

COMPARATIVE ANTIOXIDANT ACTIVITY EVALUATION AND HPTLC PHYTO-CHEMICAL FINGERPRINTING OF SOME INDIAN MEDICINAL PLANT EXTRACTS

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ABSTRACT

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidant reduces risks for chronic diseases including cancer and heart disease in the body. Present research was carried out to find potential antioxidant activity and developing HPTLC phyto-chemical fingerprint of ten important Indian medicinal plant extracts and to relate it with their medicinal and disease curing ability. Plant extracts prepared in various polarity solvents like water, methanol, chloroform, petroleum ether and screened for antioxidant activity using assays, Total Phenol content estimation, DPPH radical scavenging activity and Ferrous ion chelating activity. Comparative study of extracts prepared in different polarity solvents, successfully demonstrated the nature and polarity of antioxidant phyto-chemicals present in the studied plants and thus the results can be utilized for the value addition for future reference. Over all extracts of *Lawsonia inermis*, *Murraya koenigii*, *Piper betel*, *Curcuma longa* and *Camellia sinensis* demonstrated an excellent antioxidant activity. Study results thus supports that the medicinal property of plant is correlated with their antioxidant nature of active phyto-chemicals.

Keywords: Antioxidant, Phyto-Chemicals, Solvent Polarity, Chronic Diseases

INTRODUCTION

The main characteristic of an antioxidant is its ability to trap free radicals. Free radicals like hydroxyl, reactive oxygen radicals etc. produce in our body during the normal metabolism and are extremely reactive in nature. They damages almost every molecule found in living cells, affect human health and lead to several degenerative diseases including atherosclerosis, hypertension, heart attack, diabetes, immunosuppression, neurodegenerative diseases, parkinson and alzheimer diseases, arthritis, cancer as well as premature body aging (Porwal *et al.*, 2010).

The plant kingdom constitutes most widely distributed extremely heterogeneous groups of the substances also called as PSMs (Plant Secondary Metabolites) (Patel and Jasrai, 2009; Harborne, 1984). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Many spices, fruits, vegetables and medicinal plants contain potential antioxidant compounds, such as vitamins A, C and E, β -carotene, α -tocopherol, carotenoids, flavonoids, isoflavones, flavones, flavonols, anthocyanins, proanthocyanidins, coumarins, lignans, polyphenols, catechins, isocatechins, tannins and other phenolics constituents etc (Ghasemi *et al.*, 2009). In various *in vitro* assay studies, PSMs present in various plants and their extracts reported as excellent radical scavenger, inhibits lipid peroxidation and thereby exhibit therapeutic property (Patel and Jasrai, 2012; Nahar *et al.*, 2009).

In the present investigation, considering the highly significant therapeutic role of antioxidant phyto-chemicals, effort was made to find comparative antioxidant capacity of polar to non-polar extracts of ten Indian medicinal plants and correlate it with their therapeutic ability. Subsequently extracts were subjected for the HPTLC based finger printing to develop a digital phyto-chemical profile of plant extracts. HPTLC- High Performance Thin Layer Chromatography is a sophisticated, reliable, efficient and automated form of TLC having the latest technical developments for quality assessment and

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evaluation of botanical materials (Saraswathy *et al.*, 2010). A chromatographic fingerprint of extract represents a chromatographic pattern of pharmacologically active or chemically characteristic constituents present in the extract (Bhise and Salunkhe, 2009; Sanja *et al.*, 2009). Moreover, in HPTLC phytochemical analysis technique, many samples of divergent nature can be run in a single analysis with simultaneous processing of sample and standard (Harborne, 1984; Cimpoiu, 2006).

MATERIALS AND METHODS

Collection and Extraction of Plant Material:

Plants used in the present study were *Azadirachta indica* A Juss, *Camellia sinensis* (L) O.Kuntze, *Cassia fistula* L, *Curcuma longa* L, *Datura stramonium* L, *Lawsonia inermis* L, *Moringa oleifera* Lam, *Murraya koenigii* (L) Sprengel, *Piper betel* L and *Sphaeranthus indicus* L (Table 1). Plants *Camellia*, *Curcuma* and *Lawsonia* were purchased from local market, while other plants were collected from the campus of Gujarat University, Ahmedabad and environs. Collected plant material washed and air dried under shade (one week). The dried plant parts finely powdered using electric grinder, sieved (mesh size 500 μ) and subjected for the extraction. All plant samples extracted in four solvents of different polarity viz water, methanol, chloroform and petroleum ether. For aqueous extracts, powdered plant material (50 g) extracted in 1000 ml of distilled water at 50°C temperature until the volume reduces to half. The content then filtered through whatman filter paper (no 1). The filtrate evaporated till complete dryness in oven (40°C) (Harborne, 1984; Patel and Jasrai, 2010). While for the organic solvent extraction, solvents methanol, chloroform and petroleum ether were used. The finely powdered plant material (100 g) soaked overnight in solvent (400 ml) in air tight erlenmeyer flask. The residues repeatedly extracted (three times) in 200 ml of solvent (Souri *et al.*, 2008; Khan and Nasreen, 2010). The extracts filtered through a whatman filter paper (no 1). The filtrate was evaporated to dryness to yield a dark-residue. Each sample was then transferred to glass vials (6 ×2 cm) and % yield of extracts calculated (Table 1).

Table 1: % Extract Yield of Plant Materials Extracted in Different Solvents

Plants	Family	Plant Part Used	% Extract Yield*			
			WE	ME	CH	PE
<i>Azadirachta indica</i> A Juss	Meliaceae	Leaves	28.70	13.70	9.10	3.17
<i>Camellia sinensis</i> (L) O.Kuntze	Theaceae	Cured leaves	21.47	17.84	1.17	0.28
<i>Cassia fistula</i> L	Caesalpiniaceae	Fruit pulp	48.04	27.03	0.33	0.16
<i>Curcuma longa</i> L	Zingiberaceae	Rhizome	7.53	6.68	10.43	3.17
<i>Datura stramonium</i> L	Solanaceae	Twigs	25.47	12.00	12.00	3.27
<i>Lawsonia inermis</i> L	Lythraceae	Leaves	68.04	28.96	14.58	5.84
<i>Moringa oleifera</i> Lam	Moringaceae	Twigs	34.83	14.74	6.36	2.06
<i>Murraya koenigii</i> (L) Sprengel	Rutaceae	Leaves	29.33	16.96	17.32	7.47
<i>Piper betel</i> L	Piperaceae	Leaves	27.66	13.85	10.42	0.98
<i>Sphaeranthus indicus</i> L	Asteraceae	Aerial part	19.78	7.30	4.42	3.88

[Note: * represents g extract/100g dry powder, WE= Water; ME= Methanol; CH= Chloroform; PE= Petroleum ether]

Screening Antioxidant Activity

All plant extracts were subjected for the screening antioxidant activity by implementing standardized protocols of three different assays. The chemicals utilized were of pure and analytical grade. OD Readings were taken using UV-VIS Spectrophotometer (Elico), in six replicates per sample and calculated for their standard errors. The detailed procedure of the *in vitro* assays is mentioned below.

(a) *Phenol estimation by Folin-Ciocalteu method*: To 6 ml of double dist. water added 2 mg sample, 0.5 ml Folin-Ciocalteu reagent and 1.5 ml 20% Na₂CO₃ (Sodium Carbonate) solution. Total volume made up to 10 ml by addition of dist. water. The mixture incubated for 30 min. at 25°C and then OD taken at

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760nm. % phenol calculated with reference to standard curve, using % extract yield and Gallic acid equivalents (GAE) (Ghasemi *et al.*, 2009).

(b) *DPPH radical scavenging assay*: 2 ml 0.5 mM methanolic solution of DPPH (1,1-Diphenyl-2-picrylhydrazyl) mixed with the 2 ml methanolic solution containing 3 mg extract. The mixture shaken vigorously and allowed to incubate in dark for 30 min. and the OD taken at 517nm. BHT (Butylated Hydroxy Toluene) was used as a reference compound. The calculation performed using the formula (Ghasemi *et al.*, 2009).

$$\% \text{ DPPH Radical scavenging activity (RSA)} = \left(\frac{A_{\text{control}} - A_{\text{sample}} \times 100}{A_{\text{control}}} \right)$$

[**Note:** A control = OD of DPPH solution without extract or standard, A sample = OD of DPPH solution with extract or standard]

(c) *Ferrous ion chelating activity assay*: 3 mg of extract was mixed with the 2 ml of 0.04 mM FeCl₂ and 2 ml of 0.5 mM aqueous ferrozine solution. The mixture shaken vigorously and left standing at room temperature for 10 min. and the OD taken at 562nm. Ascorbic acid (vitamin C) was used as a reference compound. The calculation performed using following formula (Dinis *et al.*, 1994).

$$\% \text{ Inhibition of Ferrozine - Fe}^{2+} \text{ complex} = \left(\frac{1 - A_1 \text{ sample} \times 100}{A_0 \text{ control}} \right)$$

[**Note:** A₀ control = OD of FeCl₂ and Ferrozine solution without extract or standard, A₁ sample= OD of FeCl₂ and Ferrozine solution with extract or standard]

HPTLC Fingerprinting of Extracts:

A standard methodology was followed for the sample preparation and HPTLC (Camag, Muttenz, Switzerland) analysis of extracts. Each extract was redissolved at 50 mg/ml concentration in their respective extraction solvent. The sample extracts were streaked (2 µl) in form of narrow bands on the precoated silica gel 60F₂₅₄ aluminum TLC plate (Wagner and Bladt, 2007). The plates were subjected to linear ascending development, in selected solvent system and the densitometric scanning of the developed chromatograms was carried out in the absorbance mode at 200- 450 nm wavelength. The digital photographs of the chromatograms were taken at three different wavelengths i.e. 254 nm through UV lamp, 366 nm through Mercuric lamp; and 400- 800 nm through Tungsten lamp.

RESULTS AND DISCUSSION

Screening Antioxidant Activity:

(a) *Phenol estimation by Folin-Ciocalteu method*: The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers (Patel and Jasrai, 2012). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports. A fair correlation between antioxidant and free radical scavenging activity and phenolic content was observed in studies by Aquil *et al.*, 2006 and Singh *et al.*, 2009. Phenolic antioxidants present in herbs have the ability to reduce lipid peroxidation, prevent DNA oxidative damage and scavenge ROS (Reactive oxygen species) like superoxide, hydrogen peroxide and hydroxyl radicals (Yoo *et al.*, 2008). According to Nahak and Sahu (2010) polar solvents like water, methanol and ethanol are helpful to better isolate the phenolic compounds. While Bushra *et al.*, (2009) investigated in the study that, higher extract yields, high phenol content and subsequent good antioxidant activity can be obtained using aqueous organic solvents like 80% methanol and 80% ethanol for extraction, as compared to the absolute organic solvents of ethanol and methanol. Several studies with varied protocols of analysis have been conducted in this field to find newer and newer plants as a source of antioxidants. Variety of solvents has been used for plant extraction, which helps to find the nature of antioxidant phyto-chemicals and their maximum extraction. In the present study, standard Gallic acid at 0.01 mg/ml concentration exhibited 0.00010% phenol content (0.50 mg/10 ml) estimated by Folin-Ciocalteu method. The % phenol of plant extracts calculated considering the % yield of plant extracts (Table 1) with reference to standard curve of Gallic acid, and the value thus

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defined as GAE (Gallic acid equivalent). The highest % phenol recorded among medicinal plants was in water extracts of *Camellia sinensis*, followed by *Lawsonia inermis*, *Piper betel* and *Murraya koenigii* as well as; highest phenol content with methanol extracts of *Piper betel* and *Camellia sinensis* GAE, which is quite high and appreciable quantity of phenol. While comparatively lower % phenol obtained with the petroleum ether extracts of *Cassia fistula* followed by *Camellia sinensis*, *Azadirachta indica* and *Datura stramonium* GAE (Table 2, Figure 1). Thus here methanol and water proved efficient solvents to extract phenols from plant materials.

Table 2: Comparative Antioxidant Activity Values for Medicinal Plant Extracts

Plants	Extract	% Phenol	% DPPH RSA	% FICA
<i>Azadirachta indica</i>	WE	1.07 ± 0.14	40.76 ± 0.29	89.02 ± 0.14
	ME	0.52 ± 0.01	72.05 ± 0.27	81.18 ± 0.09
	CH	0.04 ± 0.01	43.41 ± 0.54	43.47 ± 0.36
	PE	0.01 ± 0.001	48.05 ± 0.23	40.91 ± 0.63
<i>Camellia sinensis</i>	WE	4.96 ± 0.05	93.27 ± 0.02	58.54 ± 0.08
	ME	2.36 ± 0.24	83.09 ± 0.05	83.58 ± 0.11
	CH	0.02 ± 0.004	65.73 ± 0.22	50.06 ± 0.21
	PE	0.001 ± 0	50.08 ± 0.42	71.15 ± 0.33
<i>Cassia fistula</i>	WE	1.45 ± 0.02	26.75 ± 0.82	78.16 ± 0.09
	ME	1.07 ± 0.01	85.60 ± 0.21	40.79 ± 0.15
	CH	0.002 ± 0.00	31.27 ± 0.44	68.41 ± 0.61
	PE	0.0004 ± 0.03	12.41 ± 0.25	39.85 ± 0.15
<i>Curcuma longa</i>	WE	0.13 ± 0.002	46.22 ± 0.56	105.71 ± 0.11
	ME	0.67 ± 0.07	83.62 ± 0.06	47.85 ± 0.25
	CH	1.50 ± 0.05	86.82 ± 0.31	65.16 ± 0.30
	PE	0.03 ± 0.0009	26.74 ± 0.50	126.15 ± 0.15
<i>Lawsonia inermis</i>	WE	3.71 ± 0.04	92.19 ± 0.09	49.28 ± 0.14
	ME	1.45 ± 0.06	87.69 ± 0.08	40.15 ± 0.08
	CH	0.19 ± 0.004	69.55 ± 0.11	55.52 ± 0.59
	PE	0.05 ± 0.002	53.13 ± 0.48	69.84 ± 0.40
<i>Murraya koenigii</i>	WE	3.04 ± 0.03	53.23 ± 0.29	83.62 ± 0.07
	ME	0.39 ± 0.02	83.30 ± 0.31	39.04 ± 0.06
	CH	0.22 ± 0.01	84.66 ± 0.19	43.33 ± 0.15
	PE	0.09 ± 0.004	84.15 ± 0.10	30.44 ± 0.26
<i>Moringa oleifera</i>	WE	0.66 ± 0.03	27.44 ± 0.69	95.36 ± 0.13
	ME	0.21 ± 0.02	68.48 ± 0.44	38.79 ± 0.05
	CH	0.04 ± 0.001	40.99 ± 0.49	45.10 ± 0.11
	PE	0.01 ± 0	40.49 ± 0.43	60.32 ± 0.26
<i>Piper betel</i>	WE	3.26 ± 0.04	81.64 ± 0.17	66.36 ± 0.21
	ME	2.96 ± 0.05	83.42 ± 0.06	52.90 ± 0.23
	CH	1.97 ± 0.02	86.10 ± 0.08	51.72 ± 0.18
	PE	0.02 ± 0.001	57.48 ± 0.30	41.37 ± 0.47
<i>Sphaeranthus indicus</i>	WE	0.67 ± 0.05	66.73 ± 1.52	65.23 ± 0.09
	ME	0.29 ± 0.05	60.67 ± 0.40	39.25 ± 0.14
	CH	0.06 ± 0.01	38.17 ± 0.22	102.23 ± 0.22
	PE	0.06 ± 0.01	16.35 ± 0.43	54.49 ± 0.55

(b) DPPH radical scavenging assay: The DPPH scavenging ability and reducing power assays provides preliminary information on the reactivity of the test compound with a free radical and its hydrogen-donating tendency and the reduction capability of the DPPH radical (Rathee *et al.*, 2006). Many researchers have reported positive correlation and observed that higher DPPH scavenging/reducing activity is related to the higher amount of antioxidants present in the sample (Ghafar *et al.*, 2010). The reduction of DPPH radicals can be observed by the decrease in absorbance at 517 nm and on the degree of discoloration due to the radical scavenging ability of antioxidant (Aquil *et al.*, 2006; Sreelatha and

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Padma, 2009). In the present study, % DPPH RSA (Radical scavenging activity) IC_{50} value for standard BHT observed at 0.08 mg/ml concentration. The highest % DPPH RSA was recorded in water extracts of *Camellia sinensis* and *Lawsonia inermis*, followed by methanol extracts of *Lawsonia inermis* and chloroform extract of *Curcuma longa* and *Piper betel*. While comparatively lower amount of % DPPH RSA recorded with petroleum ether extracts of *Cassia fistula* and *Sphaeranthus indicus* followed by water extract of *Datura stramonium* (Table 2, Figure 1).

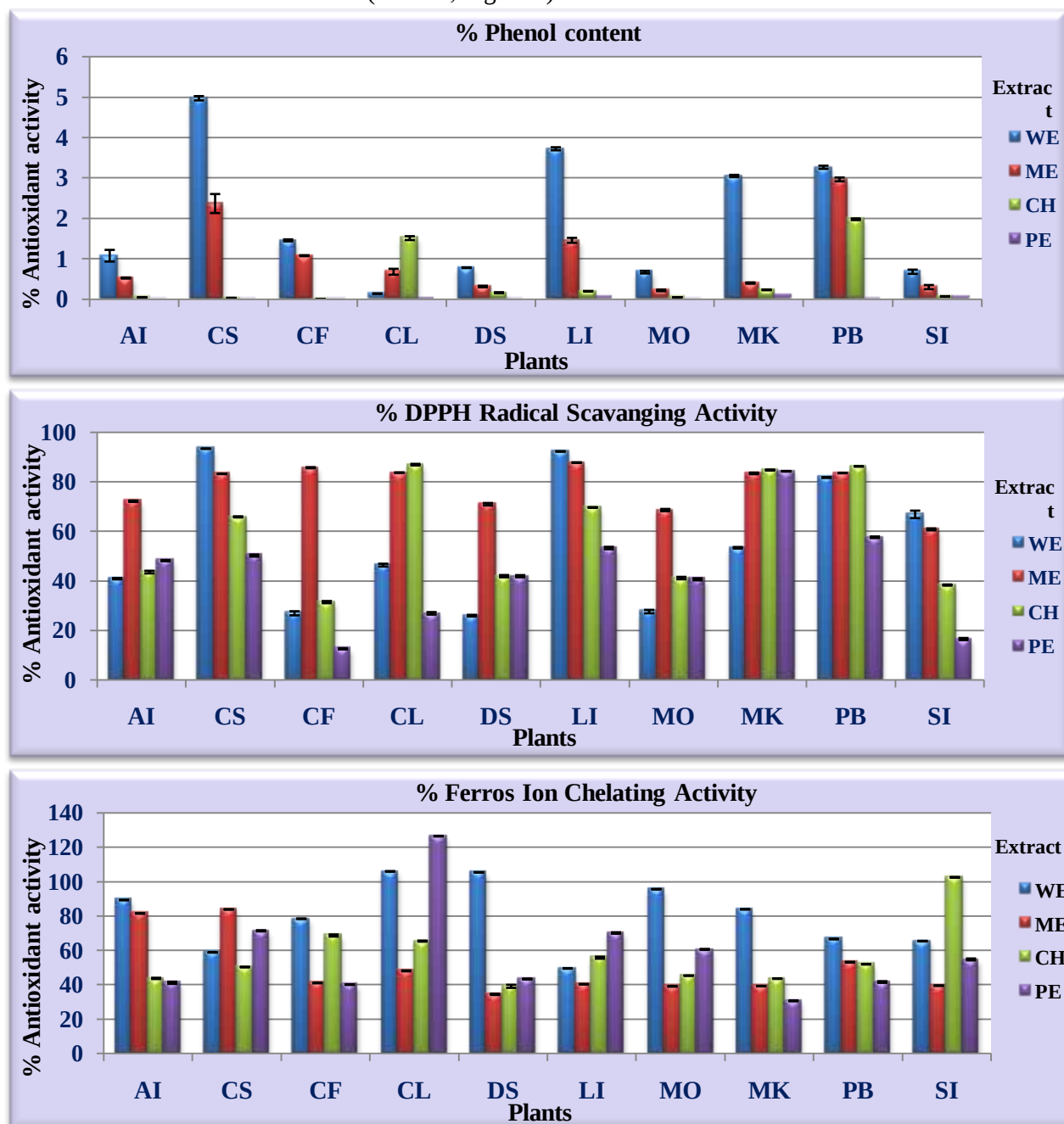
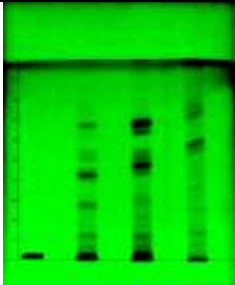
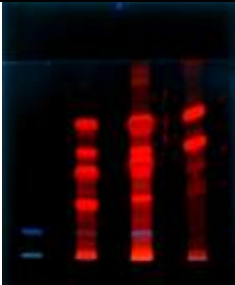
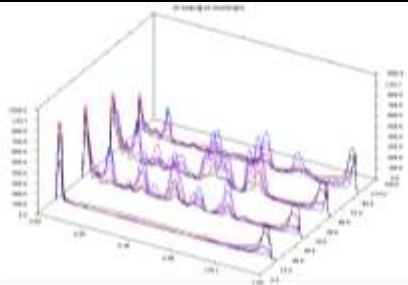
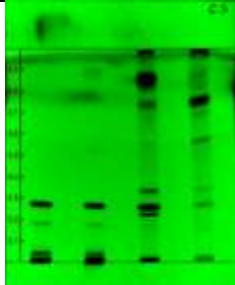
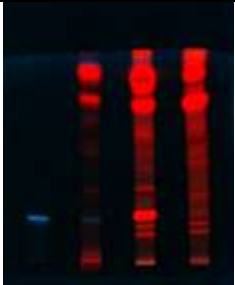
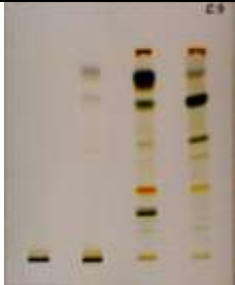
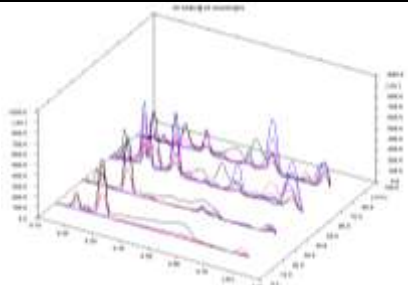
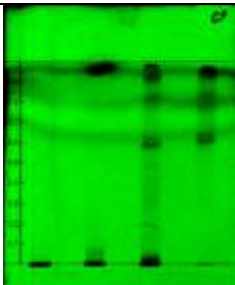
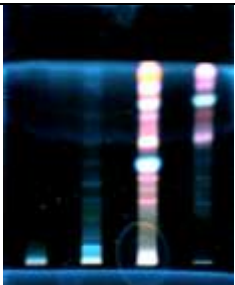
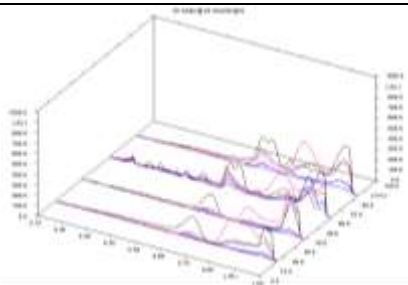
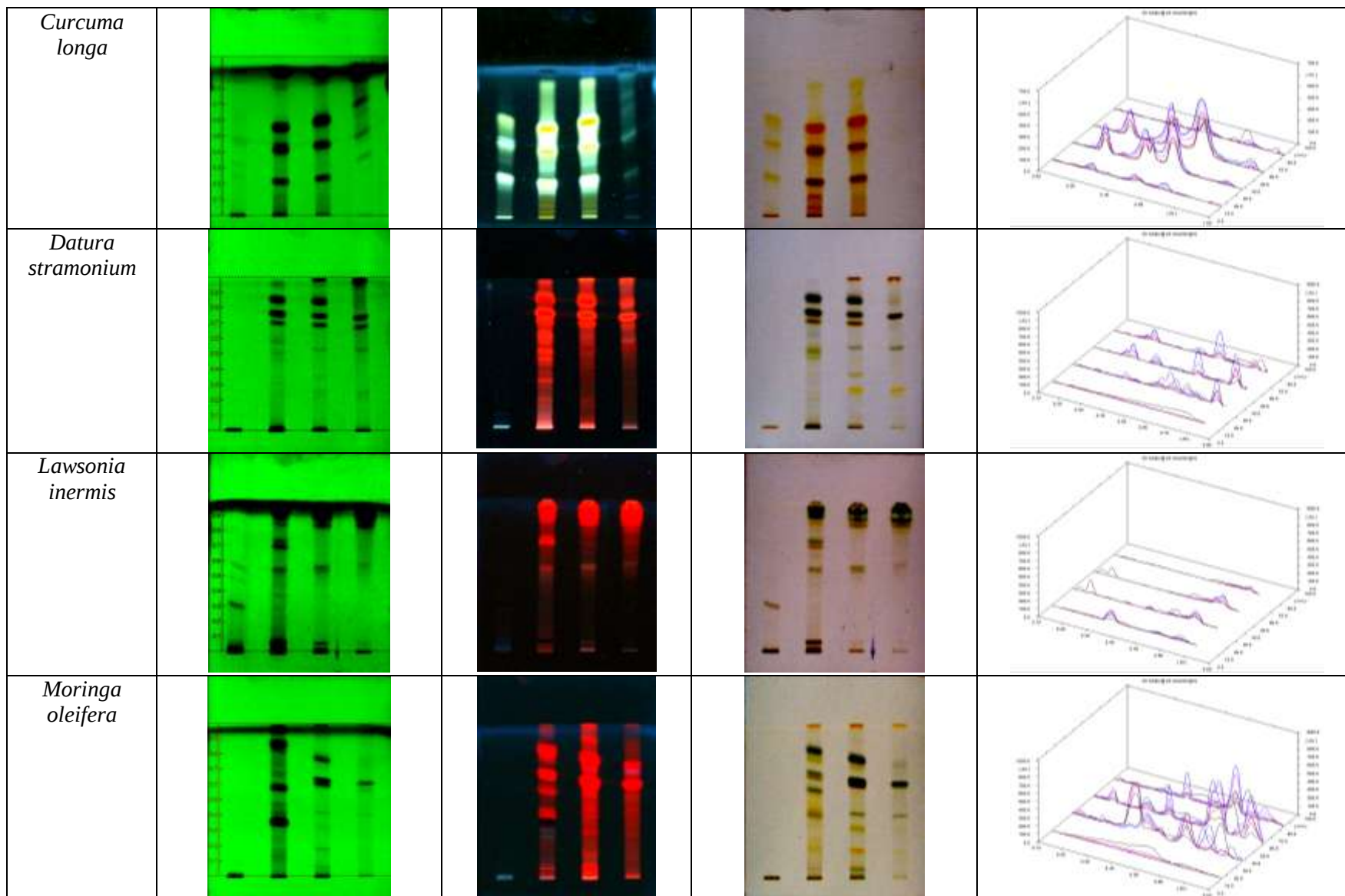


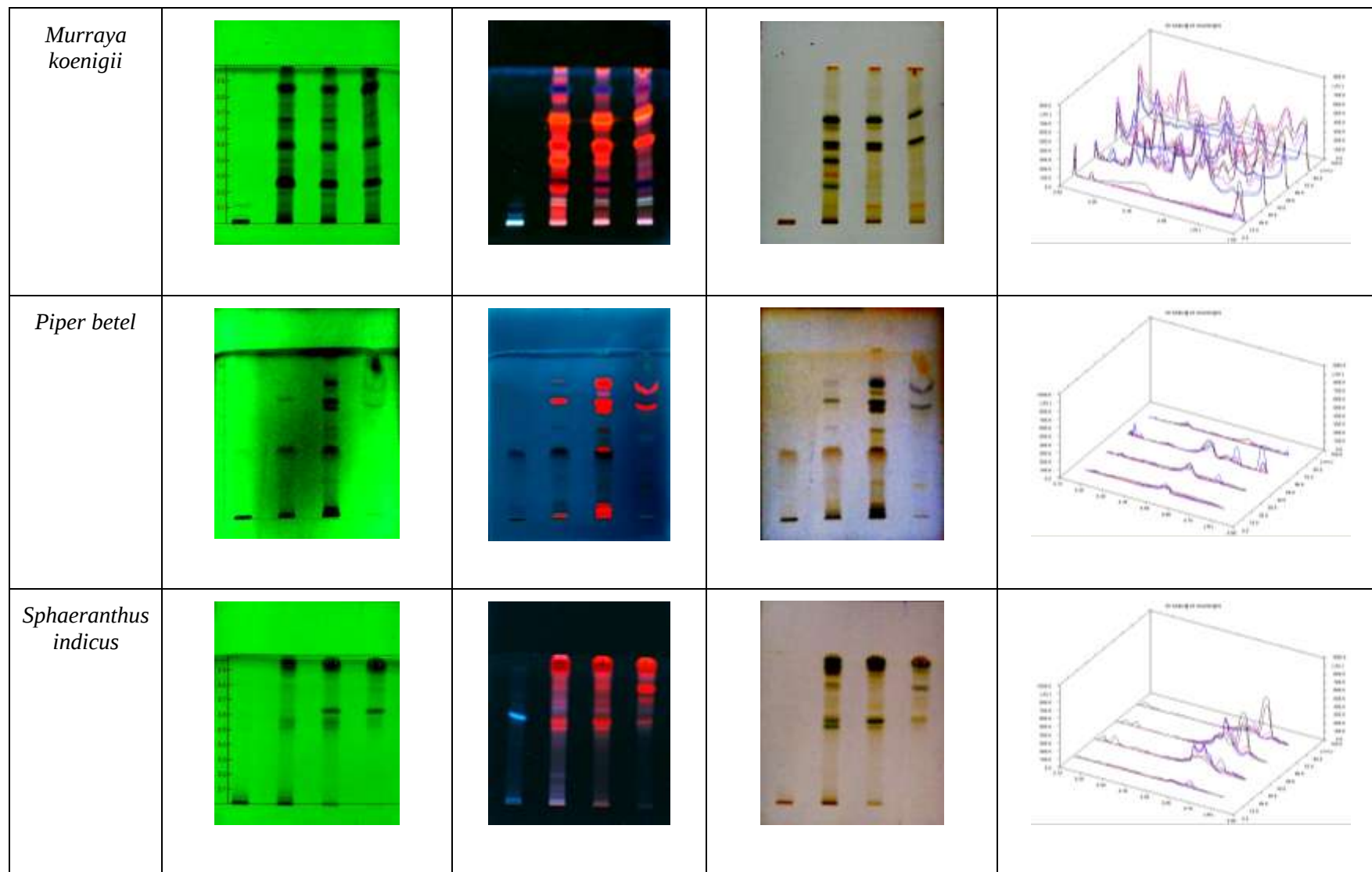
Figure 1: Antioxidant activity exhibited by plant extracts in different assays

[Note: AI= *Azadirachta indica*, CS= *Camellia sinensis*, CF= *Cassia fistula*, CL= *Curcuma longa* DS= *Datura stramonium*, LI= *Lawsonia inermis*, MO= *Moringa oleifera*, MK= *Murraya koenigii*, PB= *Piper betel* and SI= *Sphaeranthus indicus*]

Table 3: TLC Chromatograms and HPTLC Scanning results

Plant	TLC chromatogram visualized in various lights representing separated compounds			3-D graphical display of absorbance peaks (200-450 nm)
	Florescent light	UV light	Visible light	
<i>Azadirachta indica</i>	 Track 1 2 3 4			
<i>Camellia sinensis</i>				
<i>Cassia fistula</i>				





[Note: Vertical scale represents distance between two bars and indicates 0.10 R_f value]

Table 4: Densitometric scanning results and number of bands/ compounds detected and their Rf value recorded at respective scanning wavelengths (200- 450 nm) on chromatograms

Plants	Track	No. of bands and Rf value	Detection Wavelength (nm)									
			200	250	300	350	400	450				
<i>Azadirachta indica</i>	1	Bands Rf	3 (0.00, 0.47, 0.88)	4 (0.00, 0.64, 0.76, 0.85)	4 (0.00, 0.63, 0.75, 0.81)	3 (0.00, 0.11, 0.85)	2 (0.00, 0.86)	3 (0.00, 0.05, 0.86)				
	2	Bands Rf	13 (0.00, 0.05, 0.10, 0.15, 0.19, 0.24, 0.34, 0.39, 0.48, 0.62, 0.69, 0.79, 0.89)	13 (0.00, 0.06, 0.10, 0.15, 0.19, 0.24, 0.37, 0.47, 0.59, 0.63, 0.79, 0.89)	12 (0.00, 0.06, 0.09, 0.18, 0.24, 0.30, 0.37, 0.47, 0.58, 0.70, 0.79, 0.88)	12 (0.00, 0.08, 0.16, 0.20, 0.23, 0.31, 0.37, 0.47, 0.58, 0.72, 0.84, 0.96)	12 (0.00, 0.08, 0.18, 0.23, 0.31, 0.36, 0.48, 0.58, 0.72, 0.83, 0.90, 0.96)	13 (0.00, 0.08, 0.18, 0.23, 0.31, 0.36, 0.47, 0.52, 0.57, 0.64, 0.72, 0.90)				
	3	Bands Rf	14 (0.01, 0.06, 0.09, 0.16, 0.19, 0.24, 0.32, 0.35, 0.40, 0.50, 0.58, 0.65, 0.71, 0.89)	13 (0.00, 0.06, 0.11, 0.16, 0.20, 0.25, 0.32, 0.40, 0.50, 0.59, 0.65, 0.72, 0.86)	12 (0.00, 0.07, 0.10, 0.18, 0.26, 0.38, 0.45, 0.52, 0.60, 0.65, 0.86, 0.97)	11 (0.00, 0.10, 0.17, 0.26, 0.32, 0.38, 0.52, 0.60, 0.65, 0.84, 0.96)	15 (0.00, 0.10, 0.15, 0.18, 0.22, 0.26, 0.32, 0.39, 0.49, 0.52, 0.58, 0.60, 0.65, 0.84, 0.90, 0.96)	11 (0.00, 0.09, 0.20, 0.27, 0.37, 0.46, 0.49, 0.59, 0.66, 0.77, 0.90)				
<i>Camellia sinensis</i>	4	Bands Rf	11 (0.00, 0.06, 0.13, 0.23, 0.27, 0.35, 0.43, 0.51, 0.68, 0.74, 0.85)	10 (0.00, 0.07, 0.13, 0.23, 0.35, 0.43, 0.51, 0.64, 0.68, 0.75)	10 (0.00, 0.07, 0.11, 0.18, 0.23, 0.31, 0.41, 0.50, 0.66, 0.75)	7 (0.00, 0.11, 0.23, 0.30, 0.41, 0.51, 0.67)	9 (0.00, 0.04, 0.10, 0.18, 0.22, 0.32, 0.40, 0.41, 0.52, 0.67)	8 (0.00, 0.10, 0.18, 0.22, 0.34, 0.40, 0.50, 0.65, 0.75)				
	1	Bands Rf	4 (0.14, 0.22, 0.41, 0.65)	4 (0.15, 0.23, 0.51, 0.75)	4 (0.15, 0.20, 0.24, 0.74)	1 (0.18)	-	-				
	2	Bands Rf	8 (0.11, 0.14, 0.16, 0.22, 0.32, 0.38,	5 (0.14, 0.23, 0.32, 0.49,	7 (0.10, 0.15, 0.24, 0.32,	5 (0.18, 0.32, 0.50,	6 (0.32, 0.56,	3 (0.46, 0.56, 0.73)				

Cassia fistula	3	Bands Rf	0.48, 0.75) 8 (0.11, 0.14, 0.21, 0.31, 0.37, 0.46, 0.64, 0.72)	0.75) 11 (0.10, 0.13, 0.15, 0.19, 0.21, 0.24, 0.30, 0.43, 0.50, 0.72)	0.50, 0.74) 12 (0.11, 0.17, 0.21, 0.24, 0.27, 0.37, 0.44, 0.60, 0.72)	0.57, 0.75) 11 (0.11, 0.15, 0.19, 0.24, 0.27, 0.37, 0.53, 0.72, 0.75)	0.67) 13 (0.12, 0.16, 0.21, 0.24, 0.28, 0.39, 0.47, 0.60, 0.71)	10 (0.12, 0.17, 0.19, 0.24, 0.31, 0.42, 0.47, 0.60, 0.70)	0.16, 0.27, 0.42, 0.51,	
	4	Bands Rf	11 (0.11, 0.15, 0.18, 0.23, 0.33, 0.40, 0.49, 0.57, 0.64, 0.68, 0.75)	10 (0.11, 0.14, 0.19, 0.21, 0.24, 0.52, 0.57, 0.68, 0.75)	9 (0.12, 0.25, 0.32, 0.39, 0.53, 0.75)	10 (0.13, 0.22, 0.32, 0.44, 0.53, 0.68, 0.74)	11 (0.13, 0.18, 0.22, 0.28, 0.31, 0.39, 0.53, 0.74)	9 (0.14, 0.22, 0.33, 0.39, 0.45, 0.64, 0.73)	0.18, 0.33, 0.47, 0.63,	
	1	Bands Rf	3 (0.57, 0.75, 0.90)	5 (0.21, 0.59, 0.74, 0.86, 0.92)	4 (0.59, 0.71, 0.86, 0.93)	2 (0.60, 0.87)	1 (0.89)	1 (0.90)		
	2	Bands Rf	4 (0.35, 0.55, 0.74, 0.85)	4 (0.55, 0.61, 0.72, 0.83)	2 (0.55, 0.71)	2 (0.56, 0.85)	1 (0.87)	1 (0.95)		
	3	Bands Rf	9 (0.13, 0.24, 0.29, 0.45, 0.53, 0.61, 0.68, 0.73, 0.86)	9 (0.12, 0.15, 0.25, 0.38, 0.46, 0.73, 0.85, 0.95)	9 (0.16, 0.22, 0.35, 0.40, 0.44, 0.68, 0.87, 0.94)	9 (0.17, 0.21, 0.25, 0.39, 0.44, 0.52, 0.87, 0.95)	10 (0.12, 0.21, 0.35, 0.39, 0.45, 0.53, 0.67, 0.86, 0.95)	8 (0.13, 0.25, 0.38, 0.39, 0.62, 0.76, 0.85, 0.95)	0.26, 0.54, 0.75,	
	4	Bands Rf	7 (0.20, 0.48, 0.55, 0.64, 0.70, 0.75, 0.87)	5 (0.48, 0.54, 0.72, 0.80, 0.87)	4 (0.54, 0.59, 0.69, 0.87)	4 (0.56, 0.70, 0.83, 0.88)	4 (0.55, 0.70, 0.84, 0.88)	2 (0.57, 0.87)		
	1	Bands Rf	6 (0.23, 0.28, 0.37, 0.47, 0.58, 0.70)	4 (0.23, 0.29, 0.37, 0.46)	6 (0.11, 0.19, 0.24, 0.29, 0.36, 0.57)	7 (0.11, 0.20, 0.24, 0.29, 0.36, 0.42,	3 (0.30, 0.36, 0.56)	-		
Citrus limon										

<i>Curcuma longa</i>	2	Bands Rf	9 (0.11, 0.20, 0.24, 0.29, 0.37, 0.54, 0.59, 0.64, 0.71)	7 (0.12, 0.20, 0.25, 0.37, 0.67)	7 (0.11, 0.19, 0.25, 0.29, 0.37, 0.51, 0.64)	7 (0.11, 0.20, 0.25, 0.29, 0.36, 0.51, 0.65)	6 (0.11, 0.24, 0.29, 0.35, 0.45, 0.67)	4 (0.11, 0.24, 0.28, 0.66)
	3	Bands Rf	4 (0.38, 0.48, 0.52, 0.63)	4 (0.29, 0.41, 0.59, 0.68)	4 (0.20, 0.28, 0.42, 0.65)	4 (0.11, 0.23, 0.28, 0.64)	5 (0.11, 0.23, 0.29, 0.38, 0.65)	3 (0.11, 0.29, 0.65)
	4	Bands Rf	4 (0.29, 0.42, 0.55, 0.61)	5 (0.28, 0.42, 0.54, 0.60, 0.66)	5 (0.26, 0.33, 0.42, 0.54, 0.66)	5 (0.11, 0.26, 0.31, 0.42, 0.67)	5 (0.11, 0.26, 0.31, 0.40, 0.66)	1 (0.68)
	1	Bands Rf	3 (0.09, 0.42, 0.49)	3 (0.18, 0.29, 0.45)	5 (0.17, 0.44, 0.62, 0.72, 0.75)	5 (0.13, 0.18, 0.43, 0.62, 0.72)	3 (0.18, 0.43, 0.58)	3 (0.14, 0.40, 0.57)
	2	Bands Rf	6 (0.14, 0.25, 0.37, 0.45, 0.53, 0.68)	6 (0.14, 0.25, 0.34, 0.46, 0.52, 0.85)	6 (0.14, 0.33, 0.46, 0.52, 0.70, 0.79)	6 (0.10, 0.14, 0.26, 0.33, 0.46, 0.52)	7 (0.10, 0.14, 0.26, 0.33, 0.46, 0.50, 0.86)	5 (0.11, 0.26, 0.46, 0.50, 0.84)
	3	Bands Rf	5 (0.01, 0.16, 0.38, 0.55, 0.89)	5 (0.17, 0.28, 0.36, 0.55, 0.89)	3 (0.15, 0.36, 0.55)	6 (0.11, 0.16, 0.30, 0.36, 0.53, 0.89)	4 (0.15, 0.35, 0.53, 0.85)	4 (0.12, 0.28, 0.53, 0.85)
<i>Datura stramonium</i>	4	Bands Rf	8 (0.02, 0.29, 0.38, 0.42, 0.47, 0.66, 0.75, 0.92)	4 (0.33, 0.45, 0.72, 0.95)	2 (0.46, 0.73)	2 (0.46, 0.77)	2 (0.47, 0.78)	1 (0.47)
	1	Bands Rf	4 (0.31, 0.43, 0.62, 0.70)	- -	1 (0.17)	- -	- -	- -
	2	Bands Rf	7 (0.11, 0.33, 0.45, 0.54, 0.61, 0.68, 0.75)	5 (0.23, 0.47, 0.54, 0.61, 0.74)	7 (0.18, 0.24, 0.33, 0.45, 0.54, 0.66)	7 (0.19, 0.24, 0.33, 0.46, 0.54, 0.64)	7 (0.19, 0.30, 0.39, 0.45, 0.54, 0.63)	9 (0.14, 0.19, 0.24, 0.29, 0.36, 0.46)

					0.74)	0.75)	0.75)	0.54, 0.74)	0.65,
	3	Bands Rf	4 (0.45, 0.54, 0.67, 0.75)	4 (0.22, 0.37, 0.57, 0.72)	5 (0.21, 0.35, 0.56, 0.67, 0.73)	5 (0.22, 0.35, 0.56, 0.66, 0.73)	6 (0.22, 0.36, 0.46, 0.54, 0.66, 0.74)	5 (0.21, 0.35, 0.47, 0.56, 0.73)	
	4	Bands Rf	6 (0.24, 0.45, 0.56, 0.63, 0.67, 0.76)	5 (0.14, 0.19, 0.24, 0.56, 0.71)	4 (0.22, 0.50, 0.56, 0.73)	4 (0.22, 0.55, 0.65, 0.72)	5 (0.21, 0.47, 0.55, 0.64, 0.73)	5 (0.22, 0.38, 0.47, 0.56, 0.74)	
<i>Lawsonia inermis</i>	1	Bands Rf	5 (0.25, 0.31, 0.37, 0.48, 0.60)	3 (0.30, 0.50, 0.59)	3 (0.29, 0.50, 0.59)	4 (0.16, 0.29, 0.50, 0.57)	4 (0.14, 0.30, 0.50, 0.59)	2 (0.29, 0.50)	
	2	Bands Rf	7 (0.11, 0.15, 0.22, 0.39, 0.44, 0.48, 0.56)	3 (0.39, 0.46, 0.55)	4 (0.30, 0.39, 0.47, 0.54)	4 (0.30, 0.40, 0.47, 0.54)	4 (0.16, 0.30, 0.47, 0.56)	2 (0.46, 0.53)	
	3	Bands Rf	6 (0.11, 0.16, 0.44, 0.48, 0.56, 0.62)	1 (0.54)	1 (0.47)	2 (0.48, 0.58)	2 (0.48, 0.62)	3 (0.50, 0.58, 0.62)	
	4	Bands Rf	4 (0.49, 0.54, 0.56, 0.64)	1 (0.63)	2 (0.54, 0.63)	2 (0.53, 0.62)	1 (0.59)	1 (0.63)	
<i>Moringa oleifera</i>	1	Bands Rf	7 (0.22, 0.38, 0.45, 0.57, 0.61, 0.65, 0.68)	3 (0.50, 0.57, 0.62)	- -	- -	- -	- -	
	2	Bands Rf	8 (0.11, 0.21, 0.27, 0.39, 0.43, 0.49, 0.53, 0.65)	10 (0.12, 0.16, 0.21, 0.25, 0.30, 0.39, 0.44, 0.50, 0.62, 0.69)	10 (0.12, 0.16, 0.21, 0.25, 0.30, 0.39, 0.44, 0.50, 0.62, 0.69)	11 (0.12, 0.16, 0.19, 0.21, 0.24, 0.28, 0.39, 0.45, 0.50, 0.62, 0.72)	10 (0.12, 0.16, 0.21, 0.25, 0.31, 0.36, 0.50, 0.61, 0.72, 0.77)	8 (0.12, 0.16, 0.22, 0.30, 0.36, 0.50, 0.61, 0.71)	
	3	Bands Rf	9 (0.12, 0.16, 0.27, 0.37, 0.45, 0.51, 0.56, 0.63, 0.72)	8 (0.12, 0.16, 0.28, 0.39, 0.45, 0.54)	8 (0.13, 0.15, 0.28, 0.37, 0.51, 0.55)	7 (0.13, 0.22, 0.27, 0.37, 0.50, 0.54)	9 (0.13, 0.15, 0.21, 0.26, 0.37, 0.45)	8 (0.10, 0.15, 0.28, 0.60, 0.48, 0.55)	

			0.67, 0.71)	0.61, 0.66)	0.67)	0.50, 0.66)	0.56, 0.66, 0.72)	
	4	Bands Rf	7 (0.11, 0.17, 0.27, 0.42, 0.56, 0.62, 0.73)	8 (0.11, 0.38, 0.43, 0.52, 0.57, 0.64, 0.70, 0.73)	7 (0.15, 0.20, 0.37, 0.53, 0.64, 0.70, 0.74)	6 (0.15, 0.37, 0.48, 0.55, 0.64, 0.68)	8 (0.15, 0.25, 0.29, 0.37, 0.48, 0.55, 0.64, 0.68)	5 (0.28, 0.37, 0.54, 0.65, 0.70)
<i>Murraya koenigii</i>	1	Bands Rf	7 (0.00, 0.08, 0.24, 0.52, 0.56, 0.70, 0.88)	5 (0.00, 0.08, 0.42, 0.54, 0.90)	5 (0.00, 0.03, 0.08, 0.60, 0.90)	2 (0.00, 0.89)	2 (0.00, 0.90)	2 (0.00, 0.91)
	2	Bands Rf	13 (0.01, 0.03, 0.09, 0.15, 0.20, 0.31, 0.35, 0.41, 0.45, 0.56, 0.68, 0.71, 0.79)	16 (0.01, 0.03, 0.07, 0.09, 0.15, 0.21, 0.32, 0.36, 0.41, 0.44, 0.56, 0.62, 0.67, 0.71, 0.77, 0.89)	16 (0.00, 0.03, 0.07, 0.09, 0.15, 0.21, 0.32, 0.36, 0.41, 0.44, 0.56, 0.62, 0.68, 0.72, 0.77, 0.89)	15 (0.00, 0.03, 0.07, 0.09, 0.16, 0.21, 0.36, 0.44, 0.55, 0.61, 0.69, 0.72, 0.76, 0.89, 0.95)	10 (0.01, 0.07, 0.18, 0.27, 0.34, 0.45, 0.55, 0.75, 0.83, 0.92)	13 (0.01, 0.07, 0.13, 0.18, 0.27, 0.34, 0.38, 0.44, 0.49, 0.55, 0.63, 0.75, 0.83)
	3	Bands Rf	12 (0.01, 0.09, 0.15, 0.21, 0.30, 0.35, 0.45, 0.52, 0.56, 0.70, 0.78, 0.89)	14 (0.01, 0.09, 0.15, 0.21, 0.29, 0.36, 0.40, 0.44, 0.56, 0.62, 0.67, 0.71, 0.76, 0.89)	15 (0.01, 0.09, 0.15, 0.21, 0.29, 0.36, 0.40, 0.44, 0.56, 0.62, 0.68, 0.72, 0.77, 0.89, 0.96)	13 (0.01, 0.08, 0.16, 0.21, 0.31, 0.36, 0.40, 0.44, 0.56, 0.62, 0.72, 0.76, 0.89, 0.95)	10 (0.00, 0.07, 0.20, 0.28, 0.36, 0.41, 0.44, 0.57, 0.76, 0.87)	10 (0.00, 0.07, 0.17, 0.29, 0.36, 0.40, 0.55, 0.63, 0.75, 0.85)
	4	Bands Rf	10 (0.00, 0.10, 0.17, 0.22, 0.32, 0.40, 0.46, 0.59, 0.72, 0.81)	12 (0.00, 0.10, 0.16, 0.22, 0.31, 0.39, 0.45, 0.58, 0.66, 0.71, 0.80, 0.91)	12 (0.00, 0.10, 0.17, 0.22, 0.33, 0.39, 0.46, 0.58, 0.66, 0.72, 0.80, 0.91)	14 (0.00, 0.08, 0.10, 0.17, 0.22, 0.33, 0.39, 0.43, 0.47, 0.58, 0.65, 0.80, 0.91, 0.96)	9 (0.00, 0.07, 0.18, 0.23, 0.29, 0.47, 0.64, 0.81, 0.90)	11 (0.00, 0.07, 0.17, 0.23, 0.29, 0.43, 0.60, 0.79, 0.87)

<i>Piper betel</i>	1	Bands	4	3	3	2	3	2
		Rf	(0.18, 0.25, 0.48, 0.72)	(0.25, 0.46, 0.73)	(0.45, 0.56, 0.61)	(0.17, 0.46)	(0.16, 0.44, 0.71)	(0.18, 0.43)
	2	Bands	2	4	5	3	5	4
		Rf	(0.49, 0.74)	(0.22, 0.31, 0.37, 0.45)	(0.21, 0.36, 0.66)	(0.20, 0.46, 0.64)	(0.17, 0.35, 0.64)	(0.19, 0.44, 0.65, 0.77)
	3	Bands	4	7	7	7	8	8
		Rf	(0.16, 0.32, 0.47, 0.58)	(0.16, 0.32, 0.58, 0.77)	(0.19, 0.45, 0.65, 0.76)	(0.16, 0.31, 0.58, 0.76)	(0.18, 0.44, 0.63, 0.76)	(0.16, 0.18, 0.20, 0.31, 0.42, 0.58, 0.62, 0.74)
	4	Bands	6	1	3	4	5	3
		Rf	(0.27, 0.30, 0.35, 0.43, 0.57, 0.66)	(0.30)	(0.29, 0.52)	0.42, (0.29, 0.60, 0.78)	0.39, (0.16, 0.61, 0.77)	0.29, (0.28, 0.78)
<i>Sphaeranthus indicus</i>	1	Bands	8	2	2	2	1	1
		Rf	(0.11, 0.19, 0.24, 0.36, 0.45, 0.53, 0.61, 0.69)	(0.52, 0.60)	(0.50, 0.58)	(0.50, 0.59)	(0.52)	(0.51)
	2	Bands	9	7	7	4	4	6
		Rf	(0.11, 0.17, 0.27, 0.40, 0.46, 0.50, 0.56, 0.65, 0.76)	(0.14, 0.40, 0.57, 0.72)	(0.32, 0.48, 0.66, 0.72)	(0.14, 0.42, 0.57, 0.68)	(0.31, 0.47, 0.68, 0.72)	(0.43, 0.59, 0.48, 0.59, 0.71)
	3	Bands	7	3	3	3	3	3
		Rf	(0.12, 0.17, 0.36, 0.46, 0.51, 0.59, 0.63)	(0.47, 0.59, 0.66)	(0.47, 0.58, 0.67)	(0.44, 0.58, 0.68)	(0.41, 0.58, 0.68)	(0.48, 0.58, 0.68)
	4	Bands	5	3	3	3	4	4
		Rf	(0.11, 0.17, 0.51, 0.63, 0.76)	(0.50, 0.63, 0.66)	(0.52, 0.63, 0.67)	(0.52, 0.69, 0.73)	(0.55, 0.69, 0.77)	(0.52, 0.60, 0.69, 0.76)

[Note: **Track 1** =Water extract, **Track 2** =Methanol extract, **Track 3** =Chloroform extract, **Track 4** =Petroleum ether extract]

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(c) *Ferrous ion chelating activity assay*: In metal ion chelating assay, if the extract/ compounds are effective, then they interfere with the formation of ferrous and ferrozine complex and able to capture ferrous ion before the formation of ferrozine by their antioxidant- chelating activity. Ferrozine can quantitatively form complexes with Fe^{2+} . In the presence of chelating agents, the complex formation is disrupted, resulting in decrease of the red colored complex. Thus reduction of the color is equal to the metal chelating activity (Singh *et al.*, 2009). In the present study, % FICA (Ferrous ion chelating activity) IC_{50} value for standard Ascorbic acid observed at 1.5 mg/ml concentration. The maximum % FICA was observed with petroleum ether extract of *Curcuma longa* followed by water extracts of *Curcuma longa*, *Datura stramonium*, and chloroform extract of *Sphaeranthus indicus*. While comparatively lower amount of % FICA recorded in case of petroleum ether extract of *Murraya koenigii*, methanol extract of *Datura stramonium* and *Moringa oleifera*, and chloroform extract of *Datura stramonium* (Table 2, Figure 1).

HPTLC Phyto-chemical Profiling of Plant Extracts:

The phyto-chemical constituents in a plant material form a characteristic fingerprint, representing the quantity of active constituents. Moreover, this helps to standardize the mixtures like herbal drug formulations and market samples (Paramasivam *et al.*, 2008). In various reports some of the plants and various solvent extracts were analyzed by TLC method. However the HPTLC technique is having precision over TLC method and thus an effort was made in the present study to develop comparative HPTLC phyto-chemical profiles of four solvent extracts of all selected ten medicinal plants (Table 3, 4).

The chromatographic development of the TLC plates in the standardized solvent system and their HPTLC densitometric scanning results (Table 4) observed in the present study are stated below. In case of *Azadirachta indica* methanol and chloroform extracts, the separated compounds were detected very well in the entire scanned wavelength (200-450nm), and recorded maximum number of separated bands compared to other extracts prepared in water and petroleum ether. Among all four extracts, water extracts showed lowest number of bands in the solvent system toluene: ethyl acetate: methanol (45: 3.5: 1.5, v/v). *Camellia sinensis* chromatograms developed in the solvent system toluene: ethyl acetate: methanol (45: 3.5: 6, v/v). Bands of water extract were not detected in 400- 450nm range of wavelengths and showed lowest number of quench bands, followed by methanol extract. While chloroform and petroleum ether extracts revealed good number of bands and detected in entire scanned wavelengths. *Cassia fistula* plate developed in solvent system toluene: ethyl acetate: methanol: butanol (36: 4: 8: 1, v/v) where chloroform extract revealed maximum numbers of separated bands, followed by petroleum ether, methanol and water extracts. 200- 300nm wavelength detected good amount of separated bands. For *Curcuma longa*, all four extracts demonstrated good number of separated bands in the solvent system- chloroform: methanol: acetic acid (94: 5: 1, v/v). *Datura stramonium* water extract was poorly separated in the solvent system toluene: ethyl acetate (7: 3, v/v) while other extracts demonstrated average number of quench bands about 4- 7 in number. *Lawsonia inermis* chloroform and petroleum ether extracts demonstrated low amount of separated bands compared to methanol and water extracts in the solvent system toluene: ethyl acetate: formic acid (27.5: 20: 2.5, v/v).

Moringa oleifera bands in water extract, detected at 200 and 250nm wavelengths during the scan. While other extracts had shown 7- 10 number of quench bands, detected at whole range of scanned wavelengths viz. 200- 450nm with toluene: ethyl acetate: methanol (43: 4: 3, v/v) solvent system. *Murraya koenigii* other extracts apart from water extract, were having excellent bands about 9- 16 in number, in the solvent system toluene: ethyl acetate: methanol (45: 3.5: 1.5, v/v). *Piper betel* all four extracts, showed 3- 7 quenching bands on the developed chromatogram using solvent system toluene: ethyl acetate: methanol (40: 20: 1, v/v) and detected at 200- 450nm wavelengths. *Sphaeranthus indicus* chromatogram developed using toluene: ethyl acetate: formic acid (25: 22.5: 2.5, v/v) solvent system, revealed nice separation of the compounds of all four extracts. Maximum number of quench bands was detected at 200 nm range.

DISCUSSION

Plants used in the present study are medicinally useful and their therapeutic importance is been documented in ancient literature and their pharmacological effects are also been studied. The purpose of

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the present analysis was to evaluate the free radical scavenging ability of plants, to find effective extract fractions, and to study the level/mode of action, using different antioxidant *in vitro* assays. An antioxidant activity of plants is often correlated with its medicinal properties. Various scientific studies and the present findings have demonstrated that antioxidant activity exhibited by phyto-chemicals or PSMs found to play important role in various pharmacological activities by inhibition of free radical induced damage for instance, exhibit anti-aging, anti-inflammatory, anti-atherosclerosis, anti-cancer etc. activities. They also stimulate immune system, regulate gene expression in cell proliferation and apoptosis, hormone metabolism and can also effectively reduce coronary heart disease and cancer mortality (Ghafar *et al.*, 2010). More precisely, bio-chemically antioxidants function as singlet or triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors and synergists (Mandal *et al.*, 2009). Thus supplementation of antioxidants as free radical scavengers has become an attractive therapeutic strategy for reducing the risk of diseases. In this context, medicinal plants and herbal drugs have found importance in preventing the deleterious consequences of oxidative stress and treating such diseases (Aquil *et al.*, 2006).

All the selected plants in the present study, has demonstrated presence of potential antioxidant activity at one or other level, and thus approves their disease curing effect on body. On the whole, chloroform and petroleum ether extracts of studied plants demonstrated appreciable quantity of phenol content but comparatively lower than the water and methanol extracts. Present study has also revealed that, *Camellia sinensis*, *Lawsonia inermis*, *Piper betel* and *Murraya koenigii* extracts found to possess good amount of % Phenol content and noticeable amount of % DPPH RSA and % FICA activity. While extract wise comparative antioxidant activity evaluation indicates that overall, water extracts of *Curcuma longa* and *Datura stramonium* demonstrated appreciably good amount of % FICA activity but found to possess minor amount of % RSA and % phenol content. While in case of methanol extract, all plants had shown significant quantity of % phenol content, % RSA and % FICA activity. Comparatively, chloroform extract exhibited low amount of phenol content and average level of % RSA and % FICA activity. On the whole, *Curcuma longa*, *Piper betel* and *Murraya koenigii* chloroform extracts demonstrated excellent % RSA, while *Sphaeranthus indicus* chloroform extract revealed superior % FICA activity. In case of petroleum ether extract, *Murraya koenigii* and *Curcuma longa* shown good % RSA and % FICA activity respectively. While petroleum ether extracts of all other plants demonstrated low quantity of % phenol content and medium amount of % RSA and % FICA activity.

Conclusion

A comparative HPTLC analysis of different polar (water, methanol) to non-polar (chloroform, petroleum ether) solvent extracts represents comparative account of polar or non-polar nature and also amount of phyto-chemicals. Moreover, the phyto-chemical profile of the plant extracts helps in understanding the extent of antioxidant activity. Also antioxidant assays demonstrated positive antioxidant activity in all four solvent extracts. Therefore all selected ten medicinal plants proved highly significant in terms of presence of antioxidant metabolites. The study hence supports the correlation of antioxidant activity of plants with their beneficial medicinal effects on the body.

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