INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

PHYTOCHEMICAL SCREENING, FUNCTIONAL GROUPS AND ELEMENT ANALYSIS OF *TYLOPHORA PAUCIFLORA* WIGHT AND ARN

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Article Received on: 03/04/12 Revised on: 10/05/12 Approved for publication: 26/05/12

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ABSTRACT
Plants and plant-based medicines are the basis of many of the modern pharmaceuticals we use today for our various ailments. The aim of the study was to find out the bioactive chemical constituents and to analyze the various functional groups of the ethanolic extract of *Tylophora pauciflora*. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, Terpenoids, steroids, Flavonoid, tannins and reducing sugar. FTIR was applied and IR spectrum in mid infrared region 4000–400 cm^{-1} was used for discriminating and to identify various functional groups present in the medicinal plant. Generally *Tylophora* genus has been used in the Ayurvedic system for the treatment of various diseases. The present findings indicated the presence of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons, halogens in ethanolic extract of *Tylophora pauciflora*. The trace element composition of *Tylophora pauciflora* was determined by Scanning Electron Microscope (SEM) with an energy dispersive x-ray spectrometer (EDX). The Concentrations of C, O, Mg, Si, S, Cl, K and Ca were measured.

Key words: *Tylophora pauciflora*, FTIR, EDAX, Phytochemical screening.

INTRODUCTION

Human use of plants as medicinal agents predates recorded history. Ethnomedical plant use data in many forms has been heavily utilized in the development of formularies and pharmacopoeias, providing a major focus in global health care, as well as contributing substantially to the drug development process[1]. The main advantage of using medicinal plants does not produce side effects when compare with synthetic drugs, because medicinal plants contain high content of antioxidant compounds are present high content in plants[2]. Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions[3]. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity[4].

Oxidative Stress imposed by reactive oxygen species may be direct or indirect cause of tissue damage and many human diseases such as Aging, Cancer, Atherosclerosis, Cardiac hypertrophy. Natural antioxidants which are commonly present in medicinal plants scavenging radicals and inhibiting lipid peroxidation and preventing oxidative damage in animal tissue or cells[5]. *Tylophora pauciflora* is one of the vital medicinal plant belongs to Asclepiadaceae family, native of India and Southeast Asia. Generally *Tylophora* genus has been used in the Ayurvedic system for the treatment of various diseases such as antitumor, anti-inflammatory, anti-arthritis, and antilupus activity[6].

The main objective of the study is to screen the phytochemicals present in *Tylophora pauciflora* and to identify the functional groups using FTIR and elemental analysis through EDAX analysis.

MATERIALS AND METHODS

Plant Collection

The whole plant of *Tylophora pauciflora* used for the investigation was obtained from Tirunelveli district, Tamilnadu, India. The plant was identified by Dr.C. Kalidass, Botanical survey of India, TNAU Campus. And Coimbatore. The plant sample was prepared and deposited in the Herbarium of the Botany Department, Bharathiyar University, Coimbatore. The voucher number is 06155. Fresh plant material was washed under running tap water then air dried and powdered.

Extraction

100g of dried plant powder was extracted in 500ml of Ethanol for 24 hr in occasional shaker at room temperature. The supernatant was collected and evaporated to make the final volume one-fifth of the original volume. It was stored at 4°C in airtight bottles for further studies.

Phytochemical analysis

Phytochemical screening of plant extract was carried out according to the method adopted by Peach and Tracey[7].

FTIR Spectrum Analysis

The ethanol extracts of *Tylophora pauciflora* was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded on a Shimadzu FTIR Spectrometer 8000 series, between 4,000–400 cm^{-1}.

Energy Dispersive X-ray Spectroscopy (EDAX Analysis)

The crude extract derived from plant sample of *Tylophora pauciflora* were subjected to the elemental analysis using Scanning Electron Microscope (SEM) with an energy dispersive x-ray spectrometer (EDAX).

RESULTS

PHYTOCHEMICAL SCREENING OF PLANT MATERIALS

Table 1 shows the phytochemical screening of *Tylophora pauciflora.* Preliminary phytochemical screening of ten different chemical compounds (Alkaloids, Cardioglycosides, Saponin, Oils and fats, Tannin and phenolic compound, Terpenoids, Flavonoids, Aminoacids and proteins, Steroids, Carbohydrates) were tested in five different extracts. Among the five different extracts, ethanol extract show the presence of maximum number of compounds than other solvents. Ethanol extracts having high phytochemicals than other solvents. Phytochemical screening of *Tylophora pauciflora* listed in table 1.

FOURIER TRANSFORM INFRARED (FTIR) FINGERPRINTING ANALYSIS

Results of FTIR spectroscopic studies have revealed the presence of various chemical constituents in ethanolic extract of *Tylophora pauciflora* (Figure 1).

The peak at 3412 and 2926 cm^{-1} are corresponded to Hydroxyl and CH stretching frequency respectively. A band at 1722 cm^{-1} is corresponded to carbonyl carbon. The peak at 1635 cm^{-1} to assign C=C. The strong peak at 2862.36 cm–1 assigned to the CH_3 stretching vibration and the peak at 2926.01 cm–1 assigned to the C-H stretching which means that some alkane compounds existed in these rare medicinal plants. The bands between 3000 and 2800 cm-1 represent C-H stretching vibrations that are mainly generated by lipids[8,9]. The stretching assigned to the C–S linkage occur in the region at 700–600 cm–1 The weak absorption band of 601.79, 609.51, 707.88 cm–1 indicates the presence of thiol, sulphite and sulphate group in our plant extract [10]. The more intense bands occurring at 1635.64, 1722.43, 1722.42, 1834.3, 3412.08, 1635.64 corresponding to C=O stretching indicate the presence of ketones, aldehydes, carboxylic acids, esters and amide I band in *Tylophora pauciflora*. The amide band are primarily associated with the stretching motion of the C=O group. This C=O band is sensitive to the environments of the peptide linkage and also depends on the rotein's overall secondary structure[11,12].

Energy Dispersive X-ray Spectroscopy

The results of the elemental composition of *Tylophora pauciflora* using SEM and EDX technique showed in table 3. The SEM EDX spectra of the crude extract of the *Tylophora pauciflora* showed in figure 2.

Calcium, Magnesium, Silicon, Chloride, Pottasium and Carbon are present in *Tylophora pauciflora*. $CaCo_3$, SiO_2, MgO, KCl, MAD, Ca wollastonites are used as the standards.

In all these elements, Carbon and oxygen presented as high concentration while K and Ca presented as moderate amount. But Mg, Si, Cl presented only a trace quantities.

Trace elements are estimated by determining the percentage abundance (%) of elements C, Mg, Si, S, Cl, K and Ca in the sample. The concentrations of such elements, sample are reported in Table 2 and Figure 2

DISCUSSION

Plants are important source of functional components for the development of new chemotherapeutic agents. Phytochemical investigation of the ethanolic extracts of *tylophora* revealed the presence of various phytochemicals such as phenolic compounds, flavonoids, saponins, steroids, tannins. Phytomedicine have been used for the treatment of diseases as in done in cases of Unani and Ayervedic system of medicines, a natural blueprint for the development of new drugs[13]. The presence of glycosides and alkaloids in *Tylophora pauciflora* may be associated with their use by traditional medicine practitioners in healthcare systems in the treatment of cough, fever, cold and venereal diseases. Hence, phytochemicals screening serves as the initial step in predicting the types of potential active compounds[14].

FTIR Spectroscopy was useful for the compound identification and when run under IR region in the range of 400-4000 cm-1 there was a variation in the peaks in both the plant samples[15,16]. FTIR allows detecting the whole range of infrared spectrum simultaneously providing speed and accuracy in measurements of biological specimens[17]. FTIR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures, and has been used as a requisite method to identify medicines in Pharmacopoeia of many countries[18]. Based on the functional group analysis, *Tylophora pauciflora* doesn't contain any toxic compounds.

In the present study, results of EDAX showed the presence of trace elements such as C, Mg, Si, S, Cl, K and Ca in *Tylophora pauciflora*. Deficiency of these trace elements in human subjects can occur under the most practical dietary conditions and in much diseased status. In recent years, scientist and nationalists have started believing in the therapeutic role of metals in human health[19]. Trace elements play both curative and preventive role in combating diseases. Calcium is needed in the development of bone and teeth and it regulate heart rhythm, helps in normal blood clotting, maintain proper nerve and muscle functions and lower blood pressure[20]. Magnesium is important cofactor for the conversion of blood glucose into energy[21]. Potassium is essential for the transport of nutrients inside the cell. Without potassium, nutrients could not able enter into the cell that leads cell death. Silicon is also another important element to prevent the hardening of veins and arteries. Chloride works with sodium and potassium carry an electrical charge when dissolved body fluids and to regulate the pH in the body. Chloride is also important for digest the food properly and absorb many elements that what we need to survive. The presence of these trace elements in *Tylophora pauciflora* marks its usage in therapeautic purpose.

CONCLUSION

The preset study was concluded that traditional use of *Tylophora pauciflora*for human ailments and partly explained its use in herbal medicine as rich source of phytochemicals with the presence of tannins, phenols, saponins, steroids, flavinoids and terpenoids. Thus this plant can be utilized as an alternative source of useful drugs. The presence of characteristic functional groups ketones, aldehydes, carboxylic acids, esters and amide I, thiol, sulphite and sulphate, alkane, lipids are responsible for various medicinal properties of *Tylophora pauciflora.* So it contains high therapeutic content. Based on the conclusion, trace elements present in *Tylophora pauciflora*for has a lot of biological activities to prevent organs from diseases. In future, *Tylophora pauciflora*for could be used as good pharmaceutical and therapeutic agents.Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for industrial drug formulation.

ACKNOWLEDGEMENT

We, the authors are thankful to our Chancellor, Advisor, Vice-Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

REFERENCES

1. Graham JG, Quinn ML, Fabricant DS, Farnsworth NR. Plants used against cancer–an extension of the work of Jonathan Hartwell. *J Ethnopharmacol* 2000; 73 : 347–377
2. Ragavendran P, Sophia D, Arul Raj C, Gopalakrishnan VK. Functional Group Analysis Of Various Extracts Of *Aerva lanata* (L) By FTIR spectrum Pharmacologyonline Newsletter 2011; 358-364
3. Dipak k, Rupali S, Syed I, Bhadange DG. Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (ms) India. International Journal of Pharma and Bio Sciences 2010; 1(4): 253-256.
4. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and invitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complem Altern Med 2010; 10: 21.
5. Ragavendran P, Sophia D, Arul Raj C, Starlin T Gopalakrishnan V.K phytochemical screening, antioxidant activity of *Aerva lanata* (L) – an *in vitro* study. Asian J Pharm Clin Res 2012; 5: 77-81.
6. Gao W, Chen AP, Leung CH, Gullen EA, Furstner A, Shi Q, et al. Structural analogs of *Tylophora* alkaloids may not be functional analogs. Bioorg Medicinal Chem Lett 2008; 18: 704–709.
7. Paech D, Tracey MV. Modern methods of plant analysis. 4th Ed, Wieland: Springer Verlag; 1955; 373-371.
8. Wolkers, WF, Hoekstra AF. Aging of Dry Desiccation-Tolerant Pollen Does Not Affect Protein Secondary Structure. Plant Physiol 1995; 109: 907-915.
9. Wei ZL, Dong L, Tian ZH. Fourier transform infrared spectometry study on early stage of cadmium stress in clover leaves. Pak J Bot 2009; 41: 1743-1750.
10. Muruganantham S, Anbalagan G, Ramamurthy N. FT-IR and sem-eds comparative analysis of medicinal plants, *Eclipta alba* HASSK and *Eclipta Prostrata* linn. Romanian J Biophys 2009; 19: 285–294.
11. Diem M, Susie, White B, Lius, Chinboga. Infrared Spectroscopy of the Cells and Tissues: shining light onto a Noval subjects. Appl Spec 1999; 53: 148–161.
12. Jagadeesan G, Kavitha AV, Subashini J. FT-IR Study of the influence of *Tribulus terrestris* on Mercury intoxicated mice, Mus musculus liver. Tropical Biomed 2005; 22: 15–22.
13. Viji M, Murugesan S. Phytochemical analysis and antibacterial Activity of medicinal plant. *Cardiospermum halicacabum* linn. J Phytol 2010; 2: 68–77.
14. Sukumaran S, Kiruba S, Mahesh M, Nisha SR, Miller PZ, Ben CP, et al. Phytochemical constituents and antibacterial efficacy of the flowers of *Peltophorum pterocarpum* (DC.) Baker ex Heyne. Asian Pacific J Trop Med 2011; 735-738.
15. Thenmozhi M, Bhavya PK, Rajeshwari S. Compound Identification Using HPLC and FTIR In *Eclipt alba* and *Emilia sonchifolia*. Int J Engineering Sci Tech 2011; 3: 292-298.
16. Kalaiselvi M, Gomathi D, Vidya B, Uma C Evaluation of Antioxidant potential and fourier transform infrared spectroscopy analysis of *Ananus comosus* (L.) Merr peel International Research Journal of Pharmacy 2012; 3: 237-242
17. Griffiths PR, De Haseth JA. Fourier transform infrared spectroscopy. John Wiley and Sons, New York 1986; 656.
18. Liu HX, Sun SQ, Lv GH, Chan KK. Study on Angelica and its different extracts by Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy. Mol Biomol Spec 2006; 64: 321–326.
19. Udayakumar R., Begum VH. Elemental analysis of Medicinal Plants used in controlling infectious diseases. Hamdard Med 2004; 67: 35-36.
20. Bibi S, Dastagir G, Hussain F, Sanaullah P. Elemental composition of *Viola odorata* Linn. Pak J Pl Sci 2006; 12: 141-143.
21. Bahadur A, Chaudhry Z, Jan G, Danish M, Rehman A, Ahmad R, et al. Nutritional and elemental analyses of some selected fodder species used in traditional medicine. Afr J Pharm Pharmacol 2011; 5: 1157-1161.

Table 1: *In vitro* Phytochemical Screening of *Tylophora pauciflora*

Extracts	AL	SA	TP	FL	ST	CG	OF	TN	AP	CH
leum ether Ether	-	-	+	-	-	+	-	-	-	-
Chlorofom	-	-	-	+	-	-	-	+	-	+
Ethyl acetate	-	-	+	-	-	+	+	+	-	+
Ethanol	+	+	+	+	+	+	+	+	+	+
Water	+	+	+	+	-	+	-	-	+	+

'+' Present　　'_' Abesnt

AL Alkaloids
SA Saponin
TP Tannin and phenolic compounds
FL Flavonoids
ST Steroids
CG Cardioglycosides
OF Oils and fats
TN Terpenoids
AP Aminoacids and proteins
CH Carbohydrates

Table 2: The percentage of trace elements present in the *Tylophora pauciflora*

Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C	77.99	1.0094	52.98	1.20	61.82
O	28.35	0.4760	40.81	1.14	35.74
Mg	0.47	0.7393	0.43	0.09	0.25
Si	0.59	0.9126	0.44	0.07	0.22
S	0.48	0.9629	0.34	0.07	0.15
Cl	1.33	0.8300	1.10	0.10	0.43
K	4.35	1.0445	2.86	0.14	1.02
Ca	1.45	0.9627	1.03	0.11	0.36
Totals			**100.00**		

Trace elements are estimated by determining the percentage abundance (%) of elements C, Mg, Si, S, Cl, K and Ca in the sample.

Figure 1: FTIR Spectrum analysis of Ethanolic extract of *Tylophora pauciflora*

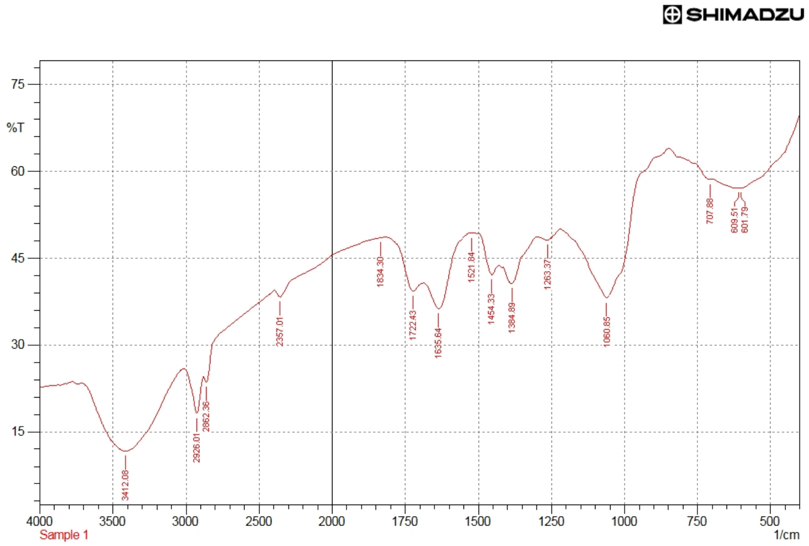
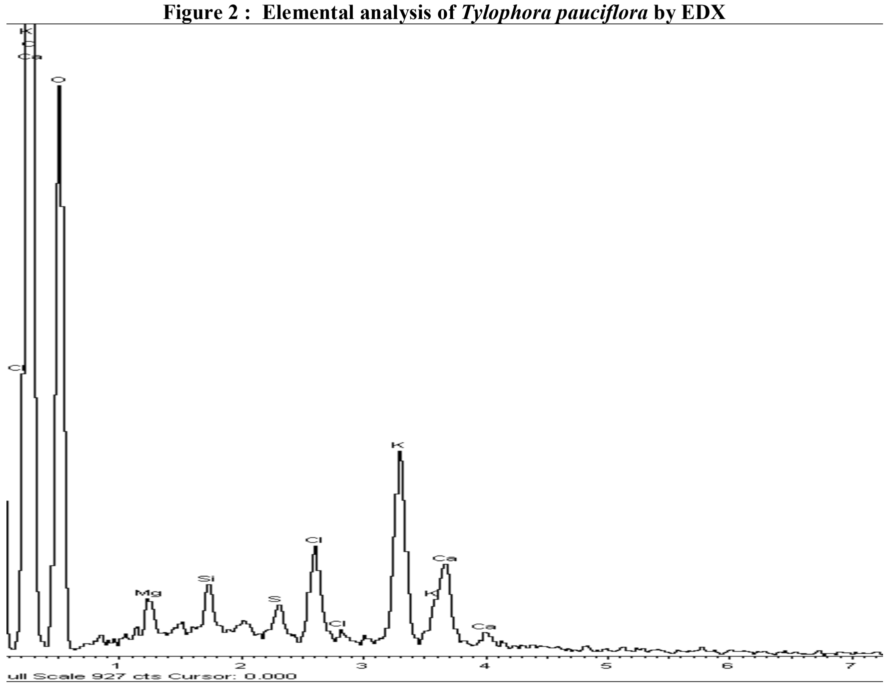


Figure 2 : Elemental analysis of *Tylophora pauciflora* by EDX



Source of support: Nil, Conflict of interest: None Declared