Research Article

Quantitative Phytochemical Evaluation of *Indigofera hirsuta* L. Plant Parts

***A. Suvarna Latha[1], N. Yasodamma[2]**

1. Department of Botany, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.
2. Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

ABSTRACT

Natural products play an important role in drug development programs in the pharmaceutical industry which are basically obtained from plants with potent bioactive compounds capable of preventing and treating most oxidative related diseases and have often been used in folkloric medicine. The most important of these bioactive constituents of plants are phenols, flavonoids, alkaloids, and saponins which provide the medicinal value to the plants in which they are present. The present study focuses on the Quantitative evaluation of important bioactive phytochemicals like phenols, flavonoids, alkaloids and saponins in different extracts of Leaf and fruit of an important medicinal plant *Indigofera hirsuta* L. belonging to family Fabaceae. *I. hirsuta* L. is an annual sub shrub and grows to a height of 1m tall and widely distributed in India with immense herbal medicinal properties. Phenols, flavonoids are known to possess wide range of biological activities like antimicrobial, antioxidant and anti-inflammatory properties, alkaloids and saponins with inhibitory properties. Till now there are no reports on quantitative studies of phytoconstituents in the selected plant. Present study revealed that phenols are the largest group of phytoconstituents in leaf methanol extracts (124 mg/g), Flavonoids are highest in fruit (32 mg/g), alkaloids are highest in fruit (18.25 mg/g) and saponins are very less in quantity in both the plant parts with (0.25 mg/g) in fruit.

Keywords: Alkaloids, flavonoids, *indigofera hirsuta* L., phenols, saponins

Received 26 Nov 2014 Received in revised form 12 March 2015 Accepted 14 March 2015

***Address for correspondence:**
A. Suvarna Latha,
Department of Botany, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.
E-mail: Suvarnalatha.2009@gmail.com

INTRODUCTION

Plants have been a variable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies [1]. Approximately 62 – 80% of the world's population still relies on traditional medicines for the treatment of common illness as given by World Health Organization (WHO) [2]. Studies have shown that many plants have chemical components and biological activities that produce definite physiological actions in the body and therefore, could be used to treat various ailments [3]. Plant materials contain many phytochemicals including compounds with antioxidant activity, which are mostly phenolic in structure. The most widespread and diverse phenolics are the flavonoids which have the same C15 (C6-C3-C6) skeleton and retard oxidation of a variety of easily oxidizable compounds [4]. Flavonoids are widely distributed in the plant kingdom as secondary metabolites of plants, with a wide spectrum of potential health benefits, such as antioxidant, anticarcinogenic, anti-inflammatory, antiobesity, antidiabetic, antiallergic, and hepato- and gastroprotective effects [5-7]. Other chronic diseases were also evaluated by flavonoid intake are asthma [8] and chronic obstructive pulmonary disease [9]. Saponins have been reported to exhibit hemolytic and foaming activity [10], antifungal [11], anti-inflammatory [12], fungistatic [13] and molluscidal [14]. The present study focuses on comparative evaluation of total phenols, flavonoids, alkaloids and saponins quantitatively in *Indigofera hirsuta* L.

Indigofera is a large genus of about 700 species of flowering plants belonging to the

family Fabaceae. It is commonly known as hairy Indigo, in Africa and Kenya, it is used as chest medicine, Leaf is used against infant immunity [15], urinary complaints [16], decoction of leaf is used in case of stomach problems [17] used against diarrhea [18]. Whole plant paste is applied as an external application for back ache [19], *I. hirsuta* leaf methanol and ethanol extracts showed effective antibacterial activity on *E. coli, B. subtilis, P. aeruginosa, S.aureus* [20]. *I. hirsuta* fruits showed nearly 14 Phyto constituents in aqueous, methanol, alcohol, ethyl acetate and chloroform extracts and effective antibacterial activity than the control drug *Ampicillin* [21]. *I. hirsuta* leaf, fruit alcohol and methanolic extracts showed effective anthelmintic activity than the control drug *Albendazole* due to the presence of tannins [22]. About 25 Phenolic compounds are qualitatively identified [23]. *I. hirsuta* leaf, fruit alcohol and methanolic extracts showed effective antifungal activity [24]. Due to its high medicinal significance it is need to identify quantitatively various important bioactive secondary metabolites.

Figure 1: ***I. hirsuta*** **- Natural Habitat**

Figure 2: ***I. hirsuta*****- Fruits**

MATERIALS AND METHODS

Collection and identification of Plant material:

Plant material *Indigofera hirsuta* L. (Fabaceae) was collected from S. V. Agricultural College during the months of September - January, and was authenticated by Prof. N. Yasodamma the voucher specimen SVUTY IH-1293 was preserved in the herbarium, Department of Botany, S.V.University, Tirupati as per the standard method [25]. Leaves and fruits (Fig : 1 & 2)were collected, thoroughly washed, cut in to pieces and further dried under shade at 28 ± 2 ^{0}C for about 10 days. The dried parts were ground well in to a fine powder in a mixer grinder and sieved to particle size of 50 – 150mm. The powders were stored at room temperatures for phenols, flavonoids, alkaloids and saponin content determination.

Extracts preparation: Shade dried leaf and fruit powders were subjected to soxhlet extractions with Alcohol and Methanol for total phenols determination. The extract were filtered and concentrated at reduced temperature on a rotary evaporator. The above obtained semisolid extracts were preserved in airtight bottles at 4 ºC in the refrigerator until further use.

QUANTITATIVE ANALYSIS

Determination of Total phenolic content

Total phenolic content was determined by Folin-Ciocalteau method [26, 27]. Gallic acid is used as standard and the total phenolic content is expressed as mg/g Gallic Acid equivalents (GAE). Concentrations from 1.0 to 100 µg/ml of gallic acid solutions were prepared in methanol. 0.2 ml solution (1mg/ml) of leaf and fruit of alcohol and methanol extracts was also prepared and 0.5ml of each sample were introduced into test tubes and mixed with Folin-Ciocalteau reagent (5 ml, 1:10 diluted with distilled water) and after five minutes aqueous Na_2Co_3 (4ml, 1M) was added. The mixture was allowed to stand for 30 minutes and absorbance was read at 765nm spectrophotometrically. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Total phenolic content values are expressed in terms of Gallic acid equivalent (mg/g) using the formula:

$C = C_1 \times V/m$.

Where; C= Total content of phenolic compounds in mg/g, in GAE (Gallic Acid Equivalent); C_1= Concentration of Gallic acid established from the calibration curve in mg/ml; V= Volume of extract in ml; m= Weight of plant extract in gm.

Determination of Total flavonoid content

Total flavonoid content was determined [28] by taking ten g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Determination of total alkaloid content

Determination of total alkaloids was done [29] by taking five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered, to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the total precipitation formed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the total alkaloid content, which was dried and weighed.

Determination of Total Saponin content

Total saponin content was determined [30] by taking plant samples, which were ground and 20 g of each powder, put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. Combined both extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-Butanol was added. The combined n-Butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight, and the total saponin content was expressed as mg/g.

Statistical analysis: All values have been expressed as mean ± standard deviation.

RESULTS

The amount of total phenols was determined with Folin - Ciocalteu reagent. Gallic acid is used as standard compound with Standard curve equation as; y= 0.0106x + 0.041, R2 =0.996 (Fig-4). The total phenol contents (Gallic acid equivalents, mg/g) in leaf, fruit alcohol and methanol extracts were calculated. It was observed that among the phytoconstituents studied Phenols are the largest group in *I. hirsuta* leaf methanol extracts (123.9 mg/g) followed by fruit methanol extract (81.15 mg/g), fruit alcohol extract (73.02 mg/g) and leaf alcohol extract (50.7 mg/g). Flavonoids, alkaloids and saponins are highest in fruit as 32.07 mg/g, 18.25 mg/g & 0.25 mg/g than Leaf (30.21, 15.20, 0.13 mg/g) (**Table 1, Fig. 3**).

Table 1: Quantitative Phytochemical Components (mg/g)

Plant Name	Plant part	Total Phenols		Total Flavonoids	Total Alkaloids	Total Saponins
		Alcohol	Methanol			
I. hirsuta	Leaf	50.7±0.12	123.9±1.3	30.21±0.04	15.20 ±0.04	0.13±0.01
	Fruit	73.02±0.2	81.15±1.8	32.07±0.03	18.25 ±0.03	0.25±0.04

All values have been expressed as mean ± standard deviation

DISCUSSION

The present investigation of quantitative analysis of bioactive compounds revealed that leaf and fruit contain significant amounts of Phytocompounds. *I. aspalathoides* methanolic extracts showed total phenolic content of 1.9 µg at 0.1ml of Pyrocatechol equivalent with radical scavenging [31]. Whole plant methanolic extract resulted 47.38 mg of total phenol and found to be more quantity than total tannins 34.59 mg [32]. Polyphenol content were observed with highest phenols in *I. colutea* 54 mg GAE/100 mg, highest flavonoids in *I.nigritana* 9.63 mg/QE/100 mg [33]. When compared with the other species of the genus, *I. hirsuta* is showing significant amounts of compounds.

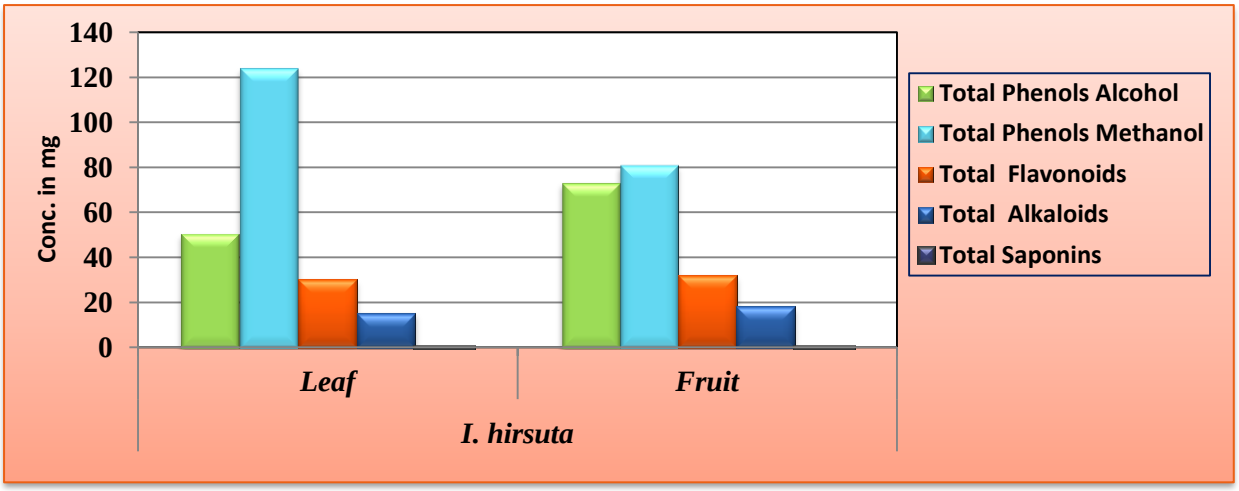

Figure 3: Comparative account of Phytochemical compounds (mg/g)

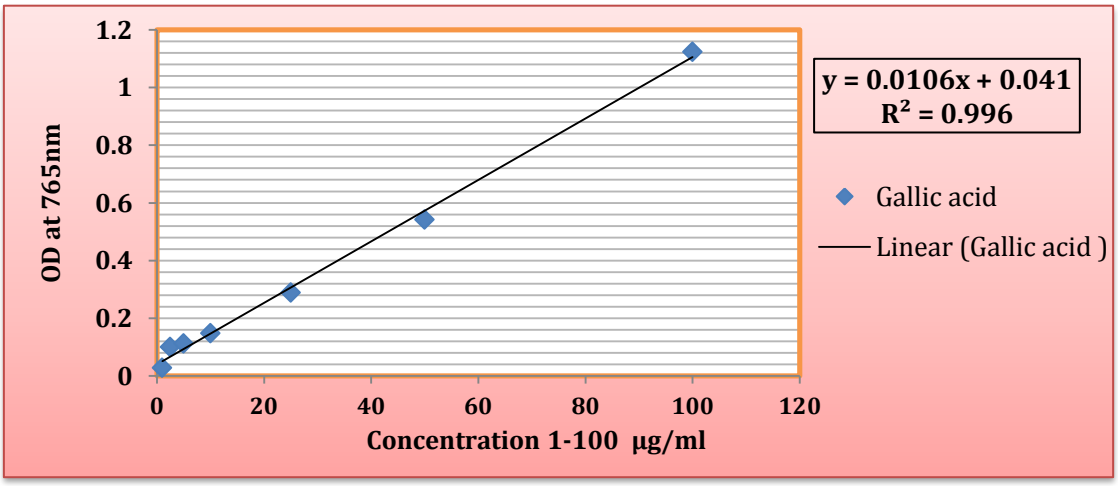

Figure 4: Standard graph for Gallic acid: Total Phenolic content

CONCLUSION

From the results obtained by the present studies it is concluded that *I. hirsuta* is a good source of natural phytoconstituents which contribute medicinal, biological and physiological properties to the plant. Hence the plant extracts are a good source of potent natural drug and require further pharmacological studies to establish the importance of phytoconstituents having protective action.

REFERENCES

1. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumder K. Gomathi P, Rajeshwar Y, Saravanan S, Kumar MS, Murugesh K, Kumar KA. Antioxidant and antimicrobial activities of Bauhinia racemosa L. stem bark. Braz. J. Med. Biol. Res 2005;38:1015-1024.
2. Zhang X. Traditional medicine, its importance and protection, In: Twarog, S., Kapoor, P.(eds). Protecting and promoting traditional knowledge system, National experiences and International Dimensions, Part-I. The role of Traditional knowledge in Health care and Agriculture New York; United Nations: 2004. 3-6.
3. Omale J, Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata. Afric. J. Biotech 2008;7(17):3129-3133.
4. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry 2001;49:5165-5170.
5. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio RG, Pizzolatti MG, Silva FR. Flavonoids: Prospective drug candidates. Mini Rev Med Chem. 2008;8:1429-1440.
6. Kampa M, Nifli AP, Notas G, Castanas E. Polyphenols and cancer cell growth. Rev Physiol Biochem Pharmacol. 2007;159:79-113.
7. Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R and Chen SS Flavonoids in food and their health benefits. Plant Foods Hum Nutr. 2004;59:113-122.

8. Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, Hakulinen T, Aromaam A. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr. 2002;76:560-568.
9. Tabak C, Arts IC, Smit HA, Heederik D, Kromhout D. Chronic obstructive pulmonary disease and intake of catechins, flavonols, and flavones: The MORGEN Study. Am J Respir Crit Care Med. 2001; 164:61-64.
10. Feroz M, Ahmad R, Sindhu STAK, Shahbaz AM. Preliminary phytochemical analysis of some plant seeds. Pak Vet J. 1993;13:4.
11. Nagata K, Tajiro T, Estsuji H, Noboyasu E Shunuchi M, Chikao N. Comellindin antifungal saponins isolated from Camellia japonica. Agric Biol Chem. 1985;49:1181-1186.
12. Takagi K, Hee PE, Histoshi K. Antibacterial Activity of Various Phytoconstituents of Neem. Agric Biol Chem Bull. 1980;28:1183.
13. Zehavi UML, Segel R J, Potential Use of Colocasia esculentum and Panicum repens as Bioindicators for Environmental Management of Linggi River, Malaysia. Phytophatol. 1986;116:338-343.
14. Sati OP, Chandra CD, Usha R. Preliminary Phytochemical Analysis of Some Plant Seeds, Planta Medica. 1987;5:981-983.
15. Suvarnalatha A, Phytochemical studies of three medicinal plants. M. Phil. Dissertation, Department of Botany, Sri Venkateswara University, Tirupati, India. 2004.
16. Gaur RD, Jyotsana Sharma, Painuli RM. Plants used in traditional healthcare of livestock by Gujjar community of Sub-Himalayan tracts, Uttarakhand. Indian J of Natural Products and Resources. 2010;1(2):243-248.
17. Faridah Hanum, Vander Maesen LJG. Plant Resources of South-East Asia 11- Auxiliary plants, LIPI Press, Jakarta, Indonesia 2007.
18. Siva R. Status of natural dyes and dye-yielding plants in India. Curr. Sci., 2007;92:916-925.
19. Burkill JH. A dictionary of Economic Products of Malaysia Peninsular, Ministry of Agriculture, Kuala Lumpur, Malaysia.1935.
20. Yasodamma N, Suvarna latha A, Santosh Kumar M, Paramageetham Ch. Phytochemical and antibacterial studies of *Indigofera hirsuta* Linn. J. Trop. Med. Plants, 2009;10(1):27 - 29.
21. Suvarnalatha A, Yasodamma N, Chaithra D. Phytochemical and antibacterial studies of *Indigofera hirsuta* L. fruit extracts. Indo American Journal of Pharmaceutical Research. 2013;3(2):4026-4032.
22. Suvarnalatha A, Yasodamma N, Alekhya C. Anthelmintic activity of *Indigofera hirsuta* L. leaf and Fruit extracts. Indo American Journal of Pharmaceutical Research. 2013;3(5): 6299-6303.
23. Suvarna Latha A, Yasodamma N. Qualitative Phenolic compounds Identification and Analysis in *Indigofera hirsuta* L., An International Journal of Advances in Pharmaceutical Sciences. 2014;5(6):2446-2451.
24. Suvarna Latha A, Yasodamma N. Antifungal activity of Leaf and Fruit extracts of *Indigofera hirsuta* L., An International Journal of Advances in Pharmaceutical Sciences. 2014; 5(6):2511-2514.
25. Jain SK, Rao RR. A Handbook of Field and Herbarium Methods. Today and tomorrow's Printers and Publishers, New Delhi, 1977. 462.
26. Ebrahimzadeh MA, Pourmorad F, Bekharadnia AR. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. Journal of Biotechnology, 2008;32:43-49.
27. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica, Mey. Pharmacology online. 2008;2:560-567.
28. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pacific Sci. 1974;48:458-463.
29. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, London. Ltd. 1973. 49-188.
30. Obdoni BO, Ochuko PO Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 2001;8b:203-208.
31. Subhashini S, Kantha D, Arunachalam, Sathesh Kumar A. Preclinical studies on the Phytochemical, Antimicrobial, and Wound healing properties of *Indigofera* aspalathoides leaves. J. of Pharmacy Research. 2011;4(9):3206-3211.
32. Tamilselvi N, Krishnamoorthy P, Dhamotharan R, Sivakumar S. Studies on Phytochemical Constituents of *Indigofera* aspalathoides (Shivanar vembu) Vahl. Ex.DC. Journal of Pharmacy Research. 2011;4(9):3052.
33. Bakasso S, Lamien-Meda A, Lamien CE, Kiendrebeogo M, Millogo J, Ouedraogo AG and Nacoulma OG. Polyphenol contents and Antioxidant activities of five *Indigofera* species (Fabaceae) from Burkina Faso. Pakistan Journal of Biological Sciences. 2008;11(11): 1429-1435.