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Original Research Article

Fluorescence analysis, UV-Vis analysis and HPTLC study of *Adansonia digitata*

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ABSTRACT

Background: Medicinal plants are important part of Indian system of medicine. Medicinal plants are used for preparation of millions of preventive and curative medicines. *Adansonia digitata* is also one of such plants having great medicinal value.

Objective: *Adansonia digitata* is a well-known plant in the traditional medicinal system and it has been employed for the treatment of various diseases. So, the present study was aimed to explore the plant by means of fluorescence study, UV-VIS spectroscopic and HPTLC study.

Material andMethods: The present work focused on some of the standardizing parameters used to assess the quality parameters of polyherbal formulation. The root bark, stem bark, leaf and fruit of *Adansonia digitata* were analyzed for fluorescence activity, UV-VIS spectroscopy and HPTLC study respectively.

Observation andResults: The results of the study revealed multifaceted fluorescence character has been displayed by plant extract. The UV-VIS spectrum showed different peaks ranging from 200-800nmwith different absorption respectively. HPTLC study shows similar spectra were obtained at R_f0.02 in all extract,

at R_f 0.12 in root bark and stem bark extract, at R_f 0.37 in stem bark and fruit extract, at R_f0.54 in stem bark and leaf extract and at 0.72 R_f in stem bark and fruit extract was obtained.

Conclusion: The results confirm the fact that this plant posses important bioactive constituents useful for our health, so further scientific investigation is needed.

KEYWORDS

Adandonia, Chromatography Floroscence, Spectroscopy, UV.

INTRODUCTION

Medicinal plants and their formulations have been used since long for primary health careto relieve and cure various diseases. The knowledge about medicinal plants at an early age was documented systematically and organized scientifically in *Ayurvedic Samhitas*, *Nighantus* and other texts in which we can get so many references of medicinal plants. There is a promising future of medicinal plants as there are about half a million plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential. The different phytocontituents present in medicinal plants are alkaloid, glycoside, terpenoids, tannins, flavanoidsand amino acids etc. give specific properties to plants[1].A variety of techniques can be used to determine and estimate the presences of such phytocontituents in medicinal plants. Chromatography and spectroscopic techniques are the most helpful and popular tools used for this purpose.Chromatography is a useful procedure for resolving a multi-component mixture of trace, minor, or major constituents in its individual fractions. UV-VIS spectra arise from transition of electrons within a molecule or an ion from a lower to a higher electronic energy level[2-3]. These are simple, cost-effective and rapid tests for detecting phytocomponents.

Adansonia digitata L. is the most widespread tree species of the genus *Adansonia* and family *Bombacaceae*.It is a deciduous, massive and majestic tree grows up to 25m high, which may live for hundreds of years. The trunk is swollen and stout, up to 10m in diameter, usually tapering or cylindrical and abruptly bottle-shaped, often buttressed. The bark is smooth, reddish brown, soft and fibrous. Leaves are alternate and foliate. Leaves of young tree are often simple. Flowers are pendulous, solitary or paired in leaf axils, large and showy. Flower bud is globose or ovoid[4-7]. Adansonia has been used in traditional medicine since ancient times. The plant parts are used to treat various ailments such as tuberculosis, fever, microbial infections, diarrhea, anemia, dysentery, diarrhea etc[4]. Hence, in this study an attempt has been made to explore the plant by means of florescence analysis, UV-visible spectroscopy and HPTLC study.

Materials and Method:

Collection, Identification, and Authentification of plant:

Root bark, stem bark, and leaves of *Adansonia digitata* were collected and identified by the local taxonomist from its natural habitat Jamnagar, Gujarat during January 2018 while fruits from Bhavnagar, Gujarat during March 2018 with the help of the local taxonomist. The herbarium was submitted to the Pharmacognosy laboratory, I.P.G.T. & R.A., Gujarat Ayurved University and authenticated by the pharmacognosist of the institute, provided with herbarium reference number no. Ph.m:6162/18-19.

Preparation of Methanolic extract of samples: About 5g of the test drug powder (Root bark powder, Stem bark powder, leaves powder and fruit powder) was macerated with methanol (100ml) in a closed flask for 24 hours where initial shaking frequently during first 6hrs and kept it for 18 hrs. After 24 hours it was filtered. The filtrate was evaporated with the help of water bath and methanolic extract was collected in solid form[8].

UV Fluorescence analysis:This method is used as a diagnostic tool for the identification of authentic samples and detecting adulterants in unknown samples with the help of specific fluorescent colours[7]. Take about 0.5gm of plant powder into clean and dried test tubes. To each tube 5ml of different organic solvents like 1N NaOH,H_2SO_4, HNO_3, HCl, Acetone, Benzene, Chloroform, Methanol, etc. were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible daylight and UV light of short wavelength (254 nm) and UV light of long-wavelength (366 nm) for their characteristic fluorescent colour[9]. Fluorescence analysis reveals that an important phenomenon exhibited by various chemical constituents present in plant material; If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by reagents, hence some crude drugs are often assessed qualitatively[10].

UV-VIS spectroscopic evaluation:UV-VIS spectroscopic is simple, costeffective and rapid tests for detecting phytocomponents. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. The colour of the chemicals involved is directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum[11].UV-visible spectrophotometric analysis was conducted on the *Adansonia digitata* extract using a UV-visible spectrophotometer (Perkin Elmer) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200-800nm for proximate analysis. Firstly 1mg/ml solution of each extract was prepared. Then sample is diluted to 1:50 with the methanol.

High performance thin layer chromatography

HPTLC Aluminium pre-coated plate with Silica gel60 GF_{254} was used as stationary phaseand the mobile phase consisted of Toluene:Ethylacetate:Methanol: Formic acid (4:4:1.5:0.5) v/v. Methanolic extract of sample 1mg/ml solution was applied on the plate by mean of CamagLinomate V sample applicator fitted with a 100µl Hamilton syringe. The Twin Trough Glass Chamber was saturated with the mobile phase for 30 minutes. The TLC plate was developed to 9cm distance. The plate was removedfrom

the chamber and air-dried at room temperature. After development, densitometric scan was performed with a Camag TLC scanner III in reflectance in absorbance mode at 254 and 366 nm under control of Win CATS Software (V1.2.1.Camag). This plate was sprayed (derivatized) with anisaldehyde sulphuric acid reagent followed by heating at 110^0C for 10 minutes[12].

RESULTS:

Fluorescence analysis: Fluorescence is an important phenomenon displayed by various phyto-constituents present in plant materials. The results of the fluorescent analysis of root bark, stem bark, leaves and fruit powder of *Adansonia digitata* were depicted in table no. 1-4

Table No. 1- Florescence analysis of root bark powderof *Adansonia digitata*

S.No.	Chemical	Root Bark		
		Visible	**254**	**366**
1.	**Powder as such**	Light Brown	Brown	Light Brown
2.	**Powder + 1N NaOH (aq.)**	Brown	Dark Green	Light Brown
3.	**Powder + 1N NaOH (alc.)**	Yellowish-brown	Green	Light Green
4	**Powder +Conc.HCl**	Dark Brown	Dark Green	Dark Brown
5.	**Powder + Conc. H_2So_4**	Dark Brown	Yellowish Green	Yellowish
6.	**Powder +50% H_2So_4**	Light Brown	Green	Yellowish Green
7.	**Powder +Conc. HNO_3**	Yellowish Green	Greenish Yellow	Dark Brown
8.	**Powder + 50% HNO_3**	Yellow	Greenish Yellow	White
9.	**Powder + 40% NaOH + 10% Lead acetate**	Dull Brown	Light Green	Whitish Green
10.	**Powder + Acetic acid**	Yellow	Light Green	Yellow
11.	**Powder + $FeCl_3$**	Brown	Fluorescent Green	Reddish Brown
12.	**Powder + HNO_3 + NH_4OH**	Dark Green	Greenish Yellow	Greenish Yellow
13.	**Powder + NH_4OH**	Light Brown	Yellowish Green	Yellowish Green

14.	**Powder + Benzene**	Brown	Light Yellow	Light Yellow
15.	**Powder + Petroleum ether**	Light Brown	Light Yellow	Light Yellow
16.	**Powder + Acetone**	Light Brown	Light Yellow	Light Pink
17.	**Powder + Chloroform**	Light Brown	Light Yellow	Yellow
18.	**Powder + Methanol**	Light Brown	Light Yellow	Yellow

Table No. 2- Florescence analysis of stem bark powder of *Adansonia digitata*

S.No.	**Chemical**	**Stem Bark**		
		Visible	**254**	**366**
1.	**Powder as such**	Light Brown	Brown	Light Brown
2.	**Powder + 1N NaOH (aq.)**	Light Brown	Light Green	Light Brown
3.	**Powder + 1N NaOH (alc.)**	Whitish	Light Yellow	Light Green
4	**Powder +Conc.HCl**	Reddish Brown	Yellow	Dark Brown
5.	**Powder + Conc. H_2So_4**	Reddish Brown	Brown	Dark Brown
6.	**Powder +50% H_2So_4**	Light Brown	Yellow	Light Yellow
7.	**Powder +Conc. HNO_3**	Yellowish Green	Greenish Yellow	Dark Brown
8.	**Powder + 50% HNO_3**	Brown	Greenish Yellow	Dark Brown
9.	**Powder + 40% NaOH + 10% Lead acetate**	Dull Brown	Greenish Yellow	Yellow
10.	**Powder + Acetic acid**	Yellow	Light Green	Yellow
11.	**Powder + $FeCl_3$**	Brown	Light Green	Reddish Brown
12.	**Powder + HNO_3 + NH_4OH**	Brown	Fluorescent Green	Greenish Yellow
13.	**Powder + NH_4OH**	Yellowish Brown	Greenish Yellow	Yellowish Green
14.	**Powder + Benzene**	Light Brown	Yellowish Green	Light Yellow
15.	**Powder + Petroleum ether**	Light Brown	Light Yellow	Transparent
16.	**Powder + Acetone**	Light Brown	Light Yellow	Pinkish
17.	**Powder + Chloroform**	Light Brown	Light Yellow	Yellow
18.	**Powder + Methanol**	Light Brown	Light Yellow	Light Yellow

Table No. 3- Florescence analysis of leaf powder of *Adansonia digitata*

S.No.	Chemical	Leaf		
		Visible	**254**	**366**
1.	**Powder as such**	Dark Green	Greenish	Greenish
2.	**Powder + 1N NaOH (aq.)**	Light Brown	Light Green	Yellow
3.	**Powder + 1N NaOH (alc.)**	Greenish	Yellowish Green	Reddish Brown
4	**Powder +Conc.HCl**	Greenish	Fluorescent Green	Yellow
5.	**Powder + Conc. H_2So_4**	Yellowish Green	Greenish Yellow	Yellow
6.	**Powder +50% H_2So_4**	Greenish Yellow	Fluorescent Green	Dark Yellow
7.	**Powder +Conc. HNO_3**	Yellowish Green	Greenish Yellow	Dark Brown
8.	**Powder + 50% HNO_3**	Yellow	Greenish Yellow	Whitish Green
9.	**Powder + 40% NaOH + 10% Lead acetate**	Light Green	Greenish Yellow	Yellow
10.	**Powder + Acetic acid**	Dark Green	Greenish Yellow	Orange
11.	**Powder + $FeCl_3$**	Brown	Fluorescent Green	Reddish Brown
12.	**Powder + HNO_3 + NH_4OH**	Greenish	Greenish Yellow	Yellowish Green
13.	**Powder + NH_4OH**	Greenish	Greenish Yellow	Yellow
14.	**Powder + Benzene**	Greenish	Light Yellow	Orange
15.	**Powder + Petroleum ether**	Light Green	Light Yellow	Light Yellow
16.	**Powder + Acetone**	Greenish	Light Yellow	Pink
17.	**Powder + Chloroform**	Greenish	Light Yellow	Dark Yellow

18.	**Powder + Methanol**	Greenish	Yellowish Greenish	Orange

Table No. 4- Florescence analysis of fruit powder of *Adansonia digitata*

S.No.	**Chemical**	**Fruit**		
		Visible	**254**	**366**
1.	**Powder as such**	Light Brown	Brown	Brown
2.	**Powder + 1N NaOH (aq.)**	Yellow	Yellowish Green	Light Brown
3.	**Powder + 1N NaOH (alc.)**	Brown	Dark Green	Light Green
4	**Powder +Conc.HCl**	Reddish Brown	Yellowish Green	Dark Brown
5.	**Powder + Conc. H_2So_4**	Reddish Brown	Brown	Dark Brown
6.	**Powder +50% H_2So_4**	Dark Brown	Dark Brown	Light Green
7.	**Powder +Conc. HNO_3**	Yellowish Green	Greenish Yellow	Dark Brown
8.	**Powder + 50% HNO_3**	Brown	Greenish Yellow	Whitish
9.	**Powder + 40% NaOH + 10% Lead acetate**	Dark Brown	Greenish Yellow	Yellow
10.	**Powder + Acetic acid**	Brown	Light Greenish Yellow	Green
11.	**Powder + $FeCl_3$**	Brown	Fluorescent Green	Reddish Brown
12.	**Powder + HNO_3 + NH_4OH**	Brown	Greenish Yellow	Yellowish Green
13.	**Powder + NH_4OH**	Dark Brown	Yellowish Green	Yellow
14.	**Powder + Benzene**	Dark Brown	Light Yellow	Green
15.	**Powder + Petroleum ether**	Light Brown	Light Yellow	Yellowish Green
16.	**Powder + Acetone**	Light Brown	Light Yellow	Whitish
17.	**Powder + Chloroform**	Brown	Light Yellow	Green

18.	**Powder + Methanol**	Brown	Light Yellow	Whitish

UV-VIS spectroscopic evaluation:

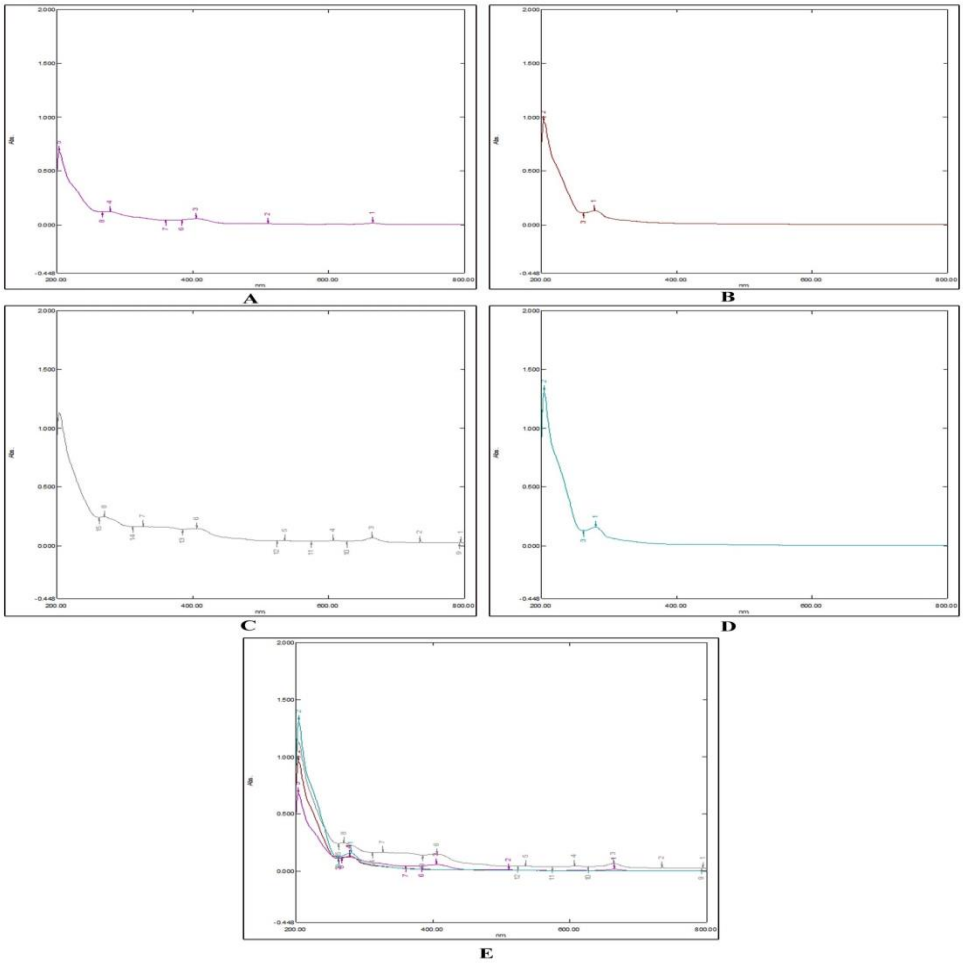

Figure no. 1: A) UV spectra of root bark extract; B) UV spectra of stem bark extract; C) UV spectra of leaf extract; D) UV spectra of fruit extract; E) Overlapping UV spectra of all extracts

The UV-VIS analysis preformed for identification of phytoconstituents present in methanolic extract of *Adansonia digitata*. Absorption bands observed pertaining to *Adansonia digitata* plant extract are displayed in table no 5 & figure no. 1.

Table No. 5- UV-VIS Spectrum Peak values of methanolic extract of *Adansonia digitata*

S.No	Name of sample	Wavelength
1.	**Root Bark Extract**	203.00, 278.50, 405.00, 510.00, 664.50
2.	**Stem Bark Extract**	203.00, 278.50

3.	**Leaf Extract**	269.50, 327.00, 405.50, 535.00, 607.00, 664.00, 734.50, 794.00
4.	**Fruit Extract**	203.50, 280.00

HPTLC Study:

HPTLC study of methanolic extract of *Adansonia. digitata* was carried out by using the solvent system of Toluene: Ethyl acetate: Methano: Formic acid (4:4:1.5:0.5) v/v was used. The respective R_f is shown in table no. 6 & Figure no. 2

Table No. 6- HPTLC study of Methanolic extracts of *Adansonia digitata* Linn.

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	3	0.03,0.13, 0.72,	4	0.03,0.12, 0.37,0.72	8	0.03,0.12, 0.18,0.25, 0.30,0.38, 0.55,0.73	6	0.03,0.20, 0.31,0.43, 0.54,0.74
366nm	2	0.03,0.72	4	0.03,0.54, 0.72,0.91	12	0.03,0.12, 0.18,0.26, 0.29,0.32, 0.37,0.45, 0.55, 0.73, 0.82,0.88	6	0.03,0.07, 0.20,0.31, 0.56,0.74,
After visualization	5	0.03,0.54, 0.76,0.90, 0.92	4	0.03,0.54, 0.76,0.90	5	0.03,0.54, 0.76,0.90, 0.92	1	0.03

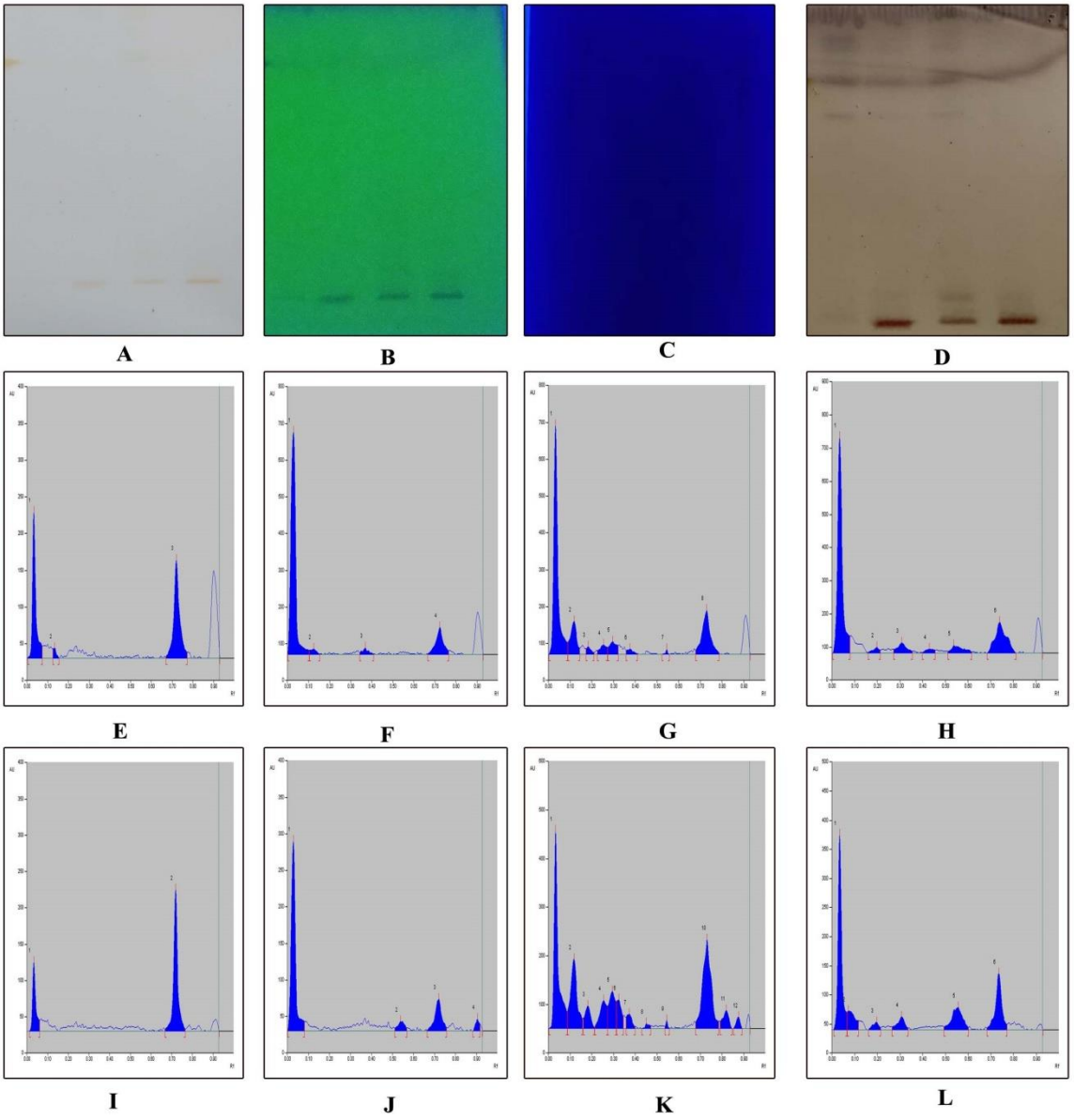

Figure no. 2: HPTLC Visualization A) Visual light; B) 254 nm; C) 366nm; D) After visualization with Anisaldehyde sulphuric acid; E) Densitogram of root bark extract at 254 nm; F) Densitogram of stem bark extract at 254 nm; G) Densitogram of leaf extract at 254 nm; H) Densitogram of fruit extract at 254 nm; I) Densitogram of root bark extract at 366 nm; J) Densitogram of stem bark extract at 366 nm; K) Densitogram of leaf extract at 366 nm; L) Densitogram of fruit extract at 366 nm

Root bark methanolic extract shows 3 spots in 254 and 2 in 366nm, stem bark methanolic extract shows 4 spots in 254 & 366nm, leaves methanolic extract shows 8 spots in 254 &12 in 366nm while fruit methanolic extract shows 6 spots in 254 nm & 366nm. Similar spectra was found common at 0.02 R_f in all the methanolic extracts, 0.12 R_f in root bark and stem bark extract, 0.37 & 0.72 R_f in stem bark and fruit extract, 0.54 in stem bark and leaf extract. After visualization root bark extract shows 5 spots, 4 spots in stem bark extract, 5 spots in leaf extract and one spot in fruit extract. Similar spectra at R_f0.02 in all extract, at R_f, 0.12 in root bark and

stem bark extract, at R_f0.37 in stem bark and fruit extract, at R_f0.54 in stem bark and leaf extract and at 0.72 R_f in stem bark and fruit extract was obtained.

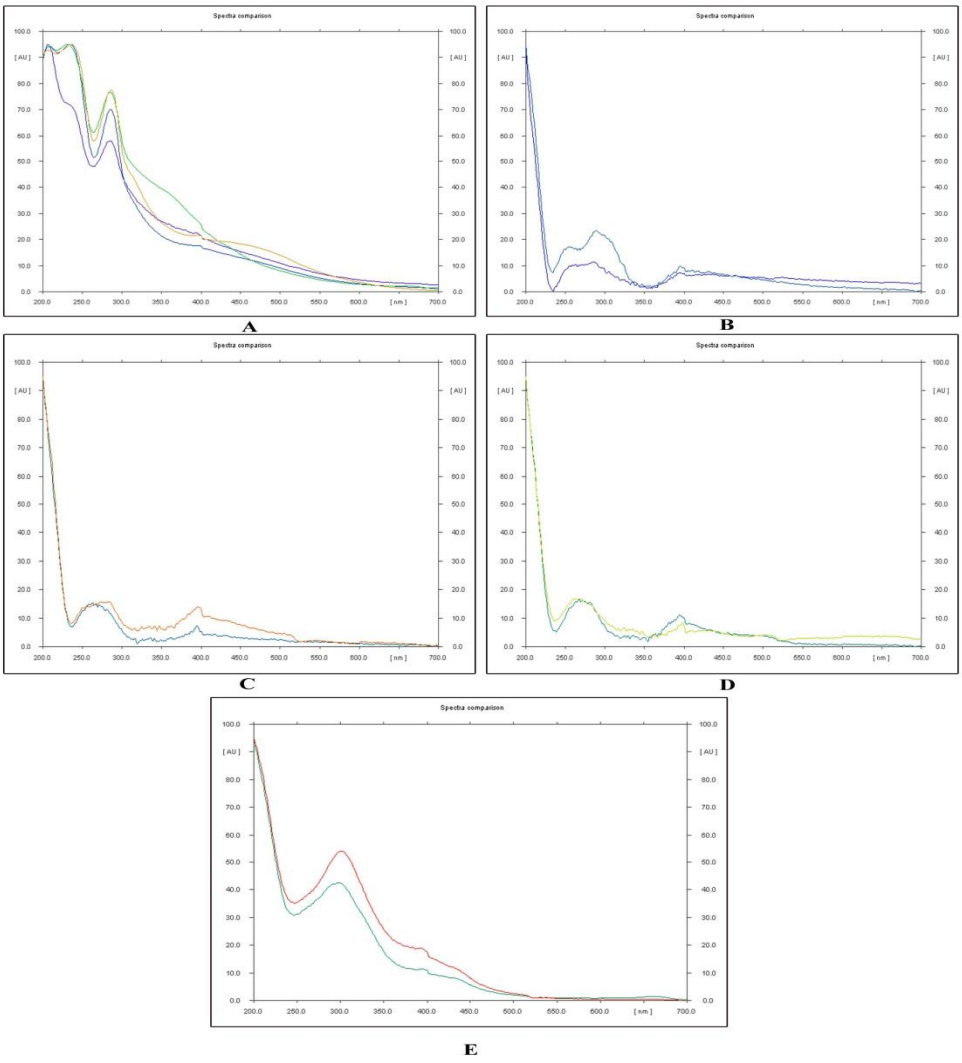

Figure no 3: A) Similar spectra at R_f0.02 in all extract; B)Similar spectra at R_f, 0.12 in root bark and stem bark extract; C)) Similar spectra at R_f0.37 in stem bark and fruit extract; D) Similar spectra at R_f0.54 in stem bark and leaf extract; E)) Similar spectra at 0.72 R_f in stem bark and fruit extract

DISCUSSION:

Fluorescence is an important phenomenon displayed by various phytoconstituents present in plant materials. Some show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products, which do not visibly fluoresce in daylight. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents and chemicals though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation. So, the herbal extract of the different parts of plant *Adansonia digitata* showed varied fluorescence character which is an essential parameter for standardization.

The herbal extract showed varied fluorescence character which is an essential parameter for standardization of herbs[13-14].

The qualitative UV-VIS profile of methanolic extract of *Adansonia digitata* was taken at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline. The root bark extract profile showed the peaks at 203, 278, 405, 510 and 664 nm, stem bark extract profile showed peaks at 203 and 278nm, leaves extract profile showed peaks at 269, 328, 405, 535, 607, 664, 734 and 794nm, fruit extract profile showed 203 and 280nm respectively. The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The absorbance in the range of 200-350 nm is associated with the n-π electronic transitions and usually comes from aromatic compounds and other chromophores, such as hydroxyl and carbonyl. The absorbance in the range of 350-500 nm are due to π-π electronic transitions[15]. All the samples except leaf extract has shown absorption at 203nm and 278 nm while at 405nm in root bark and leaf extact. This similarity may be due to the same composition of their chemical components.In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O[16]. This UV spectrum shows the presence of organic chromophoreswithin the *Adansonia digitata* extract. Nevertheless, the use of UV-visible spectrophotometery in the analysis of complex media is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system[17].

CONCLUSION

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. Characterization of secondary metabolite fingerprint by chromatography and spectroscopy provide valuable information about qualitative and quantitative information of plant. In the present study analysis of the methanolic plant extract of *Adansonia digitata* was done by using chromatographic and spectroscopic technique. Further research will be needed to find out the structural analysis of compound by use of different analytical methods such as FT-IR, NMR and mass spectrophotometer.

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