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Chromatographic determination of phenol compounds in *Tylophora pauciflora* WIGHT and ARN by HPTLC technique

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ABSTRACT

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of plants lies in some chemical substances that produce a distinct physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Phenolic compounds have been repeatedly implicated as natural anti-oxidants in fruits, vegetables, cereals and other plants. Standardization of chemical constituents in medicinal plants is an essential measurement for ensuring the quality control of herbal drugs. The HPTLC finger printing analysis of raw material serves as a primary suggestion against which unknown material can be characterized. The present investigation was undertaken to find out the phenolic compounds present in the ethanolic extract of Tylophora pauciflora by using HPTLC. The result of the present study indicates the presence of three phenolic compounds in the ethanolic extract of Tylophora pauciflora. Due to the presence of phenolic compounds in Tylophora pauciflora may be used in the prevention of various diseases such as cancer, diabetes and cardiovascular disorders.

Keywords: HPTLC, Phenolic, *Tylophora pauciflora*, Ethanolic extract

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INTRODUCTION

Traditional medicines have been used for many centuries by a substantial proportion of the population of India [1]. The World Health Organization (WHO) estimated that 80% of the populations of developing countries depend on traditional medicines for their various ailments [2]. Natural products such as herbs, fruits and vegetables become popular in recent years due to public awareness and amplifying interest among consumers and scientific community [3]. A vast diversity of bioactive components in plants has been found terpenes, lignans, sulfides, carotenoids, cumarins, saponins, phytosterols and polyphenols, including flavonoids, anthocianins and phenolic acids [4, 5]**.** Epidemiological evidence has been provided that constituents in natural products show many biological and pharmacological activities, including antioxidative, anti-inflammatory and antiviral effects [6 , 7].

The plants are distinct not only in their therapeutic properties but also in a variety of morphological characters, including those of root, stems, leaves, flower, pollen, etc. The main limitation in the use of traditional remedies is the lack of standardization of raw material, manufacturing process and the final product. A biomarker on the other hand is a group of chemical compounds which are not only unique for that plant material but also correlates with biological efficacy [8]. Development and validation of analytical methods play important roles in the discovery, development and manufacture of pharmaceuticals. HPTLC is an effective and powerful method which can be used for pharmaceuticals analysis, plant constituents, and bio macro molecules. Several samples can be run simultaneously using a small quantity of mobile phase, thus lowering analysis time and cost per analysis [9] with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products.

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 [10]. Although the preliminary phytochemical studies revealed the presence of various bioactive compounds [11], there is no detail study on phytoprofiling of *Tylophora pauciflora*. In the present study, effort has been made to elucidate the Phenolic profile of *Tylophora pauciflora* using high performance thin layer chromatography (HPTLC).

MATERIALS AND METHODS

Plant Material:

The *Tylophora pauciflora* was collected from natural habitats, Tirunelveli District, Tamil Nadu, India, and authenticated by Dr.C. Kalidass, Botanical survey of India, TNAU Campus, Coimbatore. The plant sample was collected and deposited in the Herbarium of the Botany Department, Bharathiar University, Coimbatore, Tamil Nadu. The voucher number is 006155.

Preparation of Plant Extract:

The collected fresh whole plants were air-dried at room temperature. The dried plant material was then homogenized by electric mixer grinder to obtain coarse power and stored in air-tight bottle for further studies. The shade dried, 30 g powered sample were extracted with 150 ml of ethanol for 8-12 h by using the Soxhlet apparatus [12].

HPTLC analysis:

HPTLC studies were carried out the following method by Wagner 1996 [13]. The 100mg of plant extract was dissolved in 1ml Ethanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis. 2µl of test solution and 2µl of standard solution were loaded as 5mm band length in the 3 x 10cm Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber and the plate was developoed with respective mobile phases (Phenols: Toluene-Acetone-Formic acid at the ratio of 4.5: 4.5: 1) up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm. Derivatization: The developed plate was sprayed with respective spray reagent (Phenols : 20% Sodium carbonate reagent and brief dried followed by Folin Cio-calteu reagent) and dried at 100° C in hot air oven. The plate was photo-documented in Day light mode and UV 366nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning: before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 254nm.The Rf values and finger print data were recorded by WIN CATS software.

RESULTS AND DISCUSSION

The ethanolic extract of *Tylophora pauciflora* was studied for phenolic profile in HPTLC finger printing analysis. The results are depicted in Table 1. Blue, brown colored zone was detected in UV after derivitazation in the chromatogram. Fig. 1& 2 confirms the presence of phenolic acids. The extracts were run along with the standard phenolic compounds. Rf value of the extract found to be 0.72, 0.12, 0.22, 0.33, 0.48, 0.73, 0.79, 0.88, 0.97 of peak 1, 2, 3, 4, 5, 6, 7, 8 respectively. Among them peak 1, 2, 5 showed the presence of various phenolic compounds.

Table 1: Peak table with Rf values, height and area of phenolic compounds and unknown compounds of *Tylophora pauciflora* ethanolic Extract

Peak	Rf	Height of the peak	Area of the peak	Assigned substance
1	0.72	674.4	14824.4	Phenolic standard
1	0.12	123.4	2738.0	Phenolic 1
2	0.22	209.9	6623.9	Phenolic 2
3	0.33	164.9	5131.2	Unknown
4	0.48	169.6	5630.1	Unknown
5	0.73	290.2	14073.3	Phenolic 3
6	0.79	115.7	5365.1	Unknown
7	0.88	84.3	5373.8	Unknown
8	0.97	137.8	4651.3	Unknown

Figure 1: HPTLC Chromatogram of *Tylophora pauciflora* ethanolic extract

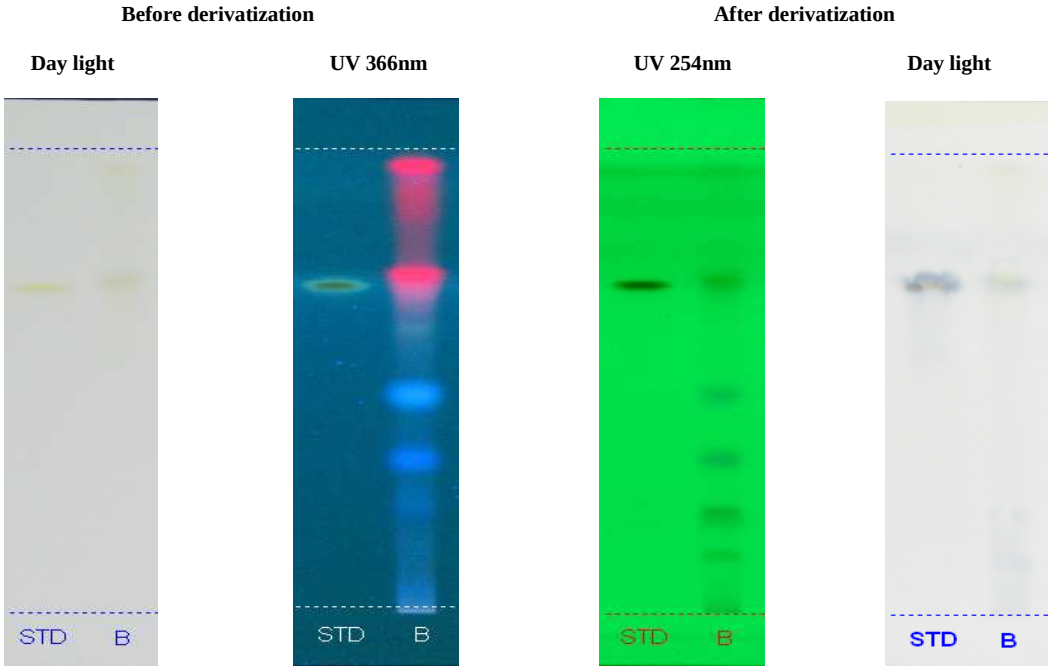
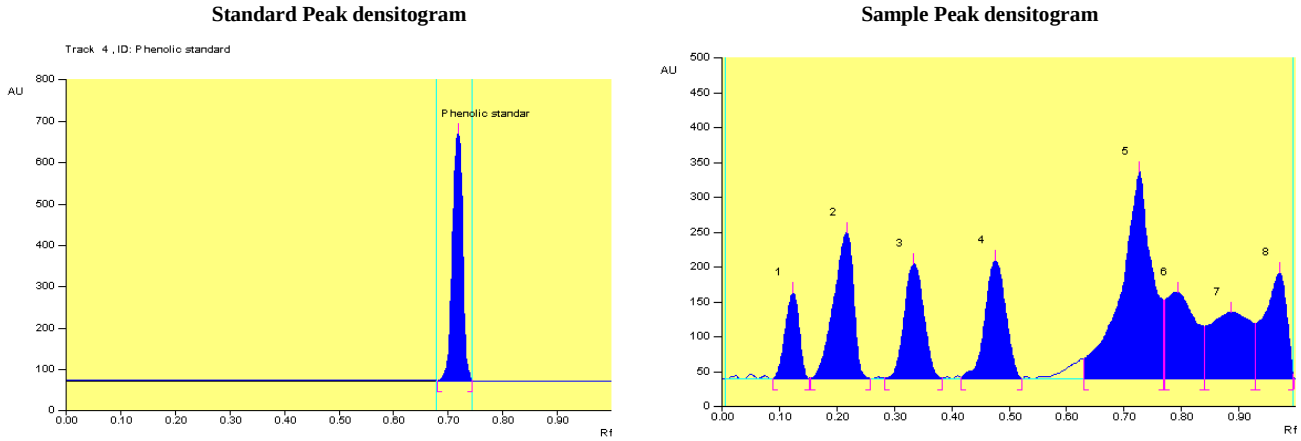


Fig 2: Peak Densitogram Display Scanned at 254 nm.



Recently, HPTLC analysis has been increasing interest in discovery of natural antioxidants, especially those of plant origin. Natural antioxidants derived from plants chiefly phenolics are of considerable interest as dietary supplements or food preservatives. In traditional medicines, medicinal plants have contributed hugely to the traditional and western medicines through providing ingredients for drugs or having played central roles in the drug discovery [14].

Flavonoids and phenolic Compounds are secondary metabolites which are widely distributed in plants [15]. They possess biological properties such as antioxidant, antiaging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities [1 6] . Earlier report suggested phenolic to be a potential iron chelator [17, 18]. Phenolic compounds could inhibit low density lipoprotein oxidation [19]. Total phenol inhibits occurrence of atherosclerosis and cancer [20].

Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties. Antioxidant activity has been directly linked to the presence of phenolic moieties present in the molecular structure of natural antioxidants. Many phytochemicals having phenolic moieties have been shown to exhibit antioxidant activity. Polyphenolic compounds have multiple applications in food, cosmetic and pharmaceutical industries [21, 22].

CONCLUSION

The present study we observed three kinds of phenolic compounds from ethanolic extract of *Tylophora pauciflora* which may help to prevent the human body against oxidative stress related disorders such as cancer, diabetes and other neurodegenerative disorders. By isolating and identification of these phenolic compounds helps in the formulation of new drugs in the treatment of various diseases.

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REFFERENCES

[1] Amit Pandey, Parul Singh, *Asian J. Plant Sci. Res*., **2011**, 1:69-80
[2] M. Prabakaran, N. Chandrakala, A. Panneerselvam, *Asian J. Plant Sci. Res*., **2011**, 1:18-25
[3] K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, J.D.H Byrne, *J. Food Compos Anal*, **2006**, 19, 669-675.
[4] E. Capecka, A. Mareczek, M. Leja, *Food Chemistry*, **2005**, 93, 223−226.
[5] I. Klimczak, M. Malecka, M. Szlachta, GA. Swiglo, *J. Food Compos Anal*, **2007**, 20,313-322.
[6] AM. Pawlowska, W. Oleszek, A. Braca, *J. Agric. Food Chem*, **2008**, 56, 3377-3380.
[7] M. Hakiman, M. Maziah, *Journal of Medicinal Plants Research*, **2009**, 3, 120-131.
[8] Y. Mariswamy, W.E. Gnaraj, M. Johnson, *Asian Pacific J of Trop Biomed*, **2011**, 428-433.
[9] S. S. Havel, S. R. Dhaneshwar, *Der Pharmacia Sinica*, **2011**, 2: 40.
[10] W. Gao, A.P Chen, C.H Leung, E.A Gullen, A. Furstner, Q. Shi, L. Wei, K.H Lee, Y. Cheng, *Bio organic & Medicinal Chemistry Letters*, **2008**, 18, 704–709.
[11] T. Starlin, C. Arul Raj, P. Ragavendran, V.K Gopalakrishnan, *IRJP*, **2012**, 36, 180-183.
[12] P.K Mukherjee, New Delhi: *Business Horizons*, **2002**, 390-403.
[13] H. Wagner, S. Baldt, E.M Zgainski, *Springer*, **1996.**
[14] P. Ragavendran, M. Muthu, C. Arul Raj, D. Sophia, T. Starlin, B. Vidya, P. Chella Perumal, V.K Gopalakrishnan, *J Pharm Biomed Sci*. **2012**, 24, 99-206.
[15] A.L Miller, *Alt. Med. Rev*, **1996**, 1,103-111.
[16] X. Han, T. Shen, H. Lou, *Int J Mol Sci*, **2007**, 8, 950-988.
[17] R.F Boyer, H.M Clark, A.P Laroche, *J. Inorg, Biochem*, **1988**, 32, 171-181.
[18] B. Havsteen, *Biochem. Pharmacol*, **1983**, 30, 1141-1148.
[19] P.L Teissedre, N. Landrault, *Food Res Intl*, **2000**, 33, 461-467.
[20] G.M Williams, M.J Iatropoulos, *Oxidants, Antioxidants and Free Radicals*, Taylor and Francis, USA, **1997**, 341-350.
[21] M.P Kahkonen, A.I Hopia, H.J Vuorela, J.P Rauha, K. Pihlaja, T.S Kujala, *Journal of Agri and Food Chem*, **1999**, 47: 3954-3962.
[22] E. Frankel, International conference on food factors. *Chemistry and Cancer Prevention*, Hamamatsu, Japan. Abstracts, **1995**, C6-2.