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***In vitro* antioxidant activity and HPTLC determination of n-hexane extract of *Emilia sonchifolia* (L.)DC.**

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ABSTRACT

The free radical scavenging activities of n-hexane extract of the whole plant of *Emilia sonchifolia* was evaluated by employing various *in vitro* assay systems like DPPH radical scavenging activity, superoxide radical scavenging activity and hydrogen peroxide scavenging activity with IC₅₀ values 180, 160 and 160 µg/ml respectively. The results of the study indicate that the n-hexane extract of the whole plant of *Emilia sonchifolia* possess a significant scavenging effect with increasing concentrations probably due to its antioxidant potential. High performance thin layer chromatography (HPTLC) analysis in the n-hexane extract of *Emilia sonchifolia* showed the presence of terpenoids which probably may be responsible for the antioxidant activity. Thus, n-hexane extract of *Emilia sonchifolia* can be used potentially as a bioactive source of natural antioxidants due to the presence of terpenoids in it.

KEY WORDS

Emilia sonchifolia, free radical, antioxidant, DPPH, HPTLC.

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INTRODUCTION

Free radicals arising from metabolism or environmental sources interact continuously in biological systems and their uncontrolled generation correlates directly with molecular level of many diseases [1]. Lots of research has clearly showed that free radicals would damage nearby structures including DNA, proteins or lipids. Radical scavenging antioxidants are particularly important in antioxidative defence in protecting cells from the injury of free radical [2]. Medicinal plants contain a variety of bioactive substances which are of considerable use against various diseases. The demand for natural food ingredients has resulted in extensive research on naturally occurring antioxidants [3] which can neutralize highly reactive free radicals.

Recently, research on phytochemicals and their effects on human health have been intensively studied. Terpenoids and isoprenoids are a structurally diverse group of natural products. More than 25,000 representatives with a variety of biological functions have been reported in the plant kingdom [4] and structurally diverse groups of secondary metabolites. Several compounds belonging to the class of terpenes have been reported to possess antioxidant properties, such as, monoterpene hydrocarbons (myrcene, terpinolene, pinene), oxygenated monoterpenes (nerol, geraniol, linalol, thymol), sesquiterpene hydrocarbons (humulene, valencene, calarene), oxygenated sesquiterpenes (trans-trans-farnesol, farnesol, farnesyl acetate, guaial), diterpene hydrocarbons (phytol, abetine), and tetraterpene hydrocarbons (carotenoids) [5,6]. From clinical studies, it is shown that terpenoids can increase the concentration of antioxidants [7].

Emilia sonchifolia (L.) DC (Asteraceae), a herbaceous plant, is found in India and other countries in Asia. It is used as folklore medicine against inflammation, rheumatism, cough, cuts and wounds in India [8]. Fresh juice squeezed from the leaves is utilized in the dressing of fresh wounds. The aqueous and methanolic extracts of *Emilia sonchifolia* leaves progressively reduced rat paw oedema [9]. The hydroethanolic extract of *Emilia sonchifolia* possess antinociceptive effect [10]. The present study was undertaken to investigate the free radical scavenging activities and also to confirm the presence of terpenoid by HPTLC analysis in the n-hexane extract of *Emilia sonchifolia*.

MATERIALS AND METHODS

Plant collection

Emilia sonchifolia was collected from Thrissur, Kerala, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/09-10/Tech/782. Fresh plant material was washed under running tap water, air dried and powdered.

Preparation of extract

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 again with the fresh solvent to ensure complete extraction.

Free radical scavenging activity

The free radical scavenging activity of the n-hexane extract of *Emilia sonchifolia* was determined by using various *in vitro* assays like 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) scavenging assay, super oxide radical scavenging assay and hydrogen peroxide radical scavenging assay.

DPPH radical scavenging activity

The free-radical scavenging activity of n-hexane extract of *Emilia sonchifolia* was tested by their ability to bleach the stable radical DPPH. The antioxidant activity using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay was assessed by the method of Blois [11]. The reaction mixture contained 100 µM DPPH in methanol and different concentrations (200-1000 µg/ml) of the extracts. Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. Butylated Hydroxyl Toluene (BHT) was used as a reference compound.

Super oxide radical scavenging activity

The measurement of superoxide scavenging activity of the n-hexane extract of *Emilia sonchifolia* is based on method as described by Liu [12] and is assayed by

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Figure 1: DPPH scavenging activity.

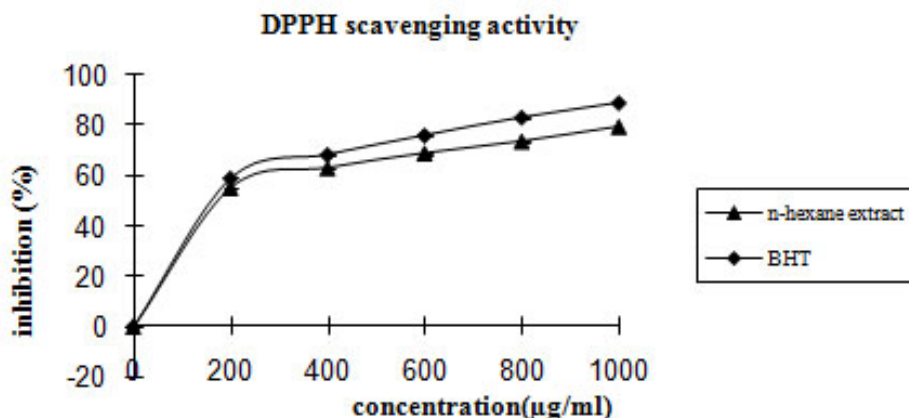
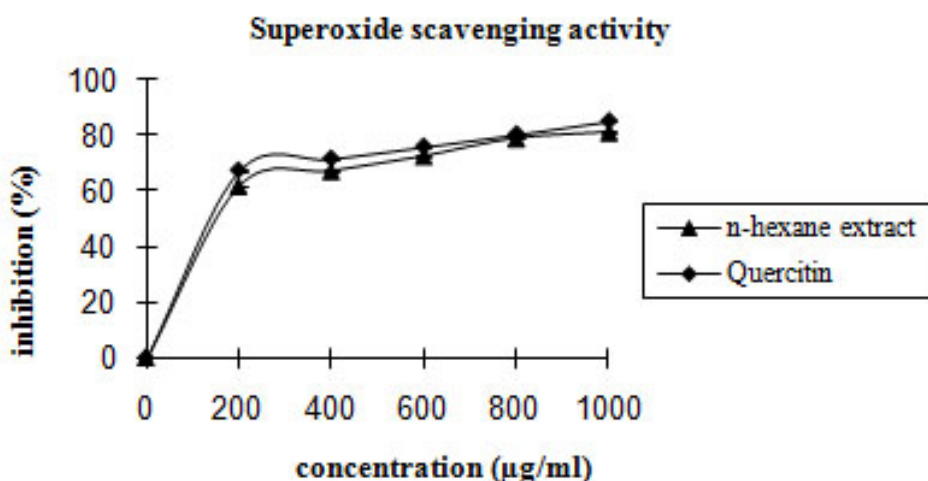


Figure 2: Superoxide scavenging activity.



the reduction of nitroblue tetrazolium (NBT). Tris HCl buffer (3 ml, 16 mM, pH 8.0) containing 1 ml NBT (50 µM) solution, 1 ml NADH (78 µM) solution and a sample solution of extract (200-1000 µg/ml) in water were mixed. The reaction was started when 1 ml of phenazine methosulfate (PMS) solution (10 µM) was added to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance was read at 560 nm against the corresponding blank samples. Quercetin was used as a reference drug. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

Hydrogen peroxide radical scavenging activity

The ability of the n-hexane extract of *Emilia sonchifolia* to scavenge hydrogen peroxide was determined according to method of Nabavi [13]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer, pH 7.4. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (200-1000 µg/ml) in distilled water were added to a hydrogen peroxide solution at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Tocopherol was used as a positive control.

Statistical analysis

The results were expressed as mean ± SD of three parallel measurements. The statistical comparison among the groups were performed with one way ANOVA test using a statistical package program (SPSS 10.0) at p<0.05 significant level.

HPTLC analysis

0.5 µl of hexane extract were loaded as 6mm band length in the 3 x 10cm Silica gel 60F254 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 (Terpenoid) and the plate was developed in the respective mobile phase (n-Hexane-Ethyl acetate (7.2: 2.9)) 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 anisaldehyde sulphuric acid 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: After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm.

RESULTS AND DISCUSSION

Our results demonstrate that the n-hexane extract of the whole plant of *Emilia sonchifolia* possess free radical scavenging activity *in vitro* models like DPPH scavenging assay, super oxide radical scavenging assay and hydrogen peroxide radical scavenging assay. DPPH is one of the free radical widely used for testing preliminary radical scavenging activity of the plant extract [14]. Scavenging of DPPH radical is related to the inhibition of lipid peroxidation [15]. The n-hexane extract of *Emilia sonchifolia* exhibited a higher free radical scavenging activity. At a concentration of 1mg /ml, the scavenging activity of n-hexane extract of *Emilia sonchifolia* reached 79.36%, while at the same concentration, the standard was 89.26%. It is found that the IC₅₀ value of the n-hexane extract of *Emilia sonchifolia* is 180 µg. This may be due to antioxidant activity of n-hexane extract of *Emilia sonchifolia* trapping free radicals by donating hydrogen atom (Figure 1)

The most prominent and generally toxic ROS are produced after infections and during inflammations by several types of inflammatory cells, e.g. neutrophils.

Figure 3: Hydrogen peroxide scavenging activity.

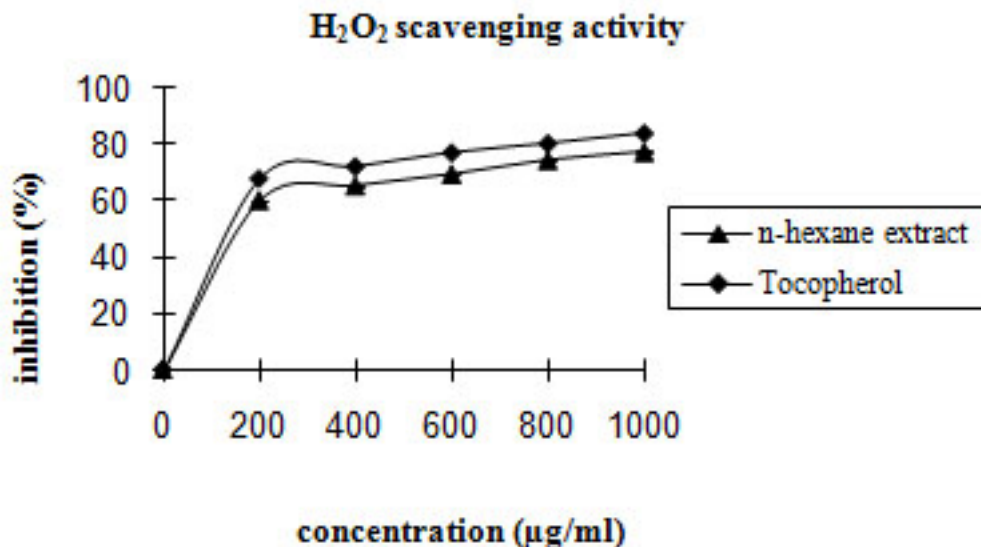
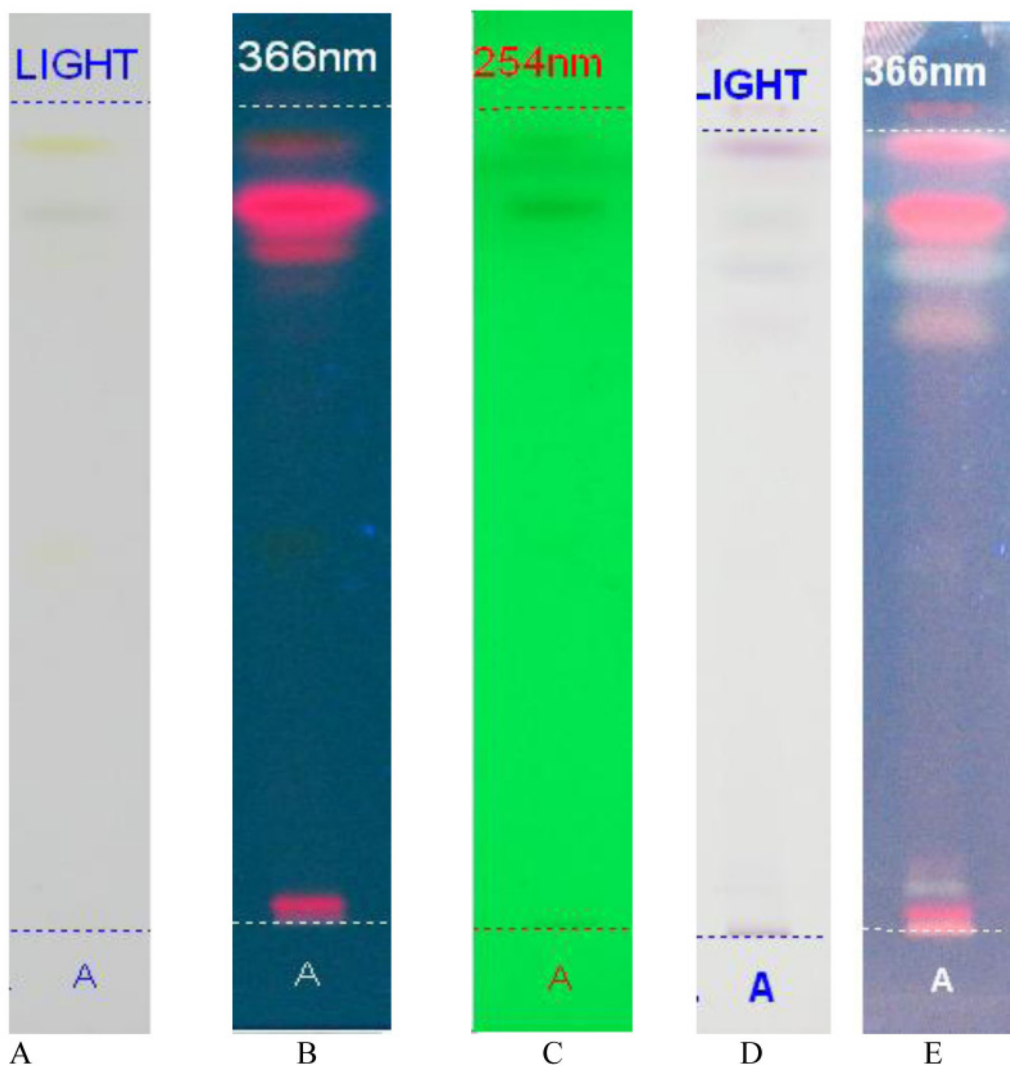


Figure 4: Chromatogram before derivatization A) Under day light B) Under UV 366nm, C) Under UV 254nm. Chromatogram after derivatization D) under day light E) Under UV 366nm.



Stimulated neutrophils release superoxide which at physiological pH undergoes dismutation to form H₂O₂ and O₂. ROS may damage surrounding tissue and so contribute to different clinical pictures or diseases such as inflammatory processes

or atherosclerosis [16]. Hydrogen peroxide itself is not very reactive, but it may induce hydroxyl radicals, which would result in great damage to cells [17]. Superoxide anion is a relatively weak oxidant, but it can generate more dangerous species,

Figure 5: Baseline display of hexane extract of *Emilia sonchifolia* (Scanned at 500nm).

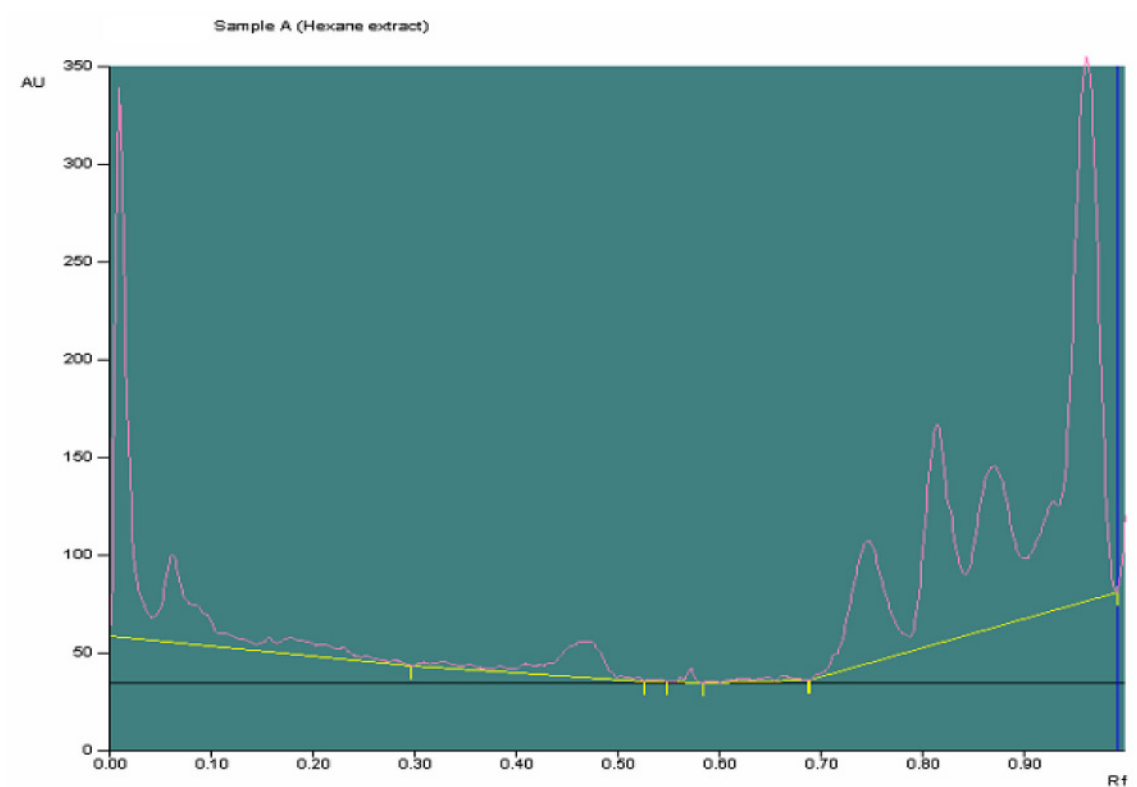
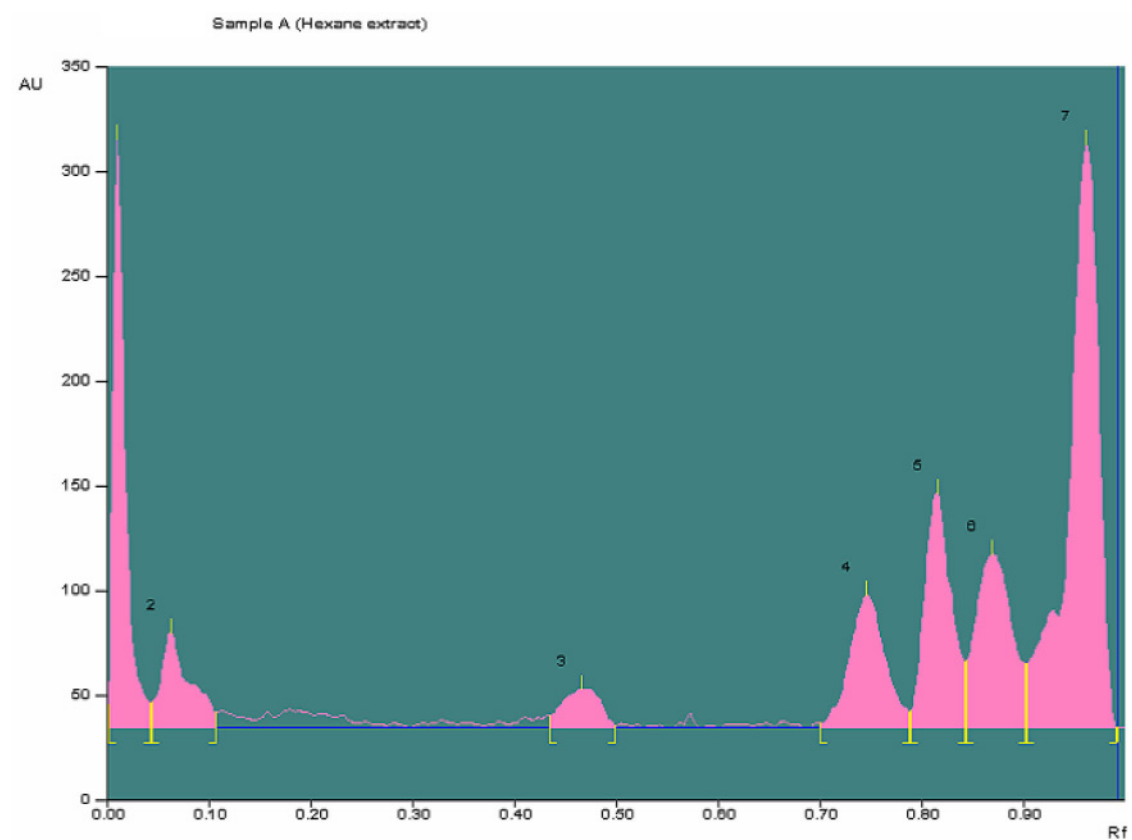


Figure 6: Peak densitogram display of hexane extract of *Emilia sonchifolia* (Scanned at 500nm).



including singlet oxygen and hydroxyl radicals, which could cause the tissue damage [18]. Therefore, it is important to discover some chemicals with good scavenging capacity on these reactive oxygen species. In this study, n-hexane extract of *Emilia sonchifolia* exhibited appreciable scavenging power and the terpenoids as the characteristic constituents may play a significant role. (Figure 2 and 3). Terpenoids

have been shown to possess antioxidative properties in different situations [19] particularly against lipid peroxidation as a result of their high lipophilicity. The antioxidant activity of terpenoids is conferred by the hydrophobic chain of polyene units that can quench singlet oxygen, neutralize thiyl radicals and combine with and stabilize peroxy radicals [20].

Table 1: Shows peak table with Rf values, height and area of Terpenoids and unknown compounds in hexane extract of *Emilia sonchifolia*.

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.01	281.1	3028.0	Unknown
Sample A	2	0.06	44.8	1163.8	Terpenoid 1
Sample A	3	0.47	18.2	630.6	Unknown
Sample A	4	0.75	62.8	2016.1	Terpenoid 2
Sample A	5	0.81	111.8	2689.8	Unknown
Sample A	6	0.87	82.3	2701.1	Terpenoid 3
Sample A	7	0.96	278.5	7503.0	Terpenoid 4

The n-hexane extract of *Emilia sonchifolia* having the potentiality to scavenge the free radical contains terpenoids which was analysed by using HPTLC. The peak table, peak display and peak densitogram were noted. Blue-violet colored zones were detected from the chromatogram after derivatization which confirmed the presence of terpenoid (Figure 4). Thus, the presence of terpenoids in the n-hexane extract of the whole plant of *Emilia sonchifolia* was confirmed by HPTLC analysis. The extract was run along with the standard terpenoid compound and it was observed that the extract showed the presence of terpenoid and it was confirmed from the chromatogram after derivatization. The Rf value of the different compounds present in the extract was found to be 0.01, 0.06, 0.47, 0.75, 0.81, 0.87 and 0.96 of peak 1, 2, 3, 4, 5, 6, and 7 respectively. Among them peaks 2, 4, 6 and 7 were found to contain terpenoids. The peak height of the respective terpenoid was also given in the Figure 5 and 6; Table 1.

CONCLUSION

Thus the results of the present study revealed the scavenging capacity of the n-hexane of *Emilia sonchifolia* and hence can be used as an antioxidant source against various diseases. This may be due to its active phytochemical constituent, terpenoid. Further, isolation and structure of the active constituent is needed which is under progress.

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