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In-vitro antioxidant activities and HPTLC study of *Adansonia digitata*: A medicinal plant

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Abstract
Background: *Adansonia digitata* L. belong to family Bombacaceae is a well-known plant in the traditional medicinal system and it has been employed for the treatment of various diseases.
Aim and Objectives: This study was aimed to establish the total phenolic content and antioxidant potential of *A. digitata* along with the HPTLC study to identify potential phytoconstituents having antioxidant activity.
Materials and Methods: Preliminary phytochemical screening and total phenol content of the samples were estimated using standard procedures and antioxidant activity of root bark, stem bark, leaves and fruits of *A. digitata* were done by *in-vitro* methods (DPPH and Nitric oxide assay). HPTLC study was also carried out using Toluene: Ethyl acetate: Formic acid (5:4:1) v/v as mobile phase.
Results: Results of the study revealed the presence of tannins, flavonoids, and steroids in all the parts of *A.digitata*. The stem bark and fruit extract produced significantly higher amounts of total phenol content compared to root bark and leaf extract. The antioxidant study demonstrated considerable effectiveness in methanolic extracts of different parts of *A.digitata* may be due to free radical scavenging activity.
Conclusion: The results of this study show that, methanolic extract of *A.digitata* has antioxidant property hence can be used as an easily accessible source of natural antioxidants.

Keywords: *adansonia Digitata*, anti-oxidant, chromatography, plant phenolics

Introduction

Antioxidants are compounds that
inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms [1]. Antioxidants as compounds that when present in low concentration in relation to the oxidant, prevent or delay the oxidation of the substrate [2]. Many plants contains a large amount of antioxidants which plays an important role in absorbing and neutralizing free radicals, quenching singlets and triplets oxygen or decomposing peroxidase. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many infectious diseases [3]. Atoms or molecules that possess unpaired electrons are called free radicals. Although free radicals are naturally produced under aerobic conditions, an excess of free radicals can damage all cellular macromolecules including proteins, carbohydrates, lipids, and nucleic acids. The free radicals start reactions such as the oxidation of DNA which can ultimately cause mutations in the genetic material. When oxidizing proteins, it has been found that the free radical can inhibit enzymes or cause proteins to denature or degrade. The free radicals have also been implicated in the pathogenesis of diabetes, liver damage, atherosclerosis, inflammation, cardiovascular disorders, neurological disorders, and in the process of aging [4].
The role of free-radical reactions in human disease, biology, toxicology, and the deterioration of food has become an area of intense interest. In the main, the free-radical reaction of lipid peroxidation is an important issue in the food industry where manufacturers minimize oxidation in lipid-containing foods by use of antioxidants during the manufacturing process; foods are produced that maintain their nutritional quality over a defined shelf life [5]. Plant secondary metabolites such as polyphenols, play an important role in the defence against free radicals. Medicinal plant parts (roots, leaves, stems, flowers, and fruits) are commonly rich in phenolic compounds, such as flavonoids, tannins, stilbenes, coumarins, lignans etc. The antioxidant properties of polyphenols are due to their redox properties, which allow them to act as reducing agents, hydrogen donators, metal chelators, and single oxygen [6].
Adansonia digitata belong to Bombacaceae family is a well-known plant in the traditional medicinal system and it has been employed for the treatment of various diseases. It has thick, angular, wide spreading branches and a short, stout trunk which attains 10-14m or more in girth [7]. The plant parts are used to treat various ailments such as diarrhoea, malaria and microbial infections [8]. Ethnobotanical studies have confirmed the high content of antioxidant vitamins in *A. digitata* fruit constituents and leaves. The main objectives of the present study is to the characterization and quantification of the phenolic fraction of methanolic extracts of different parts of the *A. digitata*; the evaluation of the antioxidant activity of extracts and HPTLC fingerprinting analysis.

Materials and Method

Collection and Authentication: Root bark, stem bark and leaves of *Adansonia digitata* L. were collected and identified by the local taxonomist from its natural habitat Jamnagar, Gujarat during the January 2018while fruits from Bhavnagar, Gujarat during the March 2018. The herbarium was submitted to the Pharmacognosy Laboratory, Institute

for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University and authenticated by the Pharmacognosist, provided with herbarium reference number no. Ph.m:6162/18-19

Preparation of plant extract: The different parts of the plant *viz*; root bark; stem bark, leaves, and fruit (cut into small pieces) were shade dried. Then dried parts were powdered individually by the mechanical grinder and sieved through 60#. The powder was stored in an airtight glass container. Five-gram powder of each sample was macerated with 100 ml methanol and water separately in a closed flask for 24 hr, shaking frequently during 6 hr and allowed to stand for 18 hr. After 24 hr samples were filtered and the extracts were collected. All the extracts were used for preliminary phytochemical screening [9].

Phytochemical analysis: The phytochemical analysis of methanolic and aqueous extracts of the plant was studied by following the standard procedures [10].

Measurement of Total Phenol content: The concentration of phenolics in plant extracts was determined using the spectrophotometric method [11]. The methanolic extracts at different concentrations (150, 300, 600, 1200µg/ml) were used in the analysis. The reaction mixture was prepared by mixing 0.5ml of methanolic solution of extract, 2.5ml of 10% Folin-ciocalteu's reagent and 2.5ml 7.5% $NaHCO_3$. The same procedure was repeated for the standard solution of Gallic acid at same concentrations and the calibration line was constructed. Blank was concomitantly prepared, containing 0.5ml methanol, 2.5ml 10% folin-ciocalteu's reagent and 2.5ml of 7.5% of $NaHCO_3$.

The samples were thereafter incubated in a thermostat at 45^0C for 45 min. The absorbance was determined using a spectrophotometer at λmax = 765nm. The samples were prepared in a triplicate manner for each analysis and the mean value of absorbance was obtained. Based on the measured absorbance, the concentration of phenolics was read (µg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent.

In-vitro Antioxidant activity

DPPH Free radical scavenging activity (DPPH assay): Free radical scavenging activity of the methanolic extracts of plant parts was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) assay. The DPPH free radical is reduced to corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a discolouration assay, which is evaluated by the addition of the DPPH solution in ethanol or methanol and the decrease in absorbance was measured[12]. The methanolic extracts at different concentrations (150, 300, 600, 1200µg/ml) were used in the analysis. The reaction mixture was prepared by mixing 3 ml of methanolic solution of extract and 1 ml solution of DPPH. The same procedure was repeated for the standard solution of Curcumin at same concentrations and the calibration line was constructed. Blank was concomitantly prepared, containing 3ml methanol and 1ml solution of DPPH. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. All the readings were performed in a triplicate manner. Then, absorbance was measured at 517nm by using a UV-Vis spectrophotometer. The percent DPPH scavenging effect was calculated by using the following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0 – A_1 / A_0 \times 100$. Where A_0 was the absorbance of the control and A_1 absorbance of the test or standard samples.

Nitric oxide radical scavenging activity: The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions [13]. The methanolic extracts at different concentrations (150, 300, 600, 1200 µg/ml) were mixed with sodium nitroprusside (10mM) in phosphate-buffered saline and incubated at room temperature for 150 mins. After the incubation period, 0.5 ml of griessreagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% N-(1-naphthyl) ethylenediaminedihydrochloride) was added. The same procedure was repeated for the standard solution of Curcumin at same concentrations and the calibration line was constructed. Blank was concomitantly prepared, containing methanol were mixed with sodium nitroprusside (10mM) in phosphate-buffered saline and incubated at room temperature for 150 mins. After the incubation period, 0.5 ml of griess reagent was added. All the readings were performed in a triplicate manner. The absorbance of the chromophore formed was read at 546nm. The percent nitric oxide scavenging activity was calculated using the following equation:

Nitric oxide scavenging activity (%) or percent inhibition = $A_0 – A_1 / A_0 \times 100$.Where A_0 was the absorbance of the control and A_1 absorbance of the test or standard.

HPTLC study

HPTLC Aluminium pre-coated plate with Silica gel60 GF_{254} was used with Toluene: Ethylacetate: Formicacid (5:4:1) v/v [14]. as mobile phase. Methanolic extract of samples 1mg/ml solution applied on the plate by using Linomat V applicator. Cammag twin trough glass chamber (10x10 cm^2) with SS lid was used for the development of the TLC plate which was previously saturated with the mobile phase for 30 minutes. The TLC plate was developed to 9 cm distance and after that removed from the chamber and air-dried at room temperature. The HPTLC fingerprint profile was snapped by Cammag scanner, under UV 254 and 366nm.

This plate was sprayed (derivatized) with vanillin-sulphuric acid reagent [15] followed by heating at 110ºC for 10 minutes, 1% w/v aluminium chloride solution [16]. and 20% Na_2CO_3 w/v followed by the folin-ciocalteu reagent [17]. The R_f was calculated manually and after scanning at a wavelength of 254 nm & 366nm.

Results

Phytochemical screening: The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. In view of the results are depicted in table no. 1, it appears that all the parts showed the presence of tannin, flavonoids, and steroids while carbohydrates is present in leaves and fruits.

Table 1: Results of Phytochemical screening of the different parts of the plant *A. digitata*

S. No	Phytoconstituents	Tests	Root Bark		Stem Bark		Leaf		Fruit	
			WE	ME	WE	ME	WE	ME	WE	ME
1.	Carbohydrates	Molisch's	-	-	-	-	+	-	+	-
2.	Proteins	Biuret	-	-	-	-	-	-	-	-
3.	Amino acids	Ninhydrin	-	-	-	-	-	-	-	-
4.	Alkaloids	Wagnor	-	-	-	-	-	-	-	-
		Mayer	-	-	-	-	-	-	-	-
5.	Tannins	$Fecl_3$	+	+	+	+	-	+	+	+
6.	Steroids	Salkowski	-	+	-	+	-	+	-	+
7.	Flavanoids	Lead Acetate	+	+	+	+	+	-	+	+
8.	Saponin glycoside	Foam	_	-	-	-	-	-	-	-

'+' Present, '-' Absent

Total Phenolic Content

Total phenolic content levels were expressed in terms of gallic acid equivalent. The total phenolic compound contents in the plant extracts are shown in table no 2. It appears that the methanolic extract of stem bark of *A. digitata* has the highest phenolic content and methanolic extract of leaves of *A. digitata* had the lowest content. Methanolic extract from the fruit of *A. digitata* shows intermediate phenolic content followed by methanolic extract of root bark of *A. digitata* extract respectively. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods [18], decrease the risk of heart diseases by inhibiting the oxidation of low-density lipoproteins. They are also known to possess antibacterial, antiviral, antimutagenic and anticarcinogenic properties [19, 20]. (Figure 1)

Table 2: Results of Total Phenolic content

S. No.	Concentration (µg/ml)	Root Bark	Stem Bark	Leaf	Fruit
1.	150	233.95±4.13	269.85±0.99	205.2±1.72	265.2±8.48
2.	300	288.55±1.44	422.7±5.72	258.15±0.03	339.8±3.79
3.	600	429.55±3.58	842.1±5.99	290.05±8.69	687.5±15.44
4.	1200	728 ±6.20	1859.05±13.89	747.5±7.93	1459.45±17.37

Data: Mean ±SD (standard deviation), (n=3)

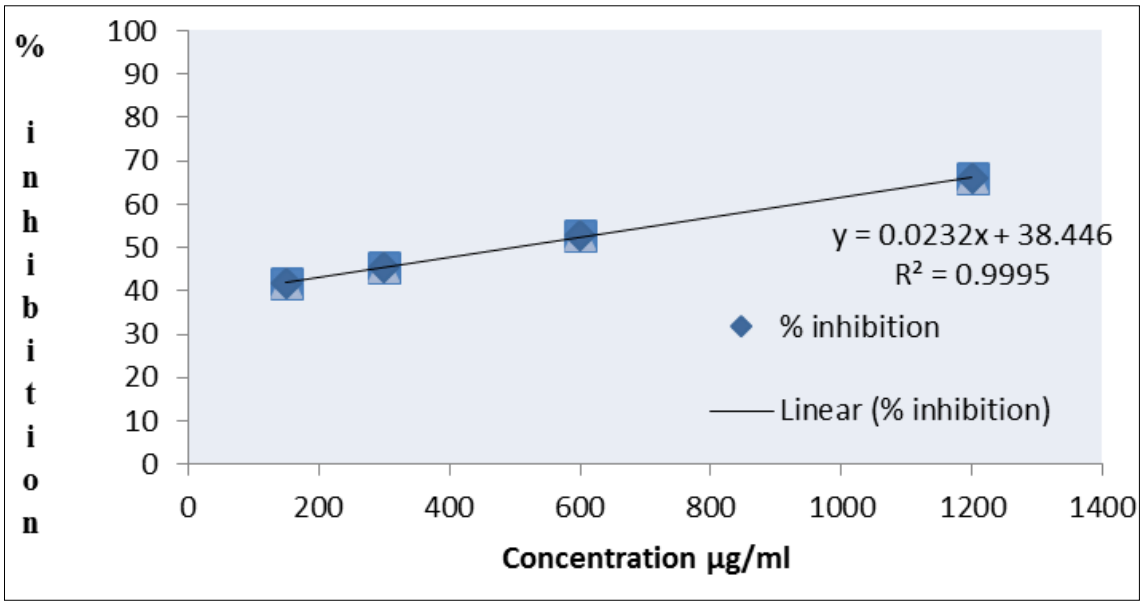

Fig 1: Total phenolic content of standard Gallic acid

The results of free radical scavenging activities DPPH assay and nitric oxide radical scavenging activity are shown in table no. 3 & 4.

The crude methanolic extract of *A. digitata* leaf and fruit show good DPPH and nitric oxide free radical-scavenging activities. (Figure 2-5)

Table 3: Results of DPPH assay

Extracts	*Adansonia digitata*		
	Conc. (µg/ml)	% inhibition	IC_{50} (µg/ml)
Root bark extract	150	5.22±0.11	1017.57
	300	10.40±0.01	
	600	24.21±0.01	
	1200	61.07±0.08	
Stem bark extract	150	3.23±0.22	1148.03
	300	6.17±0.01	
	600	26.02±0.02	
	1200	51.89±0.08	
Leaf extract	150	20.58±0.04	480.49
	300	40.39±0.08	
	600	62.97±0.09	
	1200	99.79±0.16	
Fruit extract	150	24.21 ±0.06	511.19
	300	32.71±0.08	
	600	58.23±0.16	
	1200	99.75±0.18	
Curcumin	150	41.83±0.58	362.80
	300	50.27±0.29	
	600	58.70±1.45	
	1200	67.13±2.03	

Data: Mean ±SD (standard deviation) (n=3)

Table 4: Results of Nitric Oxide assay

Extracts	*Adansonia digitata*		
	Conc. (µg/ml)	% inhibition	IC_{50} (µg/ml)
Root bark extract	150	7.25±0.14	918.98
	300	15.23±0.20	
	600	30.74±0.16	
	1200	66.41±0.15	
Stem bark extract	150	3.81±0.48	812.28
	300	7.64±0.38	
	600	28.80±0.23	
	1200	82.49±0.17	
Leaf extract	150	11.35±0.29	633.47
	300	24.03±0.28	
	600	46.85±0.21	
	1200	95.11±0.18	
Fruit extract	150	9.74±0.68	632.30
	300	16.30±0.49	
	600	56.64±0.31	
	1200	94.11±0.22	
Curcumin	150	34.72±0.20	411.67
	300	48.61±0.41	
	600	55.55±0.20	
	1200	93.05±0.61	

Data: Mean ±SD (standard deviation) (n=3)

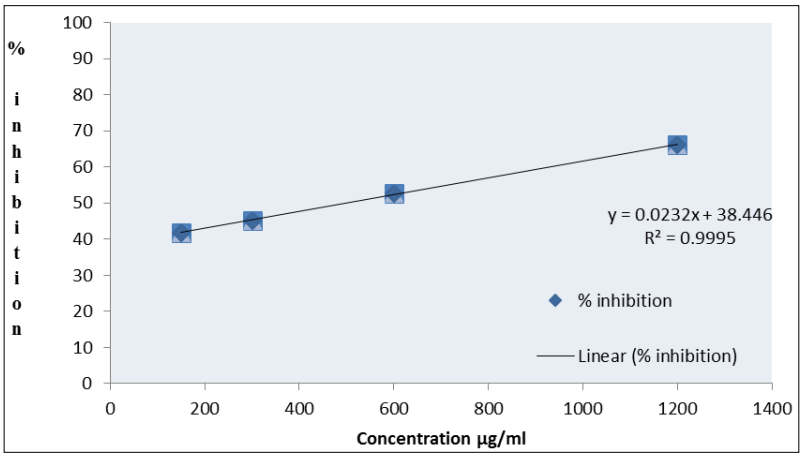

Fig 2: DPPH free radical scavenging activity of standard Curcumin

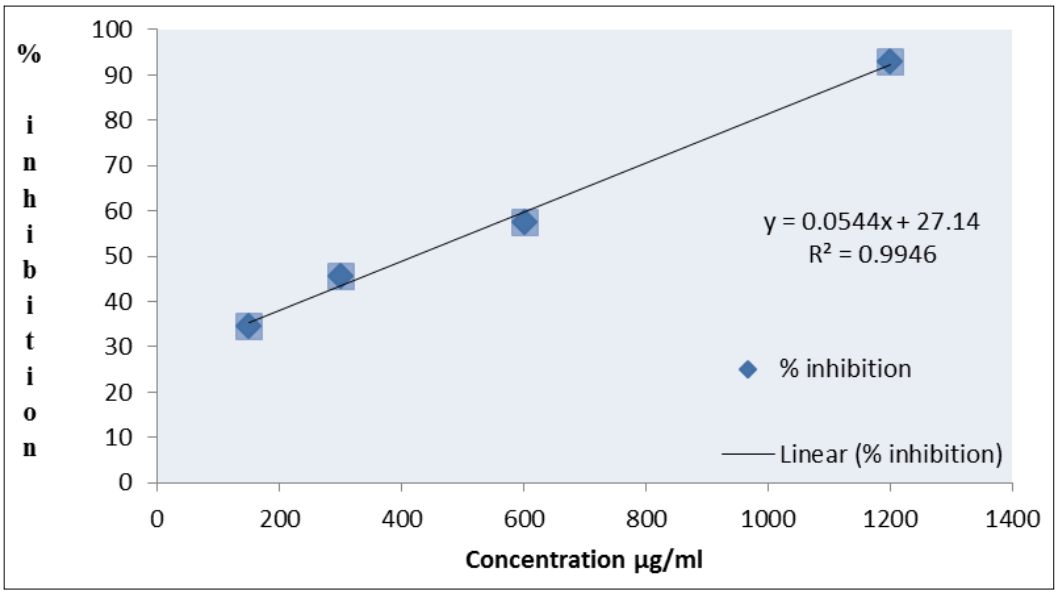

Fig 3: Nitric oxide free radical scavenging activity of standard Curcumin

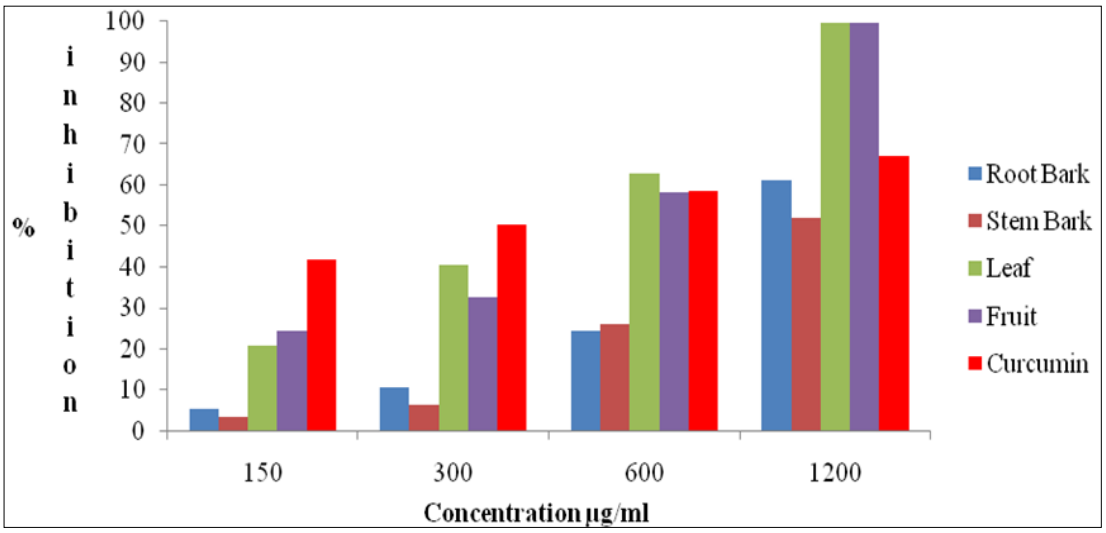

Fig 4: DPPH free radical scavenging activity of different parts of *A. digitata* and standard Curcumin

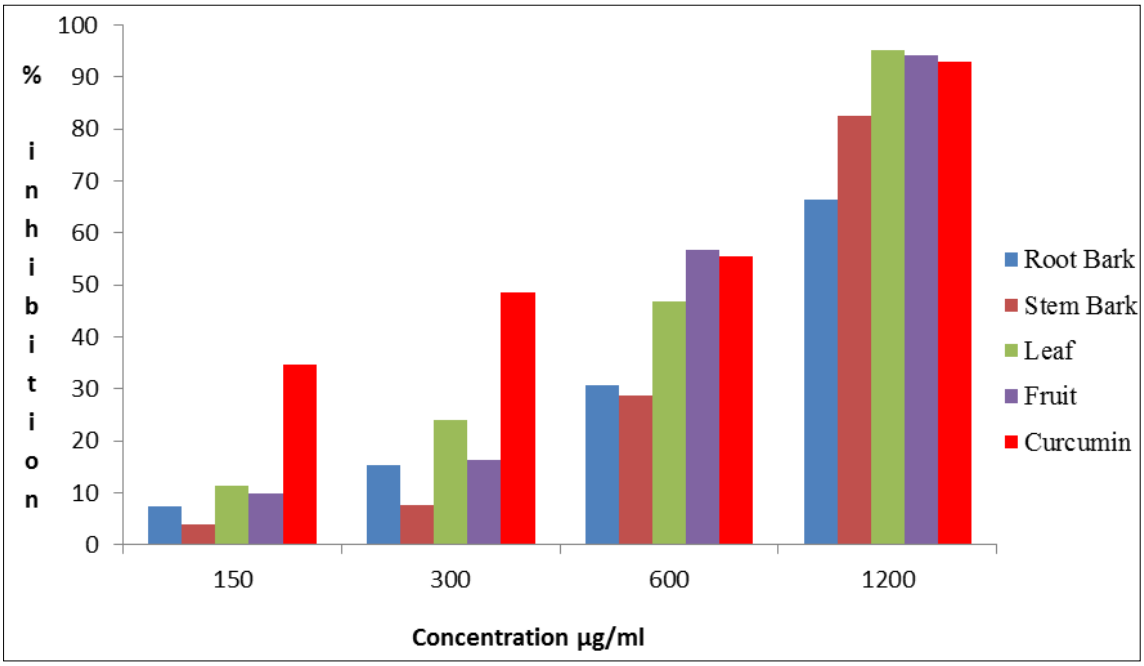

Figure 5: Nitric oxide free radical scavenging activity of different parts of *A. digitata* and standard Curcumin

HPTLC Study

High-performance thin-layer chromatography (HPTLC) is emerging as a versatile, high-throughput, and cost effective technology that is uniquely suited to assess the identity and quality of botanical materials. HPTLC study of methanolic extract of *A. digitata* was carried out by using the solvent system of Toluene: Ethyl acetate: Formic acid (5:4:1) v/v was used. The respective R_f is shown in table no. 5 (Figure 6, 7 and 8)

Table 5: HPTLC study of Methanolic extracts of *Adansonia digitata* Linn. using Toluene: Ethyl acetate: Formic acid (5:4:1) v/v.

Sample name	Root Bark		Stem Bark		Leaves		Fruit	
Toluene: Ethyl acetate: Formic acid (5:4:1) v/v								
	N0. of spots	R_f	No. of spots	R_f	No. of spots	R_f	No. of spots	R_f
254nm	8	0.02,0.43, 0.60,0.63, 0.68,0.74, 0.80,0.89	4	0.03,0.59, 0.67,0.79	10	0.03,0.29, 0.41,0.59, 0.62,0.65, 0.70,0.77, 0.84, 0.88	9	0.02,0.12, 0.16,0.30, 0.40,0.58, 0.62,0.66, 0.88
366nm	8	0.02,0.60, 0.63,0.74, 0.80,0.89, 0.90,0.94	4	0.02,0.75, 0.79,0.95	10	0.03,0.20, 0.29,0.59, 0.62,0.65, 0.70,0.77, 0.84, 0.89	12	0.02,0.16, 0.30,0.52, 0.59,0.62, 0.66,0.70, 0.77,0.85, 0.89, 0.94
Derivatization with Vanillin- Sulphuric acid								
Visual	5	0.35,0.52, 0.54,0.72, 0.77	3	0.36,0.72, 0.77	9	0.36,0.52, 0.54,0.60, 0.62,0.64, 0.77,0.78, 0.84	5	0.36,0.52, 0.56,0.72, 0.77
254nm	18	0.06, 0.12, 0.16,0.18,0.20,0.35,0.37,0.43,0.44,0.46,0.53,0.59,0.65,0.71,0.74,0.77,0.84,0.91	21	0.05, 0.14, 0.16,0.18,0.20,0.33,0.35,0.42,0.45,0.47,0.48,0.58,0.61,0.67,0.72,0.75,0.77,0.81,0.84,0.89,0.94	21	0.05, 0.07, 0.14,0.17,0.20,0.24,0.26,0.36,0.40,0.42,0.48,0.52,0.54,0.57,0.61,0.70,0.75,0.77,0.81,0.86,0.87	19	0.03,0.16,0.19,0.24,0.27,0.33,0.45,0.46, 0.57,0.60,0.70,0.72,0.73,0.76,0.81,0.85,0.86,0.90,0.96
366nm	12	0.06, 0.12, 0.18,0.20,0.33,0.43,0.45,0.64,0.69,0.75,0.85,0.91	17	0.01,0.05, 0.16,0.18,0.20,0.27,0.34,0.35,0.44,0.48,0.62,0.65,0.72,0.81,0.84,0.89,0.94	12	0.04, 0.19, 0.30,0.40,0.42,0.49,0.52,0.60,0.67,0.75,0.82,0.85,	14	0.03, 0.15, 0.19,0.30,0.43,0.48,0.58,0.61,0.67,0.71,0.74,0.80,0.85,0.90
Derivatization with 1% Aluminium Chloride Sol^{n}w/v								
Visual	2	0.02,0.55	5	0.02,0.39,0.65,0,73,0.95	3	0.02,0.73,0.95	2	0.02,0.75
254nm	6	0.02, 0.35, 0.44,0.54, 0.77,0.95	8	0.02,0.24,0.40,0.45, 0.69,0.77,0.91,0.95	11	0.02,0.14,0.24,0.32,0.43,0.52, 0.53,0.62,0.66,0.75,0.95	8	0.02,0.09,0.13,0.32,0.45,0.56,0.76,0.96
366nm	4	0.02, 0.33, 0.44,0.71,	6	0.02,0.13,0.40,0.44, 0.66,0.71	8	0.02,0.04,0.17,0.25,0.32,0.44, 0.66,0.70	7	0.02,0.14,0.32,0.43,0.58,0.71,0.82
Derivatization with 20%Na_2Co_3 w/v+ Folin-Ciocalteu reagent								
Visual	1	0.02	1	0.5	2	0.02,0.79	2	0.02,0.57
254nm	10	0.03, 0.06, 0.09,0.22,0.33,0.35,0.37,0.39,0.75,0.77	23	0.04,0.06, 0.09,0.33,0.36,0.38,0.40,0.42,0.44,0.48,0.58,0.60,0.63,0.65,0.68,0.71,0.73,0.75,0.77,0.79,0.81,0.83,0.87	12	0.07, 0.10, 0.22,0.23,0.31,0.37,0.39,0.41,0.44,0.66,0.69,0.75	28	0.11,0.13,0.18,0.20,0.23,0.25,0.26,0.29,0.30,0.37,0.38,0.42,0.45,0.52,0.54,0.55,0.57,0.62,0.64,0.65,0.68,0.70,0.74,0.76,0.79,0.85,0.87,0.92
366nm	6	0.13,0.16,, 0.33,0.35,0.37, 0.77	13	0.01, 0.04, 0.07,0.09,0.11,0.14,0.34,0.44,0.75,0.76,0.79,0.82,0.87	4	0.02,0.07, 0.10, 0.75	20	0.13,0.18, 0.20,0.23,0.25,0.26,0.31,0.40,0.43,0.45,0.47,0.52,0.55,0.57,0.62,0.64,0.65,0.68,0.71,0.75,

Root bark methanolic extract shows 8 spots in 254 and 366nm, stem bark methanolic extract shows 4 spots in 254 & 366nm, leaves methanolic extract shows 10 spots in 254 & 366nm while fruit methanolic extract shows 9 spots in 254 nm, 12 spots in 366nm. It was also found that R_f 0.02 present in track 1, 2 and 4 i.e. root bark, stem bark, and fruit methanolic Extracts after spectral detection from 200 to 800nm. After derivatizing with vanillin-sulphuric acid; root bark methanolic extract shows 5 spots in visual light, 18 spots in 254nm & 12 spots in 366nm, stem bark methanolic extract shows 3 spots in visual light, 21 spots in 254nm & 17 spots in 366nm, leaves methanolic extract shows 9 spots in visual light, 21 spots in 254nm & 12 spots in 366nm while fruit

methanolic extract shows 5 spots in visual light, 19 spots in 254 nm & 14 spots in 366nm.

After derivatizing with 1% aluminium chloride solution; root bark methanolic extract shows 2 spots in visual light, 6 spots in 254nm & 4 spots in 366nm, stem bark methanolic extract shows 5 spots in visual light, 8 spots in 254nm & 6 spots in 366nm, leaves methanolic extract shows 3 spots in visual light, 11 spots in 254nm & 8 spots in 366nm while fruit methanolic extract shows 2 spots in visual light, 8 spots in 254 nm & 7 spots in 366nm. After derivatizing with 20%Na_2Co_3 w/v + folin-ciocalteu reagent root bark methanolic extract shows 1 spot in visual light, 10 spots in 254nm & 6 spots in 366nm, stem bark methanolic extract shows 1 spot in visual light, 23 spots in 254nm & 13 spots in 366nm, leaves methanolic extract shows 2 spots in visual light, 12 spots in 254nm & 4 spots in 366nm while fruit methanolic extract shows 2 spots in visual light, 28 spots in 254 nm & 20 spots in 366nm.

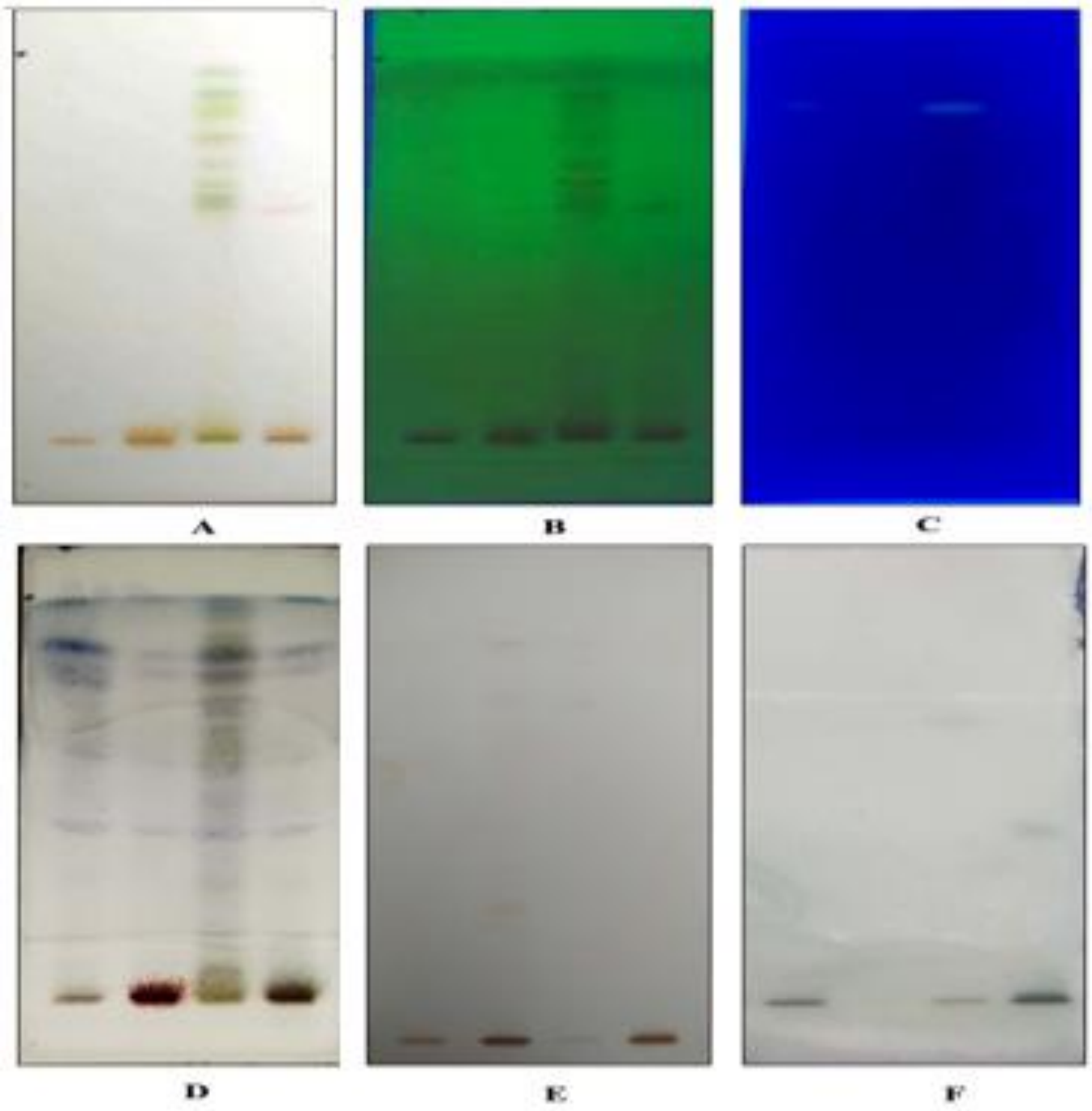

Fig 6: HPTLC plate - (A) Visual Light; (B) 254nm; (C) 366nm; (D) After derivatization with Vanillin-Sulphuric acid; ; (E) After derivatization with 1% Aluminium Chloride Sol^{n}w/v ; (F) After derivatization with 20%Na_2Co_3 w/v+ Folin-Ciocalteu reagent

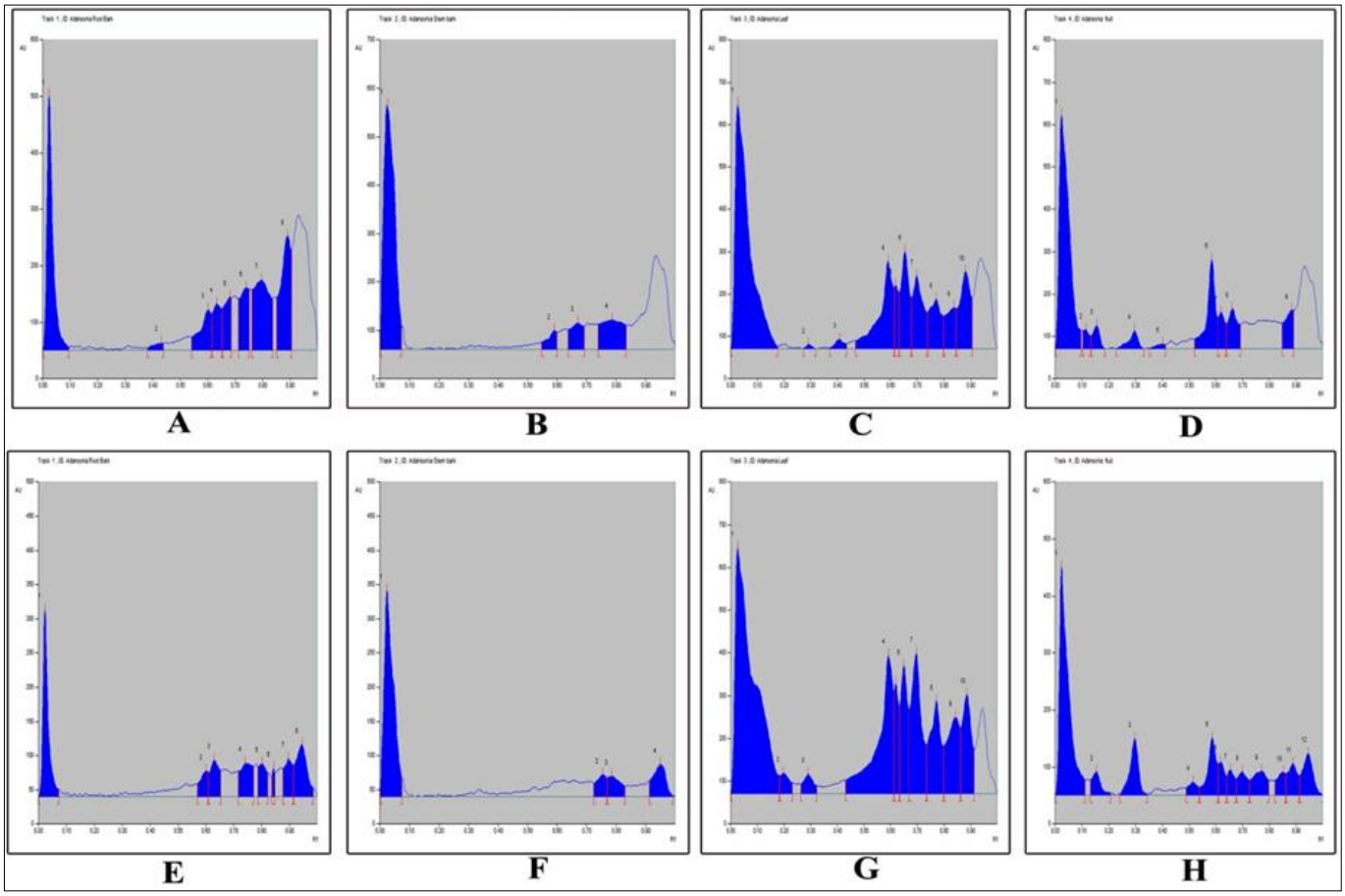

Fig 7: Densitogram at 254 nm - (A) ME of Root bark; (B) ME of Stem bark; (C) ME of Leaves; (D) ME of Fruits Densitogram at 366 nm - (E) ME of Root bark; (F) ME of Stem bark; (G) ME of Leaves; (H) ME of Fruits

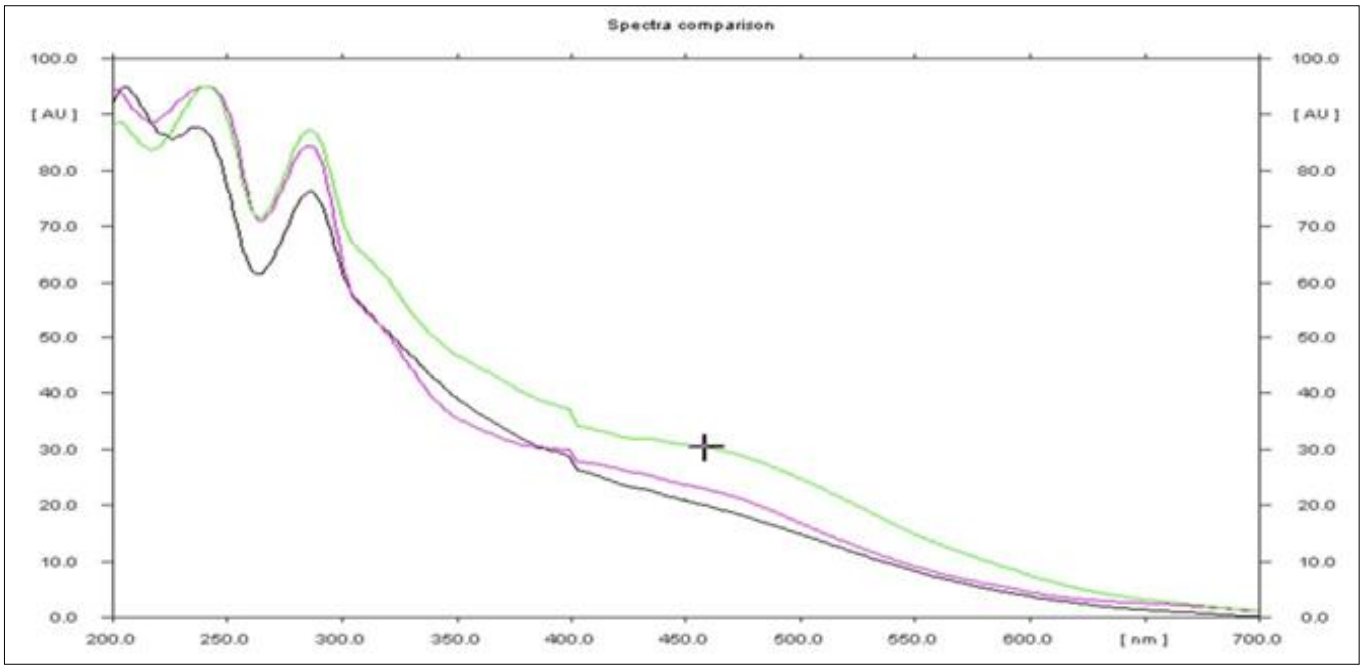

Fig 7: Similar spectra at R_f0.02 in Root bark, Stem bark and Fruit

Discussion

The use of natural antioxidants in food, cosmetics, and the therapeutic industry would be a promising substitute for synthetic antioxidants in respect of low cost, highly compatible with dietary intake and lesser harmful effects inside the human body. Many antioxidant compounds, naturally occurring in plant sources have been recognized as free radical scavengers. In the present study, attempts have been made to study the antioxidant potential of different parts of plant *A. digitata*. Major constituents that appear to be present in the plant are steroids, tannin, and flavonoids. Tannin and flavanoids are polyphenolic moieties and it has been mentioned that phenolic compounds are responsible for antioxidant activity of plants which include free radical

scavenging, inhibition of hydrolytic and oxidative enzymes and also have anti-inflammatory activity [21].The chemical substances present in the *A. digitata* have shown the good antioxidant capacity of their extracts against the free radicals in *in-vitro* study. The results obtained demonstrated the highest DPPH free radical scavenging activity was shown by leaf extract and the highest nitric oxide scavenging activity was shown by fruit extract. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract. The high scavenging property of *A. digitata* may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. Phenolic compounds are very important plant constituent with multiple biological functions including antioxidant activity much related to the radical scavenging ability of their OH groups [22]. High phenolic content of plant extracts could be responsible for their antioxidant activity [23]. Some of them are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions [24]. Chlorogenic acid [25], quercetin [26], ellagic acid [27], kaempferol [28], rutin [29], luteolin [30], apigenin [31], caffeic [32], and (−) epicatechin [33]. are some of the phenolic moieties present in *A. digitata* [34, 35]. already reported to have anti-oxidant potential. Besides, these phenol compounds possess multiple biological properties such as antitumor, antimutagenic and antibacterial properties, and these activities might be related to their antioxidant activity [36].

The HPTLC analysis of methanolic extract can provide standard fingerprints with the selected solvent system and can be used as a reference for identification and quality control of the drug. The high-performance thin-layer chromatography with toluene: ethyl acetate: formic acid (5:4:1, v/v) as the mobile phase is a reliable method for fast and easy separation of components present in methanolic extracts of different parts of plant *A. digitata*. The method can be used as a cost-effective method with reduced analysis time as compared to other alternative methods of analysis. HPTLC is a powerful analytical tool in the field of analysis. An R_f value is characteristic for any given compound (provided that the same stationary and mobile phases are used) [37]. It can provide corroborative evidence as to the identity of a compound. The mentioned R_f in the table may be used for the identification of the plant. It may show variation if the mobile phase or ratio of the mobile phase is changed. It was also found that R_f 0.02 present in track 1,2 and 4 i.e. root bark, stem bark, and fruit methanolic extracts after spectral detection from 200 to 800nm. Similar R_f denotes the presence of a similar type of component.

After visualization with 3 derivatization reagent vanillin-sulphuric acid, 1% aluminium chloride solution and 20% Na_2Co_3 followed by folin-ciocalteu reagent for detection of phenols; R_f 0.35,0.77 in root bark methanolic extract, 0.45, 0.77 in stem bark methanolic extract, 0.75 in leaves methanolic extract, 0.42, 0.76 in fruit methanolic extract at 254nm and R_f 0.33 in root bark methanolic extract, 0.44 in stem bark methanolic extract, 0.43, 0.71 in fruit methanolic extract at 366nm were found common which confirms the presence of the phenolics moieties in different parts i.e. root bark, stem bark, leaves, and fruit of *A.digitata*.

Conclusion

The results of this study show that, methanolic extract of *A.digitata* has antioxidant property hence can be used as an easily accessible source of natural antioxidants may be due to presence of phenolics type of phytoconstituents such as Chlorogenic acid, quercetin, ellagic acid, kaempferol, rutin, luteolin, apigenin, caffeine, and (−)epicatechin etc.

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