	+	+	+	-
Resin	+	-	+	-	-	+	-	-
Saponin	+	-	+	-	-	+	-	-
Tannin	+	-	-	-	+	-	-	-
Thiol	+	+	-	-	-	-	+	-

'+' represents presence of the phytoconstituent
'—' represents absence of the phytoconstituent

c) Antibacterial activity of leaf and stem extracts of I. herbstii

In the present study, antibacterial effect of the crude extracts of *I. herbstii* was quantitatively assessed on the basis of zone of inhibition. According to the Clinical and Laboratory Standards Institute (2006) guidelines, the bacterial pathogens were resistant to all three antibiotics (positive control) used in the study. Different extracts of leaf and stem of *I. herbstii*, have exhibited different degrees of antibacterial activity against bacterial pathogens (Table 3). Similarly, the inhibition zones formed by standard antibiotic disks are presented in table 4. Among the extracts, ethanolic extracts of leaf was found to be effective against the bacterial pathogens with a zone of inhibition ranging from 10.20±0.50 to 15.13±0.33 mm but the growth of *K. pneumoniae* was inhibited only at 200 mg/ml. In the case of stem, ethanolic and dichloromethane extracts exhibited antibacterial activity against bacterial pathogens except *E. coli* which was not inhibited even at the highest concentration. In the case of acetone and dichloromethane extracts, *K. pneumoniae* was susceptible only at 250 mg/ml. When the ethanolic extracts of leaf and stem of *I. herbstii* tested against the bacterial pathogens, leaf extracts were found to be more effective than stem extracts. Maximum zone of inhibition (15.13±0.33 mm) was noticed for ethanolic leaf extract against *P. aeruginosa* (Fig. 1), whereas the stem extract exhibited minimum zone of inhibition of 12.34±0.33 mm. When these results were compared with standard antibiotics, it was found that ethanolic extract is more effective against *S. aureus* than kanamycin at higher concentration. The present study on *I. herbstii* revealed the high degree of antibacterial activity against *S. aureus, E. coli*, *P. aeruginosa, E. faecalis* and *K. pneumoniae.* In general, the bacterial pathogens are known to be the cause of wounds, nosocomial infections, septicemia, pneumonia, urinary tract infections, gastroenteritis and food borne illness.

The presence of antimicrobial activity in a particular part of a plant species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins[22]. The present study indicated that the antibacterial activity of *I. herbstii* against human pathogens varied depending upon the solvent medium used for the extraction. Flavonoids are known to be produced in plants in response to microbial infections[23]. *In vitro* they have been shown to

be effective antimicrobial agents through complexing with extra-cellular and soluble proteins of bacterial cells[24].

Figure 1
Dose dependent antibacterial activity of ethanolic leaf extract against P. aeruginosa

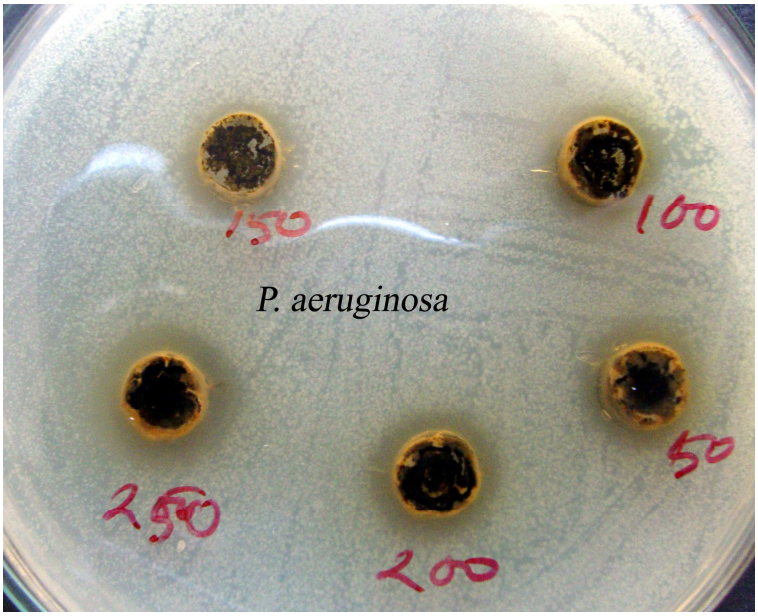

Table 4
Antibacterial activity of I. herbstii leaf and stem extracts

Extracts	Concentration (mg/ml)	Zone of Inhibition (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*E. faecalis*	*K. pneumoniae*
Leaves ethanol extract	50	-	7.27±0.57	6.68±0.75	7.33±0.12	-
	100	-	9.31±0.28	8.20±0.33	9.12±0.27	-
	150	6.52±0.34	12.21±0.33	11.33±0.56	11.30±0.33	-
	200	10.11±0.28	14.54±0.37	13.51±0.76	12.12±0.54	8.34±0.37
	250	12.33±0.65	15.13±0.33	14.12±0.21	13.24±0.26	10.20±0.50
Leaves acetone extract	50	-	-	-	-	-
	100	6.69±0.21	-	-	-	-
	150	8.71±0.56	6.52±0.22	6.71±0.38	-	-
	200	10.45±0.35	8.78±0.50	7.56±0.21	6.6±0.52	8.12±0.32
	250	12.24±0.78	10.19±0.48	9.12±0.60	8.52±0.12	10.20±0.45
Leaves dichloro-methane extract	50	6.59±0.23	-	-	-	-
	100	7.13±0.21	6.56±0.61	-	-	-
	150	9.21±0.33	7.42±0.33	-	6.50±0.17	6.72±0.33
	200	11.38±0.60	9.21±0.42	7.23±0.24	8.15±0.26	8.17±0.59
	250	13.25±0.37	11.11±0..60	8.00±0.45	9.20±0.71	10.22±0.60
Leaves petroleum ether	50	-	-	-	6.78±0.83	-
	100	6.81±0.89	6.69±0.76	-	7.12±0.22	-
	150	7.93±0.82	8.42±0.32	7.31±0.22	9.31±0.23	7.21±0.23

extract	200	10.22±0.33	9.73±0.80	8.52±0.67	10.53±0.45	9.87±0.78
	250	13.17±0.16	12.10±0.21	10.12±0.41	12.32±0.12	12.19±0.34
Stem ethanol extract	50	-	-	-	-	6.63±0.78
	100	-	-	-	6.72±0.86	7.50±0.53
	150	7.21±0.33	6.83±0.94	-	8.42±0.34	9.31±0.62
	200	9.34±0.28	9.67±0.53	-	10.31±0.45	11.84±0.33
	250	11.17±0.14	12.34±0.33	-	12.30±0.22	14.34±0.11
Stem acetone extract	50	-	-	-	6.74±0.84	-
	100	-	-	-	8.35±0.33	-
	150	-	7.23±0.33	-	9.11±0.12	-
	200	7.31±0.41	8.12±0.20	6.73±0.60	9.76±0.71	-
	250	9.23±0.33	10.08±0.12	9.21±0.28	10.50±0.50	8.23±0.34
Stem dichloro-methane extract	50	-	-	-	-	-
	100	-	6.58±0.76	-	-	-
	150	-	7.15±0.30	-	6.72±0.88	-
	200	6.53±0.60	8.74±0.66	-	7.27±0.12	-
	250	8.34±0.21	10.10±0.34	-	9.45±0.35	7.27±0.22
Stem petroleum ether extract	50	-	-	-	-	-
	100	-	-	-	-	-
	150	-	-	-	6.86±0.89	-
	200	7.10±0.12	7.12±0.20	-	8.23±0.41	7.20±0.33
	250	8.20±0.33	9.23±0.12	6.67±0.33	9.10±0.17	8.33±0.12

" - " represents absence of zone of inhibition; Values are mean ± SD of three determinations

Table 5
Antimicrobial activity of standard antibiotics

Microorganisms	**Zone of Inhibition (mm)**		
	Kanamycin (30 µg/disc)	Norfloxacin (10 µg/disc)	Ciprofloxacin (5 µg/disc)
S. aureus	8	10	12
P. aeruginosa	10	10	14
E. coli	10	14	18
E. faecalis	11	10	14
K. pneumoniae	12	16	18

CONCLUSION

The bioactive compounds extracted from this plant may form the basis for future antimicrobial research and also to control the emerging drug resistance pathogen. The results of the present study confirmed the traditional folk medicinal usage of *I. herbstii*. Traditionally water was used for extract whereas we used four different organic solvents for extraction, so it is not the exact replication of traditional knowledge. Among the extracts, the growth of the bacterial pathogens were strongly inhibited by ethanolic extracts than others, it is likely that water extracts will be more effective. Moreover, the

leaf and stem extracts have shown inhibitory effects on the growth of the bacterial pathogens with varying degrees. The sensitivity of the bacterial pathogens to the plant extracts motivates for further studies to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect and to identify the active compounds responsible for the plant biological activity.

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REFERENCES

1. Jaleel CA, Gopi R, Gomathinayagam M, Panneerselvam R. Traditional and non-traditional plant growth regulators alters phytochemical constituents in *Catharanthus roseus*. Process Biochem, 44: 205–209, (2009)
2. Ahmed I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol, 62:183-193, (1998).
3. Karthishwaran K, Mirunalini S, Dhamodharan G, Krishnaveni M, Arulmozhi V. Phytochemical investigation of methanolic extracts of the leaves of *Pergularia daemia*. J Biol Sci, 10: 242-246 (2010).
4. Tomoko N, Takashi A, Hiromu T, Yuka I, Hiroko M, Munekazu I, Totshiyuki TT, Tetsuro I, Fugio A. Iriya I, Tsutomu N, Kazuttito WW. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin resistant *Staphylococcus aureus.* J Health Sci, 48: 273-289, (2002).
5. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African J Biotechnol, 4: 685-688, (2005).
6. Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharm Biol, 37: 80-91, (1999).
7. De Feo V. Ethnomedical field study in northern Peruvian Andes with particular reference to divination practices. J Ethnopharmacol. 85: 243–256, (2003).
8. Bianchi A, Samorini G. Plants in association with ayahuasca. Year book Ethnomed, 2: 21–42, (1993).
9. Schultes RE, Hofmann A. The botany and chemistry of hallucinogens. Charles C Thompson. Springfield: USA, 1973, pp. 226.
10. Srithi K, Balslev H, Wangpakapattanawong P, Srisanga P, Trisonthi C. Medicinal plant knowledge and its erosion among the Mien (Yao) in northern Thailand. J Ethnopharmacol, 123: 335–342, (2009).
11. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Springer: New York, 2007, pp.12.
12. Vicente T, Malag´on O, Finzi PV, Vidari G, Armijos C, Zaragoza T. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. J Ethnopharmacol, 111: 63–81, (2007).
13. Harborne JB, Baxter H. Phytochemical dictionary: a handbook of bioactive compounds from plants. Taylor and Francis Ltd: 4 John St-London, 1995.

14. Kokate CK, Purohit AP, Gokhale SB. Test book of Pharmacognosy, 7th Edn. Nirali Prakashan: 105 (2003).
15. Hsu B, Coupar IM, Ken Ng. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chem, 98: 317-328, (2006).
16. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica*, and *Eugenia jambolana* Lam. Trees. Food Chem, 104: 1106-1114, (2007).
17. Harborne JB. Phytochemical methods, 3rd Edn. Chapman and Hall: London (1978).
18. Harborne JB, Baxter H. The Hand book of Natural Flavonoids. John Wiley and Sons: Chichester, 1999.
19. Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agri Food Chem, 51(25): 7292–7295, (2003).
20. Varaprasadham I, Janaky M, Marimuthu J, Nallayan S. Preliminary phytochemical and antimicrobial studies on a spike-moss *Selaginella inaequalifolia* (hook. & grev.) Spring. Asian Pacific J Trop Med, 3: 957-960, (2010).
21. Johanna WL. Spicing up a vegetarian diet: Chemopreventive effects of phytochemicals. Amer J Clin Nutr, 78: 579-583, (2003).
22. Balandrin MJ, Klocke JA. Medicinal aromatic and industrial materials from plants. Springer Verlag: Berlin, Heidelberg, 1988.
23. Dixon RA, Dey PM, Lamb CJ. Phytoalexins: enzymology and molecular biology. Adv Enzymol Relat Areas Mol Biol, 55: 1–136, (1983).
24. Tsuchiya H, Sato M, Miyazaki T, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin resistant *Staphylococcus aureus*. J Ethnopharmacol 50: 27-34, (1996).