International Journal of Phytomedicine 4 (2012) 477-485
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Phytochemical Composition of the Extracts from *Iresine herbstii* and its Therapeutic use via Antioxidant and Cytotoxic Potential by Multiple *In Vitro* Assays

Dipankar Chaudhuri[1,2], Murugan Sevanan[1*]

***Corresponding author:**

Murugan Sevanan

[1]Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimbatore-641 114, India

[2]Division of Molecular Medicine, Bose Institute, P-1/12 CIT Scheme VIIM, Kolkata - 700054, India.

Abstract

Many plants have been known to synthesize active secondary metabolites to protect themselves which have potential therapeutic applications. *Iresine herbstii* leaf and stem powders were extracted using ethanol, acetone, dichloromethane and petroleum ether. Phytochemicals were investigated by Gas Chromatography-Mass Spectroscopy (GCMS) and the total phenolic content as well as their therapeutic potentials also investigated by multiple *in vitro* assays such as radical scavenging activity, Fe^{3+}reducing power, total antioxidant capacity and cytotoxicity towards cancer cells. Ethanolic extract was subjected for spectroscopic phytochemical analysis and therapeutic uses of *I. herbstii* extracts were evaluated by different *in vitro* methods. The total phenolic content of the samples, analyzed using Folin–Ciocalteau reagent varied from 34.67±0.58 to 103.33±1.53 mg/g dry weight, expressed as gallic acid equivalents (GAE). The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the extracts was increased in a dose dependent manner and found that acetone extract of stem exhibited highest activity when compared to standard ascorbic acid. Cytotoxic effect of ethanolic extract of leaf was examined *in vitro* in HeLa cervical cancer cell line by trypan blue assay and observed over 85% reduction in live cells. The results obtained in this study clearly indicate that *I. herbstii* has a significant potential to be used as a natural antioxidant and anticancer agent.

Keyword: *Iresine herbstii*, antioxidant, radical scavenging, cytotoxicity, HeLa

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, flavours, cosmetic, ornamental, fumigants, insect deterrents and medicine. They act as best scavenger of free radicals which have been implicated in the etiology of more than one hundred disorders in human ailments, including cancer, cardiovascular diseases, ischemia and reperfusion injury of many tissues, neural disorders, diabetes mellitus, arthritis, gastritis and AIDS [4] and also the best resources from which novel bioactive substances are discovered [5]. Micronutrients such as antioxidants, gained importance in recent years as they can act as oxygen scavengers by interrupting the oxidation process by reacting with free radicals, chelating catalytic metals [6]. Among FDA approved anticancer and anti-infectious drugs from natural origin include paclitaxel, vincristine and camptothecin have a share of 60 and 75% respectively [7]. Recently, there has been a soaring interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radicals as well as cytotoxic towards cancer cell line.

Iresine herbstii belongs to the family *Amaranthaceae.* It is commonly referred to as blood leaf, chicken gizzard, beefsteak plant and herbst's blood leaf (English), Naayurivi (Tamil). Blood leaf is native to tropical South America probably first collected in Brazil. 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 [8], and also to diagnose various illnesses. *I. herbstii* was reported as an additive of ayahuasca [9], ingredient of San Pedro decoction, with possible hallucinogenic properties [10]. *I. herbstii* leaves are used as wound healing, anticancer agent [11], post-labor tonic [12], and externally against skin depurative such as eczemas, sores and pimples [8] as well as antimicrobial agent [13]. More over the plant is also used in astringent, diuretic, spasmolytic, whooping cough and roots in hemicranias [13]. Leaves and flowers are used as decoction for fever, relaxant and kidney problems [14] and also as

an antipyretic [8]. Schmidt et al [15], reported that this plant possessed anti-inflammatory, cytotoxic and apoptotic activities and also has very low antioxidant activity [16].

In accordance with the worldwide trend, the current study was undertaken to screen the phytoconstituents and its antioxidant and cytotoxic potential of leaf and stem extracts of *I. herbstii* .

Materials and Methods

Chemicals

All solvents ethanol, acetone, dichloromethane and petroleum ether were purchased from Merck, India. All chemicals used in the study were of analytical grade.

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. The plant was identified and authenticated with complete herbarium by Dr. Sasi, 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 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 ten days. The dried plant parts were pulverized, using sterile electrical blender to obtain powder. The powdered samples were stored in air tight container, protected from sunlight for further use.

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 became clear. Different extracts were concentrated under reduced pressure using rotary evaporator and they were poured into pre-weighed vial, further dried in a desiccating chamber until a constant dry weight was obtained. The residues were stored at 4 C for further studies.

Phytochemical Analysis

Gas Chromatography Mass Spectroscopy (GCMS)

Among the extracts, only ethanolic extract of leaf was selected to identify the phytochemicals using GCMS at South Indian Textile Research Association (SITRA), Coimbatore, South India by Thermo GC - Trace Ultra Version: 5.0, Thermo MS DSQ II equipment in TR 5 - Ms Capillary Standard Non - Polar Column (Dimension: 30 m, ID: 0.25 mm, Film: 0.25 µm). The chromatogram obtained from the GC was then analysed in the mass spectroscopy (MS) to get the mass of all the fractions. The identification of components was accomplished searching plant compound library.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The FTIR analysis of ethanolic leaf extract of *I. herbstii* using FTIR Shimadzu--8400S was carried out at PSG College of Arts and Science, Coimbatore, South India using KBR pellet. The FTIR was recorded in the range of 400 to 4000 cm^{-1}. The various modes of vibrations were identified and assigned to know the different functional groups present in the extract.

In vitro Antioxidant Assays

Determination of Total Phenolic Content (TPC)

The total phenolic content was determined by the Folin-Ciocalteau method [17]. 0.5ml of extract was mixed with Folin-Ciocalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 mins and then aqueous Na_2CO_3 (4 ml, 1 M) was added. The mixture was allowed to stand for 15 mins and the phenols were determined by spectrophotometric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 µg/ml solutions of Gallic acid in 50% methanol. The total phenolic content was expressed as Gallic acid (GA) equivalent (mg GA /g dry weight).

DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging assay was carried out by the method of Chang et al [18]. 1ml of 0.1mM DPPH (in ethanol) was added to different concentrations of plant extracts. The reaction mixture was well shaken and incubated in dark for 30 mins. Absorbance was checked at 517 nm against a blank (ethanol). Ascorbic acid was taken as the standard. Lower the absorbance of the reaction mixture indicates higher percentage of scavenging activity. The percentage of inhibition or scavenging of free radicals was determined by the formula

% Inhibition = [(Control OD – Sample OD)/ Control OD] 100, where control was prepared as above without extract.

Reducing Power Assay

The Fe^{3+} reducing power assay was determined by the method of Makari et al [19]. Different concentrations of the extracts (0.5 ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (0.1%). The mixture was incubated at 50 C for 20 mins. After incubation, 2.5 ml of trichloroacetic acid (10%) was added to terminate the reaction, which was then centrifuged at 3000 rpm for 10 mins. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.01%) and absorbance measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power.

Total Antioxidant Capacity

The total antioxidant capacity was assayed following the method of Preito et al [20].0.1 ml of different concentrations of plant extracts were mixed with 1 ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate; mixed in 1:1:1 ratio) respectively. The tubes were capped and incubated in a thermal block at 95 C for 90 mins. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity was expressed as equivalents of ascorbic acid.

Statistical Analysis

All the grouped data were statistically evaluated by using student's 't' test with SPSS/16 software. Values are presented as the mean ± S.D. of each three replicates in each experiment.

Cytotoxicity

Cell line and condition

HeLa cervical cancer cell line was obtained from NCCS (National Centre for Cell Sciences), Pune, India. Cells were cultured in Eagle essential medium and supplemented with 10% foetal bovine serum from Hi-Media and 20 µg/ml gentamicin procured from Nicholas Piramal, India Ltd. Routinely, the cells were maintained under 97% humidity in a biological incubator at 37 C with 5% CO_2. All cell culture procedures were carried out in a laminar flow cabinet containing a UV light. Once the 80% confluence was reached, the cells were ready for assay. Cell numbers were determined by counting with the haemocytometer and the viability was assessed using trypan blue dye exclusion.

Cell Viability using Trypan Blue Dye Exclusion Assay

The cell numbers were adjusted to 1 10^6 cells per ml by diluting with PBS. Sterile test tubes containing varying concentrations of plant extract (0.8ml) in PBS were treated with 1x10^6 cells/ ml (0.1ml). The seeded test tubes were incubated in the biological incubator at 37 C with 5% CO_2 and 97% humidity for three hours. On termination, 0.1 ml of 0.4% trypan blue: deionised water (1:1) was added to the test as well as to control tubes in order to estimate the dead HeLa. The cell viability was estimated using a haemocytometer; dead cells appear stained blue, while live cells are unstained (translucent). Cell mortality was expressed as the percentage of trypan blue positive cells compared to the total number of cells. The percentage viability was determined using the formula below-

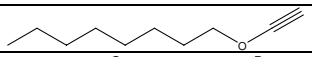
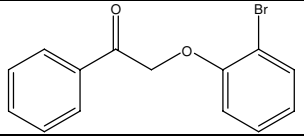
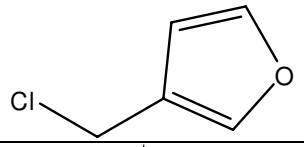
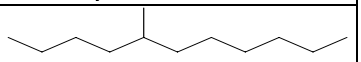
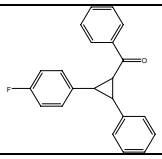
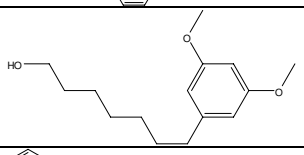
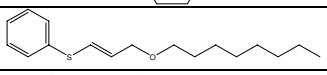
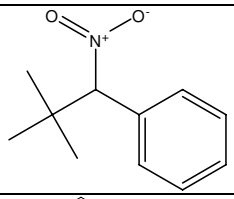
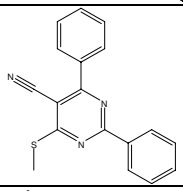
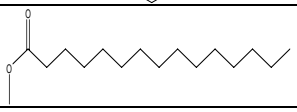
$$\% \text{ Cell mortality} = \frac{\text{Number of dead cells (stained cells)}}{\text{Total number of cells (stained and unstained cells)}} \times 100$$

Results and Discussion

Phytochemical Analysis of Ethanolic leaf extract by GCMS

Ethanolic leaf extract of *I. herbstii* was subjected to GCMS analysis (figure 1) as it can extract most of the phytochemicals than other solvents due to its higher polarity. The probable compounds present in the leaf extracts identified from plant compound library with respect to mass and retention time of each fraction, are presented in table 1. GC–MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry. It is evident from the table that the ethanolic leaf extract have a complex chemical composition. Some of the GC-MS peaks were not identified because of lack of authentic library data to corresponding compounds. A general observation is that the quantity of the aromatic compounds is more than that of aliphatic compounds in leaf extract. The identified compounds are mainly phenolic and flavonoids. Among the compounds 2-(2-Bromophenyoxy)-1-phenylethan-1-one, 5-cyno-4-methylthio-2,6-diphenylpyrimidine, 2,2-dimethyl-1-phenyl-1-nitropropane, 1-benzoyl-2-phenyl-3-(p-fluorophenyl) cyclopropane, 7(3,5-dimethyloxyphenyl)-1-heptanol and Trans-1-phenylthio-4-oxadodec-1-ene are identified as phenolic and flavonoids compounds or its derivatives. Methyl ester of undecane-5-methyl and pentadecanoic acid can be generated via-genetically from the phytol side chain of chlorophyll and may also derive from the side chain of tocopherols. 3-chloro methyl furan is a modified hydrocarbon with a hetocyclic nucleus of the furan type which can act as an inhibitor of the quorum sensing in bacteria [21]. Flavonoids and phenolic compounds have been reported to have useful properties including anti-inflammatory, enzyme inhibition, antioxidant and anticancer activity [22]. In plants, these secondary metabolites function to attract beneficial and repel harmful organisms, serve as phyto-protectants and respond to environmental changes. However, in humans, the compounds have beneficial effects including anti-inflammatory, modulation of detoxification enzymes, stimulation of the immune system, modulation of steroid metabolism, antioxidant as well as anticancer effects [23]. They were identified and reported in this plant material for the first time.

Table 1 List of probable phytochemical constituents identified by GCMS spectra

Probable compound name	Molecular Formula	M .W.	Retention time (min)	Area %	Probable compound structure
n-Octylethynyl ether	$C_{10}H_{18}O$	154	15.24	10.54	
2-(2-Bromophenyoxy)-1-phenylethan-1-one	$C_{14}H_{11}BrO_2$	290	15.76	3.59	
3-Chloro methyl furan	C_5H_5ClO	116	16.45	6.10	
Undecane,5-methyl	$C_{12}H_{26}$	170	17.23	7.15	
1-benzoyl-2-phenyl-3-(p-fluorophenyl) cyclopropane	$C_{22}H_{17}FO$	316	17.53	5.32	
7(3,5-dimethyloxyphenyl)-1-heptanol	$C_{15}H_{24}O_8$	252	18.25	14.64	
Trans -1-phenylthio-4-oxadodec-1-ene	$C_{17}H_{26}OS$	278	19.45	7.46	
2,2-dimethyl-1-phenyl-1-nitropropane	$C_{11}H_{15}NO_2$	193	20.18	5.22	
5-cyno-4-methylthio-2,6-diphenylpyrimidine	$C_{18}H_{13}N_3S$	303	23.35	6.17	
Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	22.04	4.92	

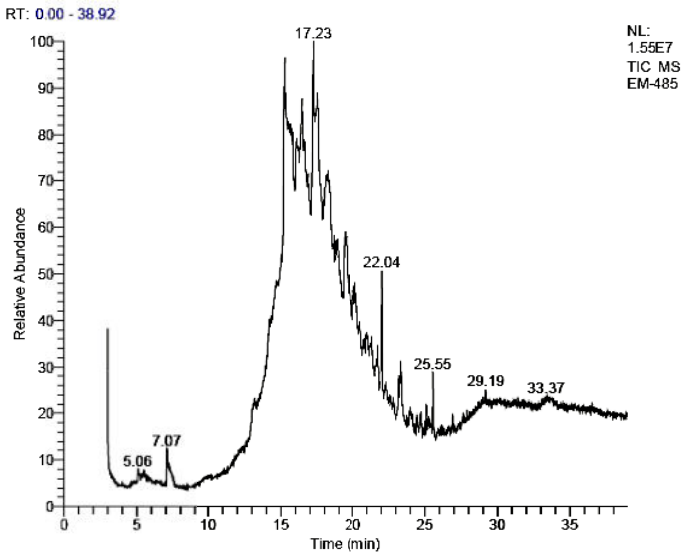


Figure 1: GCMS chromatogram of ethanolic leaf extracts of *I. herbstii*

FTIR Analysis of the plant extracts

The functional groups of the compounds present in the ethanolic leaf extract were identified by the FTIR spectra and support the identified compounds by GCMS. In the FTIR spectrum of *I. herbstii* leaf aqueous extract (figure-2) major peaks are ~1111, ~1388, ~1627, ~2924, ~3263, and ~3479 cm^{-1} and the minor peaks are ~763, ~895 and ~2376 cm^{-1}. The FTIR spectrum exhibited the characteristics finger print band features. The very strong absorption at 3479 cm^{-1} represent O-H stretching vibration and also characteristic of the presence of N-H primary amines and primary aliphatic amines was found to be at 3263 cm^{-1}. Besides, the spectrum also exhibits intense band at ~2924 assigned to the symmetric stretching vibration of sp3 hybridized $–CH_2$ groups [24] and the peak at around 1627 cm^{-1} is assigned to the amide I bonds of proteins indicating predominant–C=O functional group [25]. The wide absorption spectra at about 1620 cm^{-1} may result from stretching of vibration of -C=C-. The peak at 1388 cm^{-1} confirmed to be CH_3 deformation and can be assigned to germinal methyls and at ~1111 cm^{-1} band arises most probably from the C–O group [24] of polyols such as hydroxyflavones and catechins [26]. The absorption peak at around 1026cm^{-1} can be assigned as absorption peaks of the ether linkages or -C-O-C- or –C-O-. To a large extent, the band at ~1026 cm^{-1} might be contributed by the –C-O- groups of the polyols such as flavones, terpenoids and the polysaccharides [27]. The absorbance peak ~895 cm^{-1} indicates C-H plane deformation. The peak at ~3163 cm^{-1} is showing the primary aliphatic amines [24]. These groups indicate that they are representing some proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes and carboxylic acids [28]

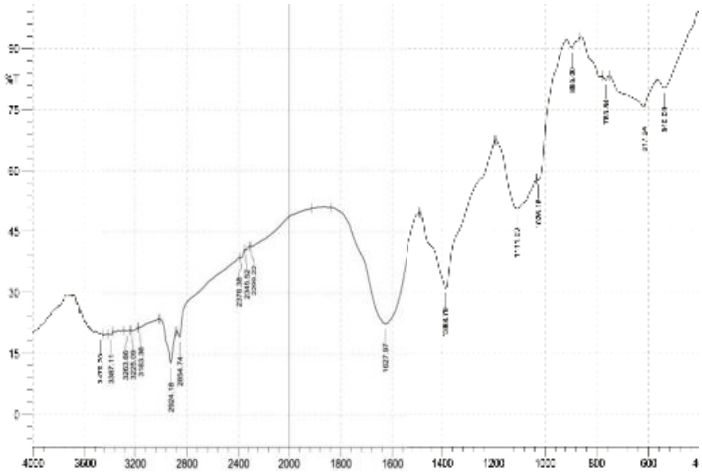


Figure 2: FTIR spectra of *I. herbstii* leaf aqueous extract

In vitro antioxidant activity

Total phenolic content of I. herbstii leaf and stem extracts

Phenolic compounds are a class of antioxidant agents which act as free radical terminators [29]. Total phenolic content of the plant extracts were determined against Gallic acid equivalent (GAE). The total phenolic content of *I.herbstii* leaf and stem extracts are shown in table 2. The phenolic content ranging from 34.67±0.58 to 108.67±1.15 mg GA/ g of dry weight and considering all the extracts, the acetone extracts of leaf (IHLA) were found to contain the highest phenolic content. The lowest amounts of phenolics were found in the petroleum ether extract of stem (IHSEt). These results indicate that the higher levels of antioxidant activity are due to the presence of phenolic compounds in the extracts. Phenols are very important phytochemicals because of their scavenging ability [30]. The interests on phenols and polyphenolic compounds, such as flavonoids which possess significant antioxidant activities, are increasing in the food industry because they slow down the oxidative degradation of lipids thus improving the quality and nutritional value of food [31].

DPPH assay of I. herbstii leaf and stem extracts

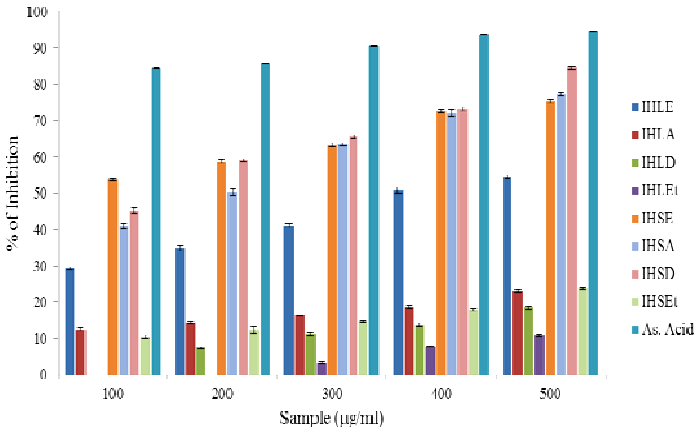
Positive DPPH test recommends that the leaf and stem extracts were free radical scavengers. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised. The degree of colour reduction from deep violet to light yellow indicates the radical scavenging power of the extract [32].

Table 2: Total phenolic content of *I. herbstii* leaf and stem extracts

Plant Parts	Phenol Content (mg GA/g dry extract)
I. herbstii Leaf Ethanol extract (IHLE)	79.00±1.00
I. herbstii Leaf Acetone extract (IHLA)	108.67±1.15
I. herbstii Leaf Dichloromethane extract (IHLD)	73.67±0.58
I. herbstii Leaf Petroleum ether extract (IHLEt)	86.00±2.00
I. herbstii Stem Ethanol extract (IHSE)	105.67±1.53
I. herbstii Stem Acetone extract (IHSA)	103.33±1.53
I. herbstii Stem Dichloromethane extract (IHSD)	101.33±0.58
I. herbstii Stem Petroleum ether extract (IHSEt)	34.67±0.58

(Values are mean ± SD, n=3. GA-Gallic Acid)

The DPPH activity of the extracts was found to increase in dose dependent manner (figure 3). When compared among the leaf extracts, IHLE exhibited more than 50% scavenging activity at 500µg/ ml, whereas low scavenging activity was observed for IHLEt. On the other hand, stem extracts exhibited good % of inhibition with more than 75% scavenging activity of DPPH except IHSEt. When the leaf and the stem extracts were compared, it was found that the stem extracts possessed higher scavenging activity than leaf extracts. The radical scavenging activity of leaf and stem extracts were compared with standard ascorbic acid and all the extracts exhibited lower activity than ascorbic acid. This may be due to the occurrence of reactive concentration of bioactive constituents and mixture of other nutrients in the extract. Phytochemicals, able to perform this reaction can be considered as natural antioxidants and therefore radical scavengers [33]. This result showed that DPPH scavenging activity was totally relative to the total phenolic content of the plant extracts. Usually, higher level of total phenol and flavonoids contents in plant extracts lead to better DPPH-scavenging activity [17].



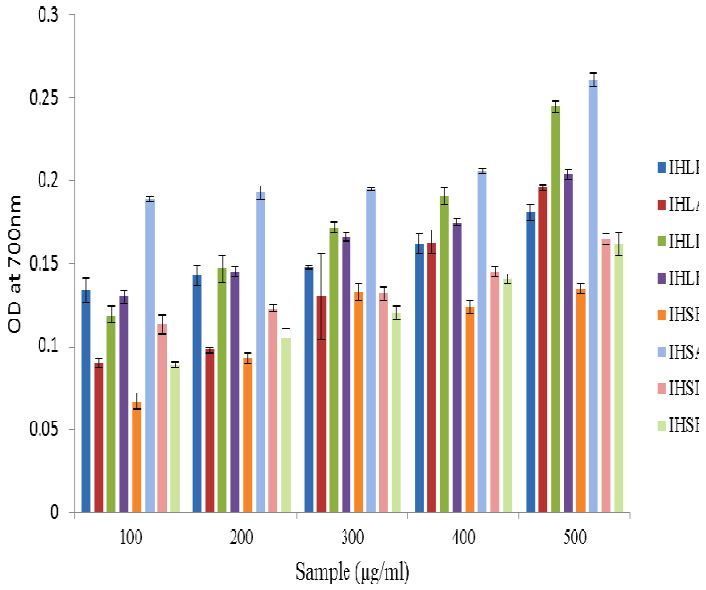
(Values are mean ± SD n=3)

Figure3: DPPH radical scavenging assay of *I. herbstii* leaf and stem extracts

Reducing power assay of I. herbstii leaf and stem extracts

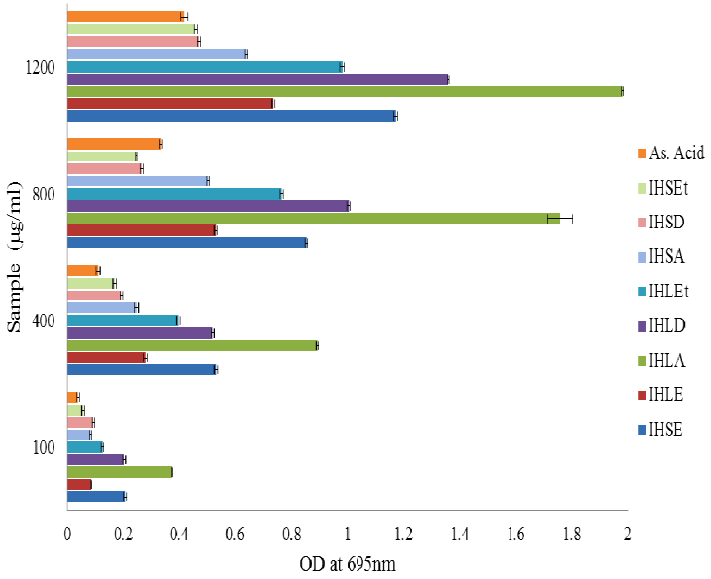
In the reducing power assay, the presence of antioxidants in the samples would resulted in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Increasing absorbance at 700 nm indicates an increase in reductive ability. The reductive property of plant extracts generally depends on the presence of phyto-reductants, which have exhibited anti-oxidative potential by breaking the free radical chain, donating a hydrogen atom [34]. Fe^{3+} reduction is often used as an indicator of electron donating activity of the extracts. Phenolic compounds present in the extracts are mainly responsible for this kind of action [35].

Figure 4 shows the dose-response bar chart for the reducing powers of the *I. herbstii* plant extract. The figure also recommends that at lower concentrations, the association between concentration and the decrease in the absorbance is linear. It was found that the reducing powers of the extracts increased with the increasing concentrations. The trend for ferric ions reducing activities of the extracts did not differ distinctly from their DPPH free radical scavenging activities, when a relationship between figures. 3 and 4 was made. Interestingly, reducing power was consistently higher than those obtained for DPPH scavenging for all the extracts. Surprisingly all extracts exhibited comparatively good reducing power than the ascorbic acid (Vitamin C). Decrease in the reducing power among the *I. herbstii* extracts were found to be in the order of IHSA, IHLD, IHLEt and IHLA. Among the extracts, IHSA exhibited higher activity than standard ascorbic acid.



(Values are mean ± SD n=3)

Figure 4: Reducing power assay of *I. herbstii* leaf and stem extracts



(Values are mean ± SD n=3)

Figure 5: Total antioxidant assay of *I. herbstii* plant parts extracts

Total antioxidant assay of I. herbstii leaf and stem extracts

The total antioxidant capacity of the extracts was calculated based on the formation of the phosphomolybdenum complex. The absorbance was increased with the increasing concentration. Total antioxidant capacity of various extracts of the plants expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm Total antioxidant assay of *I.herbstii* leaf and stem extracts were compared with ascorbic acid (figure 5). The antioxidant activity determined by this method was different according to the extracts analysed, but in general it was also higher in the acetone extract of stem, thus coinciding with the results of other assays. When the absorbance of the extracts was compared with standard ascorbic acid, they were found to possess higher level of antioxidant activity than ascorbic acid with highest activity noticed in IHLA. Decrease in total antioxidant activity among the *I. herbstii* extracts were found to be in the order of IHLA, IHLD, IHLE, IHLEt. According to the present study, *I. herbstii* clearly exhibited higher antioxidant activity and contain significantly more phenolics than the common vegetables and fruits (nutritional plants).

In vitro cytotoxicity assay

Cytotoxic activity is extremely sensitive to a wide range of compounds and may be due to any number of phytochemicals within the crude preparation [36]. Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties [37]. An initial screening for cytotoxicity of ethanolic leaf extract was performed on the HeLa cell line using the trypan blue assay (figure-6) as it exhibited most potent antioxidant potential among the extracts. IHLE induced over 85% death of Hela cells at a treatment concentration of 2 mg/ml (LC_{50}-240 µg/ml). Several plant species rich in flavonoids are reported to have disease preventive and therapeutic properties. This relates the importance of many vegetables and fruits consumption, since flavonoids are major ingredients of those to reduce the risk of cancer [38]. Cytotoxicity activity recorded in the present study is in accordance with earlier findings, since the phytochemical evaluation from the previous literature indicated the presence of flavonoids in the plant extracts with promising activity.

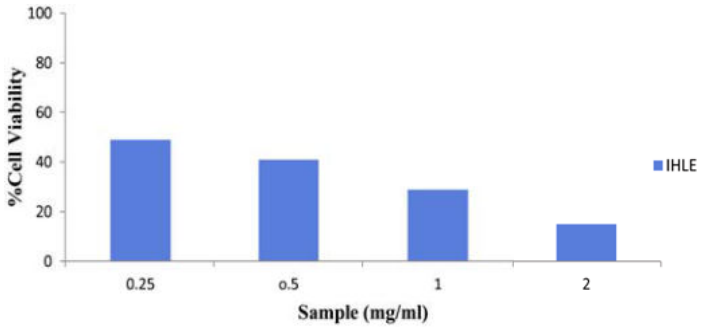


Figure 6 Cytotoxicity assay of leaf ethanolic extract of *I. herbstii*

Conclusions

Traditional uses of this plant are to cure diseases like skin disorders, diarrhoea, jaundice, fever, abdominal pain, skin burns, menstrual problems, urinary problems, sores, wounds, to expel intestinal worms and so on. According to the percentage of area in GCMS spectra with a high complex profile, approximately 10 major phytocomponents were dominant which possessed multiple pharmacological activities.

In the current study, among the extracts, IHLA showed high phenolic content as well as highest percentage of inhibition in DPPH scavenging activity and the antioxidant activity of different extracts varied with the different assays. Because of the complexity of the phyto-antioxidant and their mode of actions, no single test can provide detail picture of the antioxidant profile of a studied samples and a combination of different methods is necessary. Ethanolic extract of leaf possessed cytotoxic activity against HeLa cervical cancer cell line and this would lead to undertake further study to reveal actual anticancer property of *I. herbstii in vitro* as well as *in vivo* and to identify the active compounds responsible for the plant biological activity.

Acknowledgments

The authors would like to thank Mr. Sukumar Choudhury (Barasat, Bankura, West Bengal, India) for his kind help in plant collection, Dr. Sasi (Post Graduate and Research, Department of Botany, PSGR Krishnammal College for Women, Coimbatore, India) for providing the identification of *I. herbstii*. Authors are also grateful to the Chancellor (Dr. Paul Dhinakaran), the Vice Chancellor (Dr. Paul P Appasamy) and The Registrar (Dr. Joseph Kennady), Karunya University, Coimbatore, India for providing laboratory facilities and kind support to carry out this publication.

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. 2009; 44 (2): 205–209.

[2]. Ahmed I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol. 1998; 62 (2): 183-193.

[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. 2010; 10 (3): 242-246.

[4]. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi S, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physician India. 2004; 52:794–804.

[5]. Tomoko N, Takashi A, Hiromu T, Yuka I, Hiroko M, Munekazu I, Toshiyuki T, Tetsuro I, Fujio A, Iriya I, Tsutomu N, Kazuhito W. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin resistant *Staphylococcus aureus.* J Health Sci. 2002; 48 (3): 273-289.

[6]. Cadenas E, Packer L. Hand Book of Antioxidants, Plenum, New York, 1996; pp-203.

[7]. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod. 2003; 66(7): 1022-1037.

[8]. De Feo V. Ethnomedical field study in northern Peruvian Andes with particular reference to divination practices. J Ethnopharmacol. 2003; 85 (2-3):243–256.

[9]. Bianchi A, Samorini G. Plants in association with ayahuasca. Year book Ethnomed. 1993; 2: 21–42.

[10]. Schultes RE, Hofmann A. The Botany and Chemistry of Hallucinogens. 1973. Charles C Thompson. Springfield: USA 226.

[11]. Sebold, DF. Ethnobotanical survey of medicinal plants in the city of Campo Bom, Rio Grande do Sul, Brazil.Porto Alegre. Federal University of Rio Grande do Sul. Master thesis. (2003) 107.

[12]. 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. 2009; 123 (2): 335–342.

[13]. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Spinger: 2007. New York; 12.

[14]. 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. 2007; 111 (1):63–81.

[15]. Schmidt C, Fronza M, Goettert M, Geller F, Luik S, Flores EMM, Bittencourtd CF, Zanettie GD, Heinzmanne BM, Lauferb S, Merfort I. Biological studies on Brazilian plants used in wound healing. J Ethnopharmacol. 2009; 122 (3): 523–532.

[16]. Cai Y, Sun M, Corke H. Antioxidant activity of betalains from plants of the *Amaranthaceae*. J Agri Food Chem. 2003; 51 (8): 2288 2294.

[17]. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant activities of Iranian corn silk.Turkic J Biol. 2008; 32: 43-49.

[18]. Chang WC, Sei CK, Soon SH, Bong KC, Hye JA, Min YL, Sang HP, Soo KK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavanoids by assay guided

comparison. Plant Sci. 2002; 163 (6): 1161-1168.

[19]. Makari HK, Haraprasad N, Patil HS, Ravi kumar. *In Vitro* Antioxidant activity of the hexane and methanolic extracts of *Cordia wallichii* and *Celastrus paniculata.* Internet J Aesthet Antiaging Med. 2008; 1 (1): 1-10.

[20]. Preito P, Pineda M, Aguilar M. Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdnum complex: specific application of vitamin E. Anal Biochem. 1999; 269 (2): 337-341.

[21]. Slaughter JC. The naturally occurring furanones: formation and function from pheromone to food. Biol Rev Camb Philos Soc. 1999; 74 (3):259-276.

[22]. Harborne JB, Baxter H. The Hand book of Natural Flavonoids. 1999. John Wiley and Sons: Chichester.

[23]. Johanna WL. Spicing up a vegetarian diet: Chemopreventive effects of phytochemicals. Amer J ClinNutr. 2003; 78 (3 Suppl): 579S-583S.

[24]. Silverstein RM, Bassler GC, Morrill TC. Spectrometric Identification of Organic Compounds (4th Ed). John Wiley and Sons: New York. 1981.

[25]. Shivshankar S, Ahmad A, Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol Prog. 2003; 19 (6): 1627-1631.

[26]. Jain D, Daima HK, Kachhwaha S, Kothari SL. Synthesis of plant-mediated silver nanoparticles usingpapaya fruit extract and evaluation of their antimicrobial activities. Dig J Nanomaterials Biostruct. 2009; 4 (3): 557 - 563.

[27]. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Nanotechnol. 2007; 18 (10): 105104-105114.

[28]. Song JY, Jang HK, Kim BS. Biological synthesis of gold nanoparticles using *Magnolia kobus*and *Diopyros kaki* leaf extracts. Process Biochem. 2009; 44 (10): 1133–1138.

[29]. Shahidi F, Wanasundara PK. Phenolic antioxidants. Crit Rev Food SciNutr. 1992; 32 (1): 67-103.

[30]. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T. Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on 1, 1-diphenyl 2-picrylhydrazyl radical. Chem Pharm Bull. 1989; 37 (8), 2016–2021.

[31]. Aneta W, Jan O, Renata C. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007; 105 (3): 940–949.

[32]. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical screening and antioxidant Activities of some selected medicinal plants used for malaria therapy in South western Nigeria. Trop J Pharma Res. 2008; 7 (3): 1019-1024.

[33]. Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activity of methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas Aceites. 2009; 60(4): 405-412.

[34]. Gordon MH, Hudson BJE. The mechanism of the antioxidant action *in vitro*. Introduction to food antioxidants. 1990. London: Elsevier.p 1-18.

[35]. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Phcog Mag. 2009; 4 (18): 123-127.

[36]. He XG, Mocek U, Floss HG, Caceres A, Giron L, Buckley H, Cooney G, Manns J, Wilson BW. An antifungal compound from *Solanum nigrescens*. J Ethnopharmacol. 1994; 43 (3): 173-177.

[37]. Cardellina II JH, Fuller RW, Gamble WR, Westergaard C, Boswell J, Munro MHG, Currens M, Boyd M. Evolving strategies for the selection dereplication and prioritization of antitumorand HIV-inhibitory natural products extracts.(999.In: Bohlin, L., Bruhn, J.G. (Eds.), Bioassaay Methods in Natural Product Research and Development. KluwerAcademic Publishers, Dordrecht, p. 25–36

[38]. Kanadaswami C, Lee L, Lee PH, Hwang J, Ke F, Huang YT, Lee MT. The antitumor activities of flavonoids. In Vivo. 2005; 19(5): 895-909.