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Research Article

CHROMATOGRAPHIC FINGERPRINT ANALYSIS OF DITERPENOIDS AND SESQUITERPENOIDS IN N-HEXANE EXTRACT OF *Emilia sonchifolia* (L.) DC BY HPTLC TECHNIQUE

PRATIBHA PRABHAKARAN[1] AND VELLIYUR KANNIAPPAN GOPALAKRISHNAN[1,2]

[1]Department of Bioinformatics, [2]Department of Biochemistry, Karpagam University, Coimbatore, Tamil Nadu, India 641021. Email: vkgopalakrishnan@gmail.com

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ABSTRACT

Objective: The main objective of the study is to investigate the chemical fingerprint profile of diterpenoid and sesquiterpenoids of *E. sonchifolia* (L.) DC. using High Performance Thin Layer Chromatography (HPTLC) technique.

Methods: HPTLC profile of diterpenoid and sesquiterpenoids were done and profiles were developed for authentication.

Results: The n-hexane extract of *E. sonchifolia* showed the presence of diterpenoids in the standard but absent in the sample with different R_f values in the range of 0.01 to 0.61 but the sesquiterpenoids is present in both the standard as well as the sample with different R_f values in the range of 0.01 to 0.93.

Conclusion: Based on the results it can be concluded that the n-hexane extract of *E. sonchifolia* is the potential natural resources for pharmacology and functional foods. The development of such fingerprinting from the aerial parts of *E. sonchifolia* is useful in differentiating the species from the adulterant and act as biochemical markers in the pharmaceutical industry and plant systematic studies. In future, the bioactive compounds will be isolated from this plant which may lead to the formation of new drugs against various diseases.

Keywords: *Emilia sonchifolia* (L.) DC., HPTLC fingerprinting, Diterpenoid, Sesquiterpenoid.

INTRODUCTION

India has a rich diversity of plant-based knowledge on healthcare. In different countries plants are used medicinally as a source of many potent and powerful raw drug and they possess varied medicinal properties [1]. According to World Health Organization approximately 80% of the people in developing countries rely chiefly on traditional medicines for the primary health care needs of which a major portion involves the use of plants extracts or active principles originating from plant parts [2]. The medicinal value of these plant lies in some chemical substances that produce a definite physiological action on the human body [3]. Many of the indigenous medicinal plants are used as spices and food plants. Plants have traditionally served as man's novel weapon against different ailments [4]. Medicinal plants, also known as herbs, herbal medicines, pharmacologically active plants or phytomedicine are the dominant form of medicine in most countries [5]. Isolation of biologically active compounds from plant has always been of great interest for scientists, as these drugs have come from botanical sources [6]. Modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening; plants remain a major source of medicinal compounds. Synthetic drugs causes side effects as a result people are more favourable to use natural compounds obtained from plants [7].

E. sonchifolia (Asteraceae), is an herbaceous plant which is found in India and other countries of Asia [8]. It is an edible plant used in the Ayurvedic system of medicine for the treatment of gastropathy, diarrhea, ophthalmia, nyctalopia, cuts and wounds, intermittent fevers, pharyngodyma and asthma [9]. The root is used for controlling diarrhea. The juice of fresh leaves is used for sore ears, sore eyes and night blindness [10]. It has been reported that the methanolic extract of *E. sonchifolia* exhibited anticancer property, anti-inflammatory and antioxidant activity. Previous study revealed that the *E. sonchifolia* contains alkaloids, flavonoids and terpernes, including γ humulene, kaempherol-3-d-galactoside, quercitrin, quercetin, rutin, ursolic acid, senkirkine, doronine, β-sitosterol, and stigmasterol [11]. Some known compounds like simiral, beta-sitosterol, stigmasterol, palmitic acid and honey acid were obtained from the whole plant of *E. sonchifolia* [12]. The main objective of this study is aimed to analyze and determine the HPTLC fingerprinting profile for diterpenoids and sesquiterpenoids in the n-hexane extract of *E. sonchifoli*a.

MATERIALS AND METHODS

Plant collection and Authentication:

The whole plant material of E. *sonchifolia* was procured from Thrissur District, Kerala, India and the plant was taxonomically authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, TNAU campus Coimbatore, with the voucher number BSI/SRC/5/23/09-10/Tech/782.

Preparation of n-hexane extract

The whole plant material was washed thoroughly under running tap water, air dried, finely powdered and stored in airtight bottles. The powder soaked in n-hexane solvent was kept in the shaker for 48 h at room temperature. The extract was collected and concentrated at 40°C under reduced pressure using rotary evaporator. The dried extract was stored at 4°C until further use. The remaining residue was extracted 3 times with the fresh solvent to ensure complete extraction.

HPTLC analysis

HPTLC analysis for diterpenoids

2µl of test solution and 2µl of standard solution (Gibberellic acid) was loaded as 5mm band length in the 3 x 10 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 (after saturated with Solvent vapor) with respective mobile phase (Diterpenoid) and the plate was developed in the respective mobile phase Chloroform-Toluene-Methanol (6 : 2.5 : 1.5) 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 Visible light, UV 254nm and UV 366nm. Derivatization: The developed plate was sprayed with respective spray reagent Anisaldehyde sulphuric acid reagent and dried at 100°C in hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning: After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm.

HPTLC analysis for Sesquiterpenoids

2µl of test solution and 2µl of standard solution (dihydroxyartemisinin) was loaded as 5mm band length in the 3 x 10 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 (after saturated with Solvent vapor) with respective mobile phase (Sesquiterpenoid) and the plate was developed in the respective mobile phase Cyclohexane-Ethyl acetate-Acetic acid (20 : 10 : 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 Visible light, UV 254nm and UV 366nm. Derivatization: The developed plate was sprayed with respective spray reagent Anisaldehyde sulphuric acid reagent and dried at 100°C in hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning: After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm.

RESULT AND DISCUSSION

Natural products are the main origin of bioactive molecules and have played a major role in discovery of lead compounds for the development of drugs for treatment of human diseases [13]. High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images [14]. The major advantage of HPTLC is in reducing analysis time and cost per analysis. Another advantage is that several samples can be estimated using a small quantity of mobile phase. It also facilitates repeated detection of the chromatogram with same or different parameters. Thus it can be said that HPTLC method is simple precise, specific, sensitive and accurate [15]. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders [16]. In HPTLC profile each and every metabolite has played specific role and function in harmony with other metabolites within the organization framework of the cells in the defense mechanism of the plants. Chromatographic finger printing of phytoconstituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern [17]. According to WHO, it has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards [18].

Terpenoids represent one of the largest families of natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolism. The terpenoids show significant pharmacological activities such as antiviral, antibacterial, antimalarial, anti-inflammatory inhibition of cholesterol synthesis and anticancer activities [19]. Scientific research demonstrates that in sesquiterpenes, the lactones deserve special interest. They are mainly found in several genera of Asteraceae. However these compounds are of interest not only from chemical and chemotaxonomical standard points, but also many of them possess biological, and therapeutic activity including anti-inflammatory, anti-tumoural, antimicrobial, anthelminthic and anti-feeding[20].

HPTLC analysis for Diterpenoids

The HPTLC peak table, peak display and peak densitogram were observed and found that the blue coloured fluorescent zone at UV 366nm mode was present in the track, it was observed from the chromatogram after derivatization which confirmed the presence of diterpenoid in the given standard and may not be in the sample. (Figure 1-6).

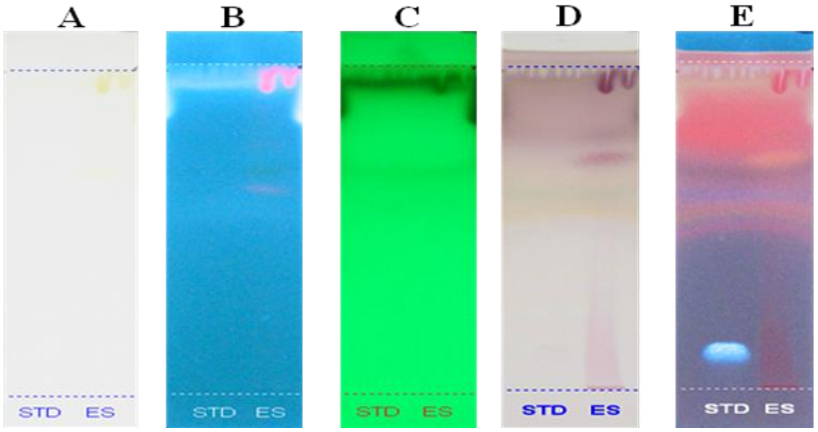

Fig. 1: Chromatogram before derivatization A) Under day light B) Under UV 366nm, C) Under UV 254nm. Chromatogram after derivatization D) under day light E) Under UV 366nm.

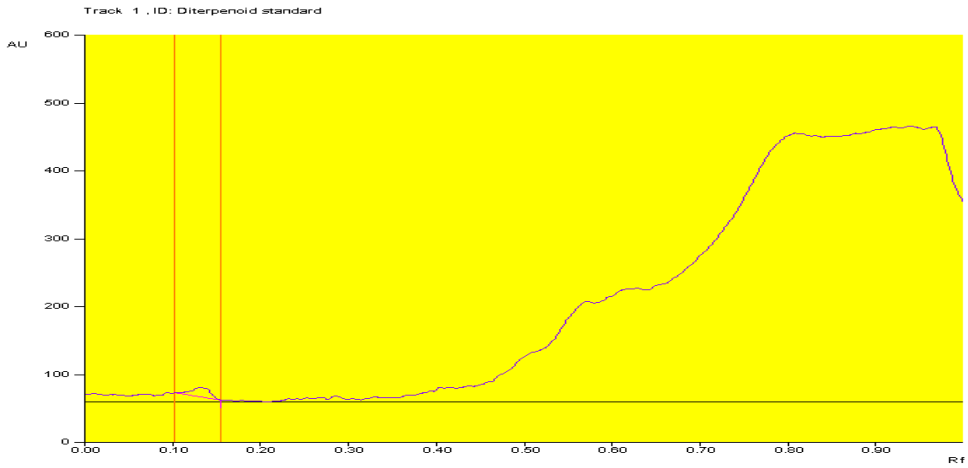

Fig. 2: Diterpenoid standard Baseline display (Scanned at 500nm)

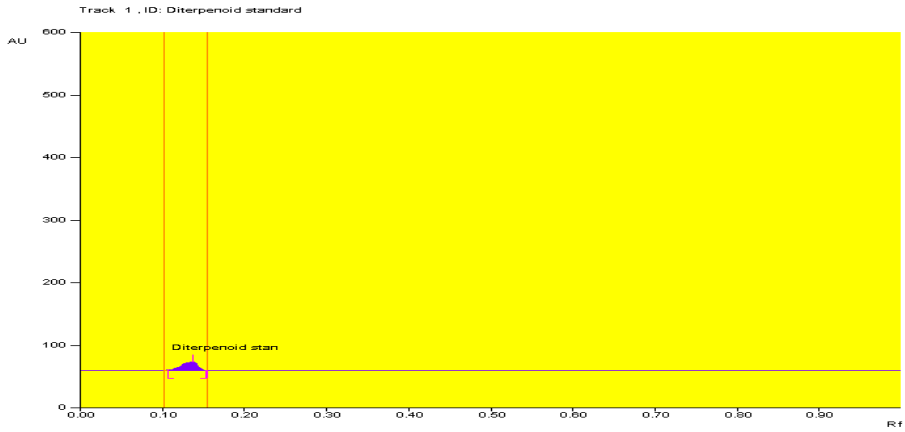

Fig. 3: Diterpenoid standard Peak densitogram display (Scanned at 500nm)

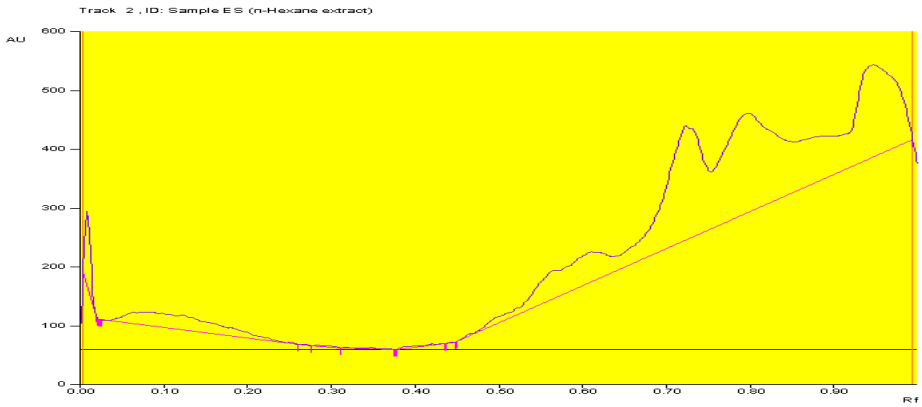

Fig. 4: Sample *E. sonchifolia* n-Hexane extract sample Baseline display (Scanned at 500nm)

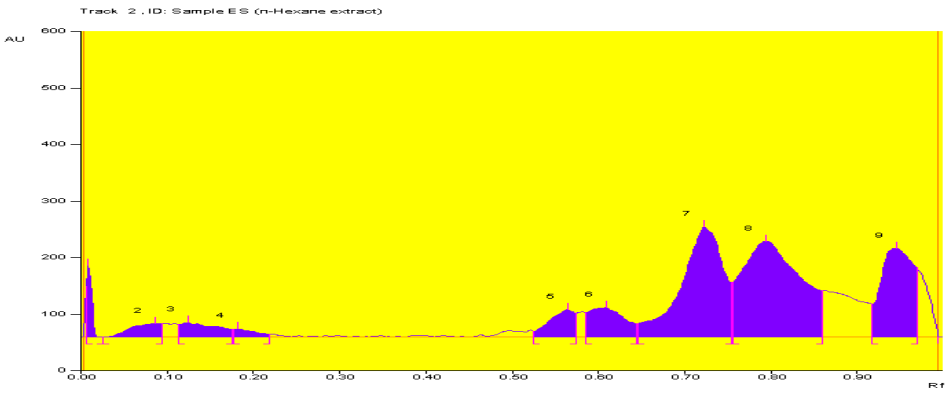

Fig. 5: Sample *E. sonchifolia* n-Hexane extract sample Peak densitogram display (Scanned at 500nm)

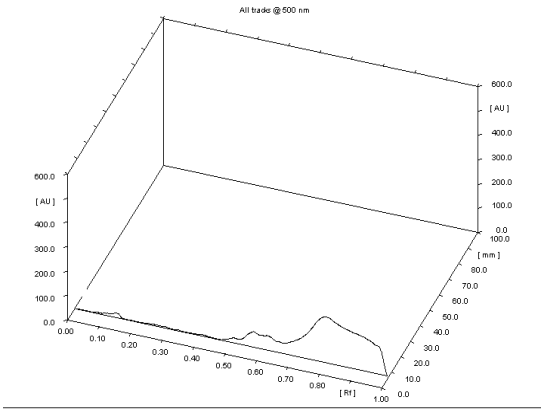

Fig. 6: 3D display of HPTLC chromatogram of aerial parts of *E. sonchifolia*

Thus, the presence of diterpenoids in the given standard and may not be in the sample (n-hexane extract of the whole plant of *E. sonchifolia*) was confirmed from the chromatogram after derivatization by HPTLC analysis. The R_f value of the different compounds present in the extract was found to be 0.01, 0.09, 0.12, 0.18, 0.56, 0.61 respectively and the diterpenoids standard is 0.14. Among all the peaks only the standard is having diterpenoids whereas it's absent in the case of all sample ES from 1 to 9 respectively which is indicated in Table 1.

Table 1: Shows peak table with R_f values, height and area of Diterpenoids and unknown compounds in hexane extract of *E. sonchifolia*

Track	Peak	R_f	Height	Area	Assigned substance
STD	1	0.14	14.2	293.8	Gibberellic Acid
Sample ES	1	0.01	126.8	708.5	Unknown
Sample ES	2	0.09	24.1	778.3	Unknown
Sample ES	3	0.12	25.5	994.5	Unknown
Sample ES	4	0.18	14.3	338.4	Unknown
Sample ES	5	0.56	46.2	1238.4	Unknown
Sample ES	6	0.61	48.9	1854.1	Unknown
Sample ES	7	0.72	189.9	8267.3	Unknown
Sample ES	8	0.79	163.2	10053.8	Unknown
Sample ES	9	0.94	148.3	4988.7	Unknown

HPTLC analysis for Sesquiterpenoids

The HPTLC analysis assessed that the n-hexane extract of *E. sonchifolia* has two Sesquiterpenoids. The peak table, peak display and peak densitogram were noted. Pink coloured zone at visible light mode was present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of sesquiterpenoid in the given standard (dihydroxyartemesinin) and may be in the sample. (Figure 7-12).

Thus, the presence of sesquiterpenoids in the n-hexane extract of the whole plant of *E. sonchifolia* was confirmed by HPTLC analysis. The R_f value of the different compounds present in the extract was found to be 0.01, 0.07, 0.51, 0.57, 0.60, 0.68, 0.75, 0.87 and 0.93 of peak 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively and that of sesquiterpenoid standard was 0.53, Among the peaks the standard and the sample ES 7, and 9 respectively were found to contain sesquiterpenoids which is indicated in Table 2.

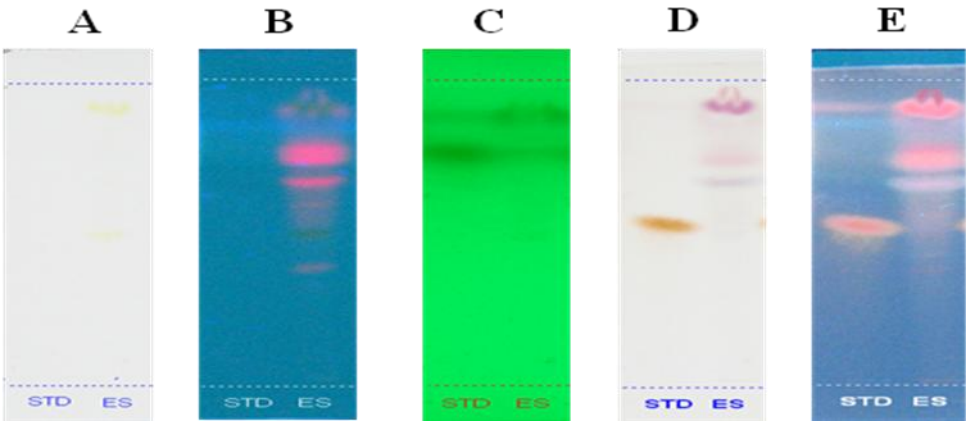

Fig. 7: Chromatogram before derivatization A) Under day light B) Under UV 366nm, C) Under UV 254nm. Chromatogram after derivatization D) under day light E) Under UV 366nm.

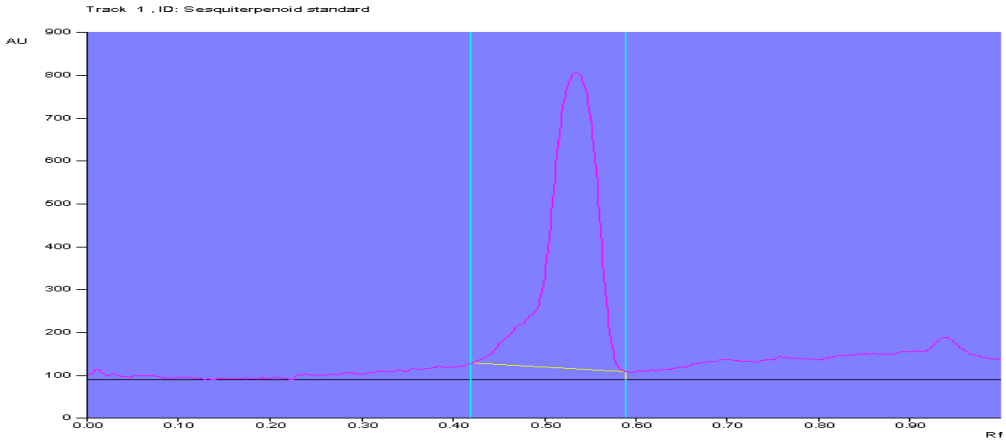

Fig. 8: Sesquiterpenoid standard Baseline display (Scanned at 500nm)

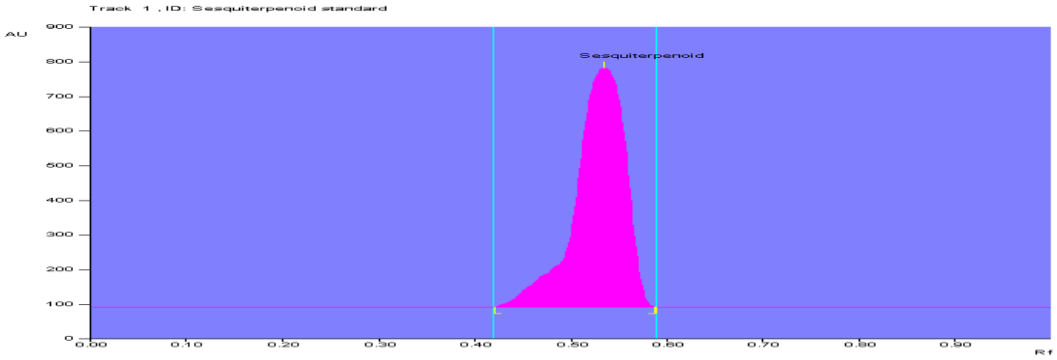

Fig. 9: Sesquiterpenoid standard Peak densitogram display (Scanned at 500nm)

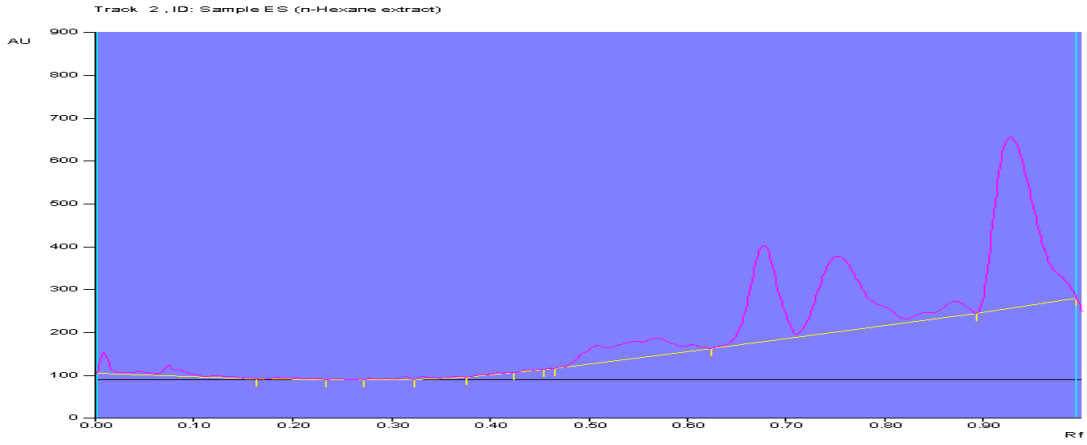

Fig. 10: Sample *E. sonchifolia* n-Hexane extract sample Baseline display (Scanned at 500nm)

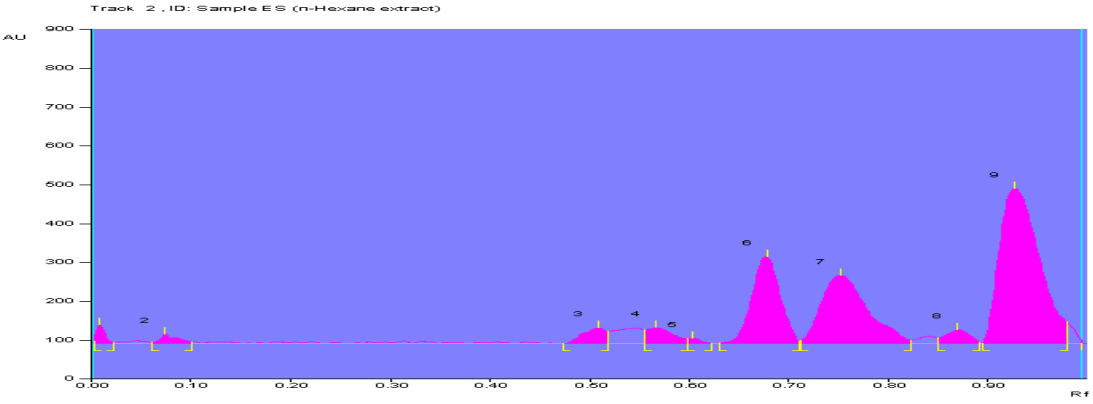

Fig. 11: Sample *E. sonchifolia* n-Hexane extract sample Peak densitogram display (Scanned at 500nm)

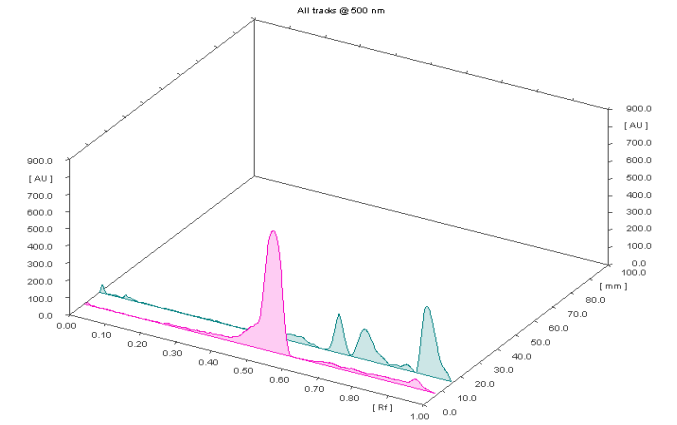

Fig. 12: 3D display of HPTLC chromatogram of aerial parts of *E. sonchifolia*

Table 2: Shows peak table with R_f values, height and area of Sesquiterpenoids and unknown compounds in hexane extract of *E. sonchifolia*

Track	Peak	R_f	Height	Area	Assigned substance
STD	1	0.53	701.1	35789.7	Dihydroxyartemesinin
Sample ES	1	0.01	48.9	372.4	Unknown
Sample ES	2	0.07	24.6	378.6	Unknown
Sample ES	3	0.51	40.1	872.7	Unknown
Sample ES	4	0.57	40.3	1007.4	Unknown
Sample ES	5	0.60	13.9	152.7	Unknown
Sample ES	6	0.68	224.0	5784.0	Unknown
Sample ES	7	0.75	175.9	7503.6	Sesquiterpenoid 1
Sample ES	8	0.87	35.0	771.4	Unknown
Sample ES	9	0.93	399.6	14653.0	Sesquiterpenoid 2

CONCLUSION

The HPTLC fingerprinting results showed that sesquiterpenoid is present within the n-hexane extract of *E. sonchifolia.* On the other hand, the diterpenoid is absent in this plant. Therefore this study can be concluded that the n-hexane extract of *E. sonchifolia* (L.) DC has numerous biological functional of sesquiterpenoid. In future, these fingerprinting images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy.

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REFERENCES

1. Mahesh B, and Satish S. Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. World J Agri Sci 2008; 4: 839-843.
2. Ahmad FB, and Ismail G. Medicinal Plants Used By Kadazandusun Communities around Crocker Range. ASEAN Rev Biodivers Environ Conserv 2003; 1-10.
3. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005; 4: 685-688.
4. Samuel JK and Andrews B. Traditional medicinal plant wealth of Pachalur and Periyur hamlets Dindigul district, Tamil Nadu. Indian J Tradition Knowl 2010; 9: 264-270.
5. Sultana N, Rahman MM, Ahmed S, Akter S, Haque MM, Parveen S, *et al.,*. Antimicrobial Compounds from the Rihzomes of *Sansevieria hyacinthoides*. Bangladesh J Sci Ind Res 2011; 3: 329-332.
6. Kuete V, Metuno R, Ngameni B, Tsafack AM, Ngandeu F, Ngandeu GW *et al.,*. Antimicrobial activity of the methanolic extracts and compounds from *Treculia obovoidea* (Moraceae). J Ethnopharmacol 2007*;* 112: 531–536.
7. Gunalan G, Saraswathy A and Vijayalakshmi K. HPTLC fingerprint profile of *Bauhinia variegata* Linn. leaves. Asian Pac J Trop Disease 2012; S21-S25.
8. Sophia D, Ragavendran P Arulraj C and Gopalakrishnan VK. *In vitro* antioxidant activity and HPTLC determination of n-hexane extract of *Emilia sonchifolia* (L.)DC. J Basic Clin Pharm 2011; 2:179-183.
9. Gayathri Devi D, Lija Y, Cibin TR, Biju PG, Devi VG and Abraham A. Evaluation of the protective effects of *Emilia sonchifolia* Linn. (DC) on perchlorate-induced oxidative damage. J Biol Sci 2006*;* 6: 887-892.
10. Raj M, Natural antioxidant (flavone glycoside) from *Emilia sonchifolia* DC. and its potential activity. Int J Pharm Pharm Sci 2012*;* 4: 159-162.
11. Lan YH, Chiang JH, Huang WW, Lu CC, Chung JG, Wu TS, *et al.,*. Activations of Both Extrinsic and Intrinsic Pathways in HCT 116 Human Colorectal Cancer Cells Contribute to Apoptosis through p53-Mediated ATM/Fas Signaling by *Emilia sonchifolia* Extract, a Folklore Medicinal Plant. J Evid Based Complementary Altern Med 2012; 10:1-13.
12. Rahman MA, Akter N, Rashid H, Ahmed NU, Uddin N and Islam MS. Analgesic and anti-inflammatory effect of whole Ageratum conyzoides and *Emilia sonchifolia* alcoholic extracts in animal models. Afr J Pharm Pharacol 2012; 6: 1469-1476.
13. Alphonso P and Saraf A. Chemical profile studies on the secondary metabolites of medicinally important plant *Zanthoxylum rhetsa* (Roxb.) DC using HPTLC. Asian Pac J Trop Biomed 2012; S1293-S1298.
14. Mariswamy Y, Gnaraj WE and Johnson M. Chromatographic finger print analysis of steroids in *Aerva lanata* L by HPTLC technique. Asian Pac J Trop Biomed 2011; 428-433.
15. Hussain S, Fareed S and Ali M. Simultaneous HPTLC-UV530 nm analysis and validation of bioactive lupeol and stigmasterol in *Hygrophila auriculata* (K. Schum) Heine. Asian Pac J Trop Biomed 2012; S612-S617.
16. Sampathkumar S and Ramakrishnan N. Chromatographic finger print analysis of *Naringi crenulata* by HPTLC technique. Asian Pac J Trop Biomed 2011; S195-S198.
17. Mariswamy Y, Gnaraj WE and Antonisamy JM. Chromatographic fingerprint analysis on flavonoids constituents of the medicinally important plant *Aerva lanata* L. by HPTLC technique. Asian Pac J Trop Biomed 2012; S8-S12.
18. Hariprasad P and Ramakrishnan N. Chromatographic finger print analysis of *Rumex vesicarius* L. by HPTLC technique. Asian Pac J Trop Biomed 2012; S57-S63.
19. Gomathi D, Ravikumar G, Kalaiselvi M, Vidya B and Uma C. HPTLC fingerprinting analysis of *Evolvulus alsinoides* (L.) L. J Acute Med 2012; 2:77-82.
20. Merfort I. Review of the analytical techniques for sesquiterpenes and sesquiterpene lactones. J. Chromatogr 2002; 967:115-130.