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PHARMACOGNOSTICAL, PHYTOCHEMICAL & PHARMACOLOGICAL EVALUATION OF BARK OF PLANT *MIMUSOPS ELNGI*: RESEARCH

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ABSTRACT

In Pharmacognostic study of bark of Mimusops elengi Linn. Macroscopic, microscopy, powder characteristic, physical parameters, and extractive values were studied. Microscopy of bark shows the presence of epidermis, covering trichomes, cholenchyma, and spongy parenchyma powder microscopy showed presence of starc grains, calcium oxalate crystals, and fibres. Bark powder was subjected to soxhlet extraction with organic solvents of increasing polarity like petroleum ether, chloroform, methanol and water. Preliminary phytochemical screening of various extracts of bark of Mimusops elengi Linn shows the presence of steroids, alkaloids, glycosides, flavonoids, tannins, saponins and phenolic content. The methanolic extract shows valuable amount of Total Phenolic and Total Flavonoid content. Two phytoconstituent (Comp-A) & (Comp-B) was isolated from ethyl acetate soluble fraction of methanolic extract by column chromatography Both isolated compound were evaluated by qualitative and an attempt was made to characterize the isolated compound by U.V, IR, 1 H NMR and MASS spectroscopic studies. Charecterization revealed that comp-A may be flavonoid and comp-B may be tannins. The assessment of antioxidant activity was done by DPPH assay, Nitric oxide scavenging activity, and Reducing power determination. The Methanolic & aqueous extract shows significant antioxidant activity as compare to petroleum ether and chloroform extract. Ascorbic acid was used as standard for comparing results.

Anthelmintic activity of Methanol, petroleum ether, chloroform aqueous extracts & ethyl acetate soluble fraction of methanolic extracts of bark of Mimusops elengi Linn were investigated against Pheretima posthuma at Various concentrations (10,25,5050 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of worms. Albendazole was included as standard reference and distilled water as control. The methanolic and ethyl acetate shows more potent activity.

KEYWORDS Mimusops elengi, epidermis, cholenchyma, scavering

INTRODUCTION

Food is consumed in combination, in relatively larged, unmeasured quantity under highly socialized condition. The view that food can have an expand role that goes well beyond providing a source of nutrient truly applied to traditional functional food. The systemic consumption of such traditional functional provides excellent preventive measures to ward of many diseases. At present recommendations are warranted to support the consumption of food rich in bioactive component such as herbs and species. There is tremendous interest and research in health promoting and protective properties of herbs and species. The real challenge lies not in providing whether the functional foods have health benefits, but in defining what these benefits are and developing the method to expose them by scientific means. Also, it lies in investigating the bioactive properties of this functional food beyond nutritional context, whether or not such properties are evident at level at which herbs and species are consumed. It is estimated that an adult in India consumes 80-200 mg per day of curcumin, the bioactive component of turmeric, and 50g of garlic in week [(1)].

Traditional medicine has been used for treatment of human illness since long time and is mainly based on component derived from natural products from herbs, plants, and animals. Medicinal natural products are very frequently used in sudan and also are widely consumed in Africa and all over the world. About 80% of the population in Africans country depend on traditional medicine for their primary health care. In Sudan 90% of populations depends mainly

on traditional medicine since admissions to hospitals and obtaining modern synthetic drugs are limited and high percentage of the population is nomads. Sustainability of the use of medicinal plant is important concern. The demand for medicinal plant is increasing in Africa as the population grows and pressure on medicinal plant resources will become greater than ever. Interest in plant derived medicine has also increased in the developed countries among the pharmaceutical companies. In contrast due to their minor side effect, the medicinal plants are widely used to treat many human diseases. The increasing cost of health care and the failure of allopathic medicine to treat some disease have also participated to the increasing consumption of traditional to fight disease [(2)].

MATERIALS AND METHODS

STANDARDISATION OF PLANT MATERIAL

Mimusops elengi. Linn (Sapotaceae) has taken for dissertation work and on the basis of literature survey; bark of plant has been selected

Collection:

The bark of *Mimusops elengi* was collected from local area of Dhule district, Maharashtra, India, in July 2012, cleaned and dried at room temperature in shade and away from direct sunlight. The dried aerial part was coarsely powdered in grinder. Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60-120 mesh to remove fine and the powder was subjected for further study.

Authentication:

The plant authenticated by Dr J- jayanthi scientist 'C' H.O.D Deputy Director Botanical Survey of India, Koregaon Road Pune, by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (Voucher specimen number ANSMIE2). (Annexure 1)

PHARMACOGNOSTIC STUDY

Macroscopic evaluation

Different parameters were studied in macroscopic evaluation of the bark of *Mimusops elengi,* which are color, odor, taste, size and shape**.**

Microscopic evaluation.

The part of plant selected in this study is confounding as bark. Therefore, microscopic study was carried out on fresh sample to ascertain its correct nature.

Chemicals and equipments.

The staining reagents such as Phloroglucinol, Conc. Hydrochloric acid and dilute iodine solution were used. Digital images captured using a Motic Digital microscope fitted with DCM(USB 2.0) resolution 350k pixels camera imaging accessory and using Motic analysis software.

Transverse section of the Bark.

The bark was thoroughly washed with water to remove the debris. Free hand sections were prepared from fresh plant material and finally stained with various staining reagents as per standard procedures.

Procedure:-

The sections were taken by placing the bark portion cut along with the midrib in between the two flat surfaces of pith. Pith is usually a piece of potato in which the longitudinal slit of 2 cm was deep is made, into which and sections were then placed and sections are taken. Transferred the sections into watch glass containing water, filtered and the sections were stained with Phloroglucinol and conc. hydrochloric acid(1:1); and then mounted in glycerin and observed under low power. Thin transverse section of middle part of fresh bark was taken, stained with Phloroglucinol conc. HCL (1:1), observed under 10X and 45X. The transverse sections were studied.

Microscopic Powder Characteristics.

The bark powder is boiled with chloral hydrate for 5-10 minutes, and then stained with phloroglucinol & conc. hydrochloric acid, dil. iodine, dil. sulphuric acid & dil. acetic acid. and observed for the microscopic features under high power.

EXTRACTION METHODOLOGY

The bark of *Mimusops elengi* was collected and dried in the shade and then pulverized in a grinder. The powdered bark was utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. A method described in Mukherjee (2002) was used for extraction of powdered plant

Technique. Hot soxhlet extraction.

Solvents: Solvent or extraction agents used in the preparation of phytopharmaceuticals must be suitable for dissolving the important therapeutic drug constituents and thus for separating them from the substance containing the drug which are to be extracted. The extraction was carried out by using methanol as a solvent and employing soxhlet extraction method. Using different solvents in increasing order of polarity, Petroleum ether (60-80^{0}C), Chloroform & methanol.

Procedure: The extraction was carried out in soxhlet extractor till all the constituents were extracted. The completion of extraction was indicated by taking sample out of siphon tube on TLC plate and placing it in iodine chamber. Absence of colored spot on plate indicates complete extraction. After completion of extraction, solvent was distilled off and concentrated extract was air-dried. The extract was stored in airtight container.

RESULT & DISCUSSION

Pharmacognostic studies:

In Pharmacognostic study of Bark of *Mimusops elengi* (linn) macroscopy, microscopy, powder characteristic, physical parameters, and extractive values were studied.

Macroscopy:

Figure No.5: Photographs of *Mimusops elengi* bark part

Morphology of bark of *Mimusops elengi*:

Morphological chararecters	**Observation**
Color	Glossy dark green
Odour	Not charecterstics
Taste	Astringent
Size	Varying in size
Shape	Oval or Elliptical
Apex	Monocronate
Venation	Dicotyledonous
Margine	Entire
Lamina	Thick
Petiole	Pulvinus

Table No 8:. Morphological characteristics of the Bark of *Mimusops eleng*

Microscopy:

Part . Microscopic Characters of bark of *Mimusops elengi.*

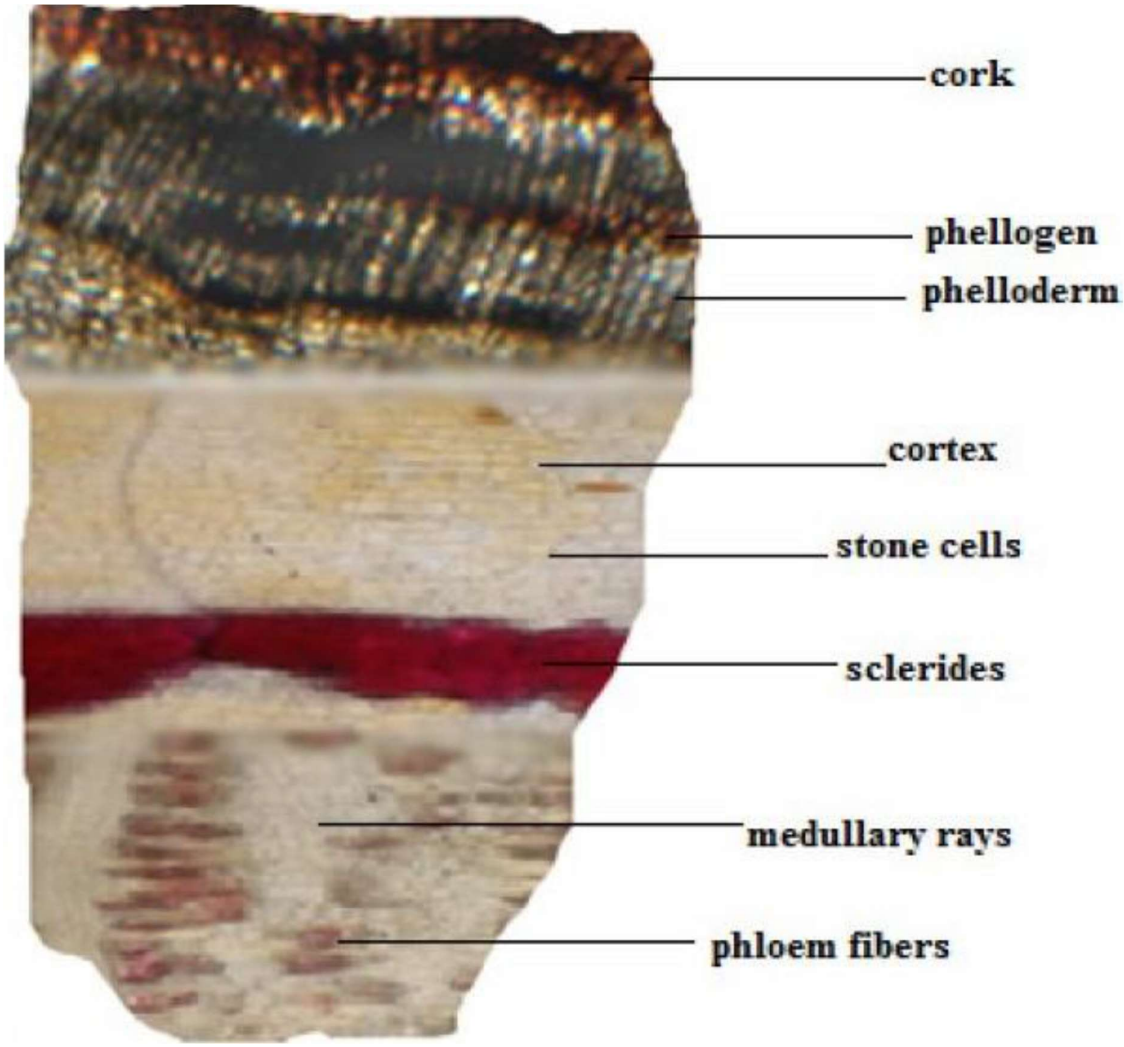


Figure No.6:. : T.S. Of bark of *Mimusops elengi* stained with phloroglucinol + conc. HCL

Part II :- Microscopic Powder Characteristics of bark of *Mimusops elengi.*

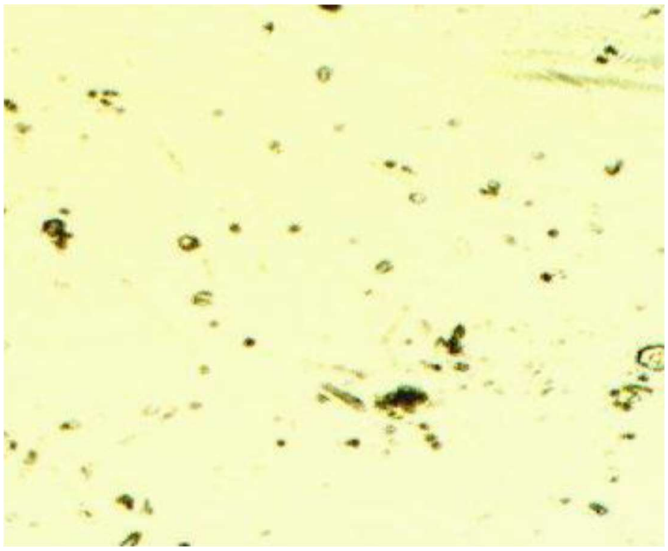


Figure No. 07 Starch grains stained with dil. iodine solution

Figure No.08 Fibre Stained with Phloroglucinol+conc HCL.(1:1)

Figure. No.9 calcium oxalate crystals stained with dil. Acetic acid.

Sr.No	Reagents	Observation	Inference
1.	Phloroglucinol + Conc HCL (1:1)	Pink	Presence of a) Epidermis. b)Covering trichome c) Vascular bundle.

Table No.9: Microscopic Characters of Bark of *Mimusops elengi.*

Sr.No	Test	Result
1.	Powder + Dilute iodine solution	Starch grains observed.
2.	Powder + Phloroglucinol + onc.HCL (1:1).	Lignified phloem fibres observed.
3.	Powder + Dilute H_2SO_4	Calcium oxalate crystals observed

Table No. 10: Microscopic Powder Characteristics of Bark of *Mimusops elengi.*

Determination of foreign organic matter:

Sr.No	Parameters	Values (%w/w)
1.	Foreign organic matter	0.15

Table No.11:- Result of foreign organic matter.

Determination of loss on drying:

Sr.No	Parameters	Values (%w/w)
01	Loss on drying	12.0%w/w

Table No.12:-Observation of Determination of loss on drying

Determination of Ash value:

Sr.No	Parameters	Values (% w/w)
1	Total ash	9.2
2	Water- soluble ash	4.5
3	Acid insoluble ash	2.3

Table No.13:- Ash value of powdered Bark of *Mimusops elengi.*

Determination of extractive value:

Sr.No	Extractive	Extractive value (%w/w
01	Alcohol soluble	25.4
02	Water soluble	23.0

Table No.14: – Extractive Values (%w/w) of the bark powder of *Mimusops elengi.*

Extraction

Sr.No	Extract	Color of extract	Yield (%)w/w
01	Petroleum ether	Dark green	2199
02	Chloroform	Dark green	2311
03	Methanol	Brown	10132
04	Aqueous	Yellowish green	14.45

Table No.15: -Yield of various extracts obtained from the bark of *Mimusops elengi* after extraction.

Preliminary Phytochemical Screening of extract:

Sr.No	Chemical test	Petrolium Ether Ext.	Chloroform Extract	Methanol Extract	Aqueous Extract
1.	**Test for Carbohydrates**				
	a) Molisch Test	-	+	+	+
	b) Fehilings Test	-	-	+	+
	c) Benedicts Test	-	-	+	+
	d) Barfoed's Test	-	-	+	+
2.	**Test for Proteins**				
	a) Biuret Test	-	-	-	-
	b) Millions Test	-	-	-	-
	c) Xanthoprotien Test	-	-	-	-
3.	**Test for Amino Acids**				
	a) Ninhydrin Test	-	-	+	+
4.	**Test for Steroids**				
	a) Salkowski Test	+	+	+	-

	b) Liebermann – Burc reaction	+	+	+	+
	c) Liebermann's reaction	+	+	+	+
5.	**Test for Glycosides**				
	a) Deoxysugares (Killer-K aniTest)	+	-	+	+
	b) Legal's Test	+	-	+	+
	c) Brontrager's Test	-	-	-	-
	d) Modified Brontrager's.	-	-	-	-
6.	**Test for Alkaloids**				
	a) Drogendroff's Test	-	-	+	+
	b) Mayers Test	-	-	+	+
	c) Hagers Test	-	-	+	+
	d) Wagners Test	-	-	+	+
7.	**Test for Flavonoids**				
	a) Lead Acetate	-	+	++	+
	b) Sodium Hydroxide	-	-	++	++
	c) Ferric Chloride Test	-	-	++	++
8.	**Test for Tannins**				
	a) 5% Ferric Chloride Test	-	+	++	++
	b) Lead Acetate Test	-	-	++	+
	c) Dilute Iodine Test.	-	+	+ +	+ +
	d) Dilute Nitric acid Test.	-	-	++	-
	e) Potassium Permanga Solution.	-	-	++	++
9.	**Test for Triterpenoids**				
	a) LibermannBurcha reaction	-	-	-	+
10.	**Test for saponins**				
	a) Foam test	-	-	+	+

Table No 16: Observation Table For Chemical Test

(-) Absent

(+) Less color intensity

(++) More color intensity

Result:- From preliminary phytochemical screening, it was found that petroleum ether extract contains steroids & glycosides; the chloroform extract contains steroids, tannins, flavonoids.

The methanolic extract contains steroids, flavonoids, alkaloids, tannins & phenolic compounds, and the aqueous extract contains Alkaloids, flavonoids, tannins, saponins & phenolic compounds.

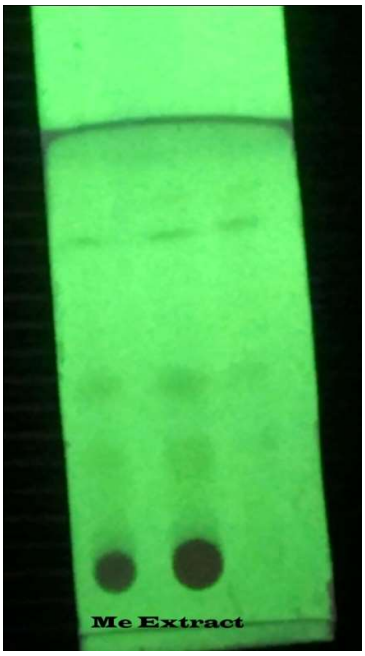
CHROMATOGRAPHIC STUDIES:

Thin layer chromatography.

Evaluation of Methanolic extract and Solvent Ether soluble fraction of methanolic extract by TLC.

Stationary phase **:** Silica gel g.
Mobile phase **:** Toluene: Ethyl acetate: Formic acid
Proportion **:** 5 : 4 : 1
Detection **:** Under U.V at 254nm, Ferric chloride reagents

Fig no 10: TLC of Methanolic extract &Solvent Ether soluble fraction of methanolic extract.

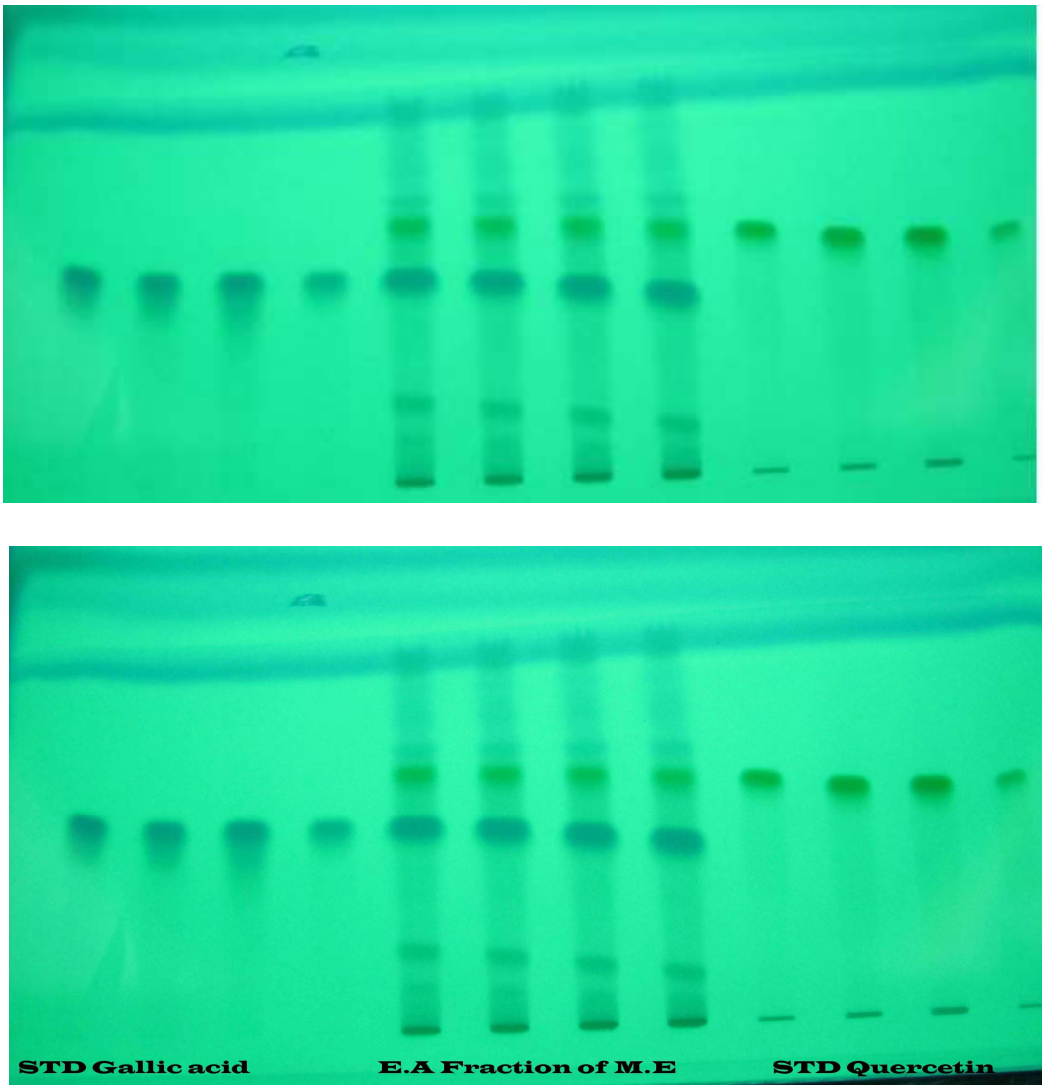


TLC of Methanolic extract visualized
Under U.V at 254 nm.

High performance thin layer chromatography(HPTLC)

Stationary phase **:** Silica gel g.
Mobile phase **:** Toluene: Ethyl acetate: Formic acid
Proportion **:** 5: 4: 1
Detection **:** Under U.V cabinet at 254 nm.
Solvent front **:** 10cm
No of tracks **:** 12

HPTLC plate of E.A fraction of Methanolic extract compared with standard gallic acid and quercetin.



Solvent ether

Figure No.11 HPTLC Plate on U.V at 254 nm.

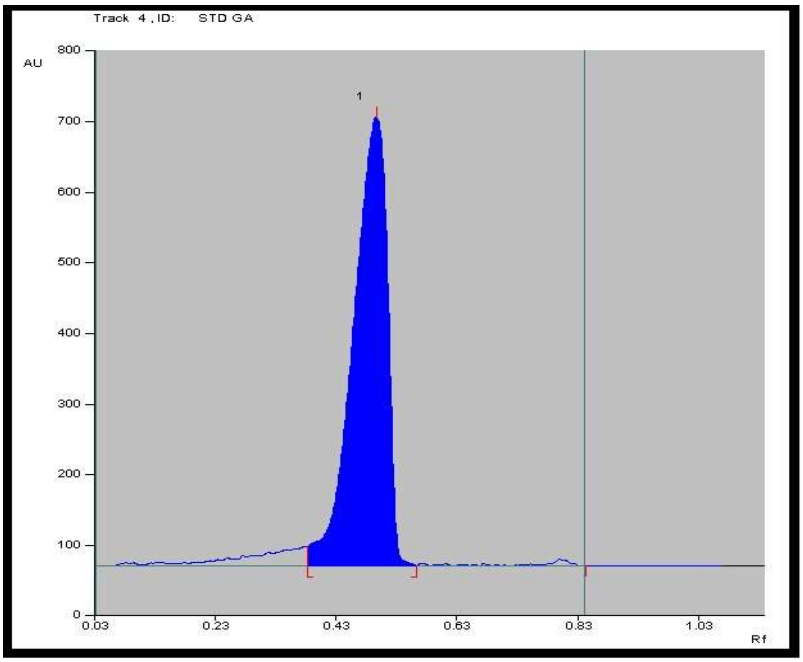


Figure No 12 HPTLC Chromatogram of Gallic acid

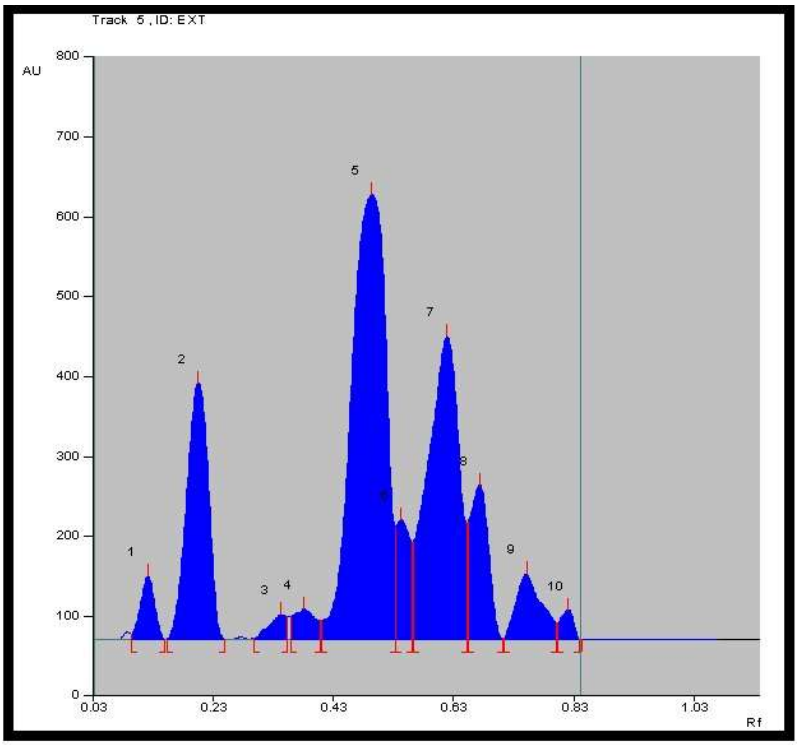


Figure No.13 HPTLC Chromatogram for Extract

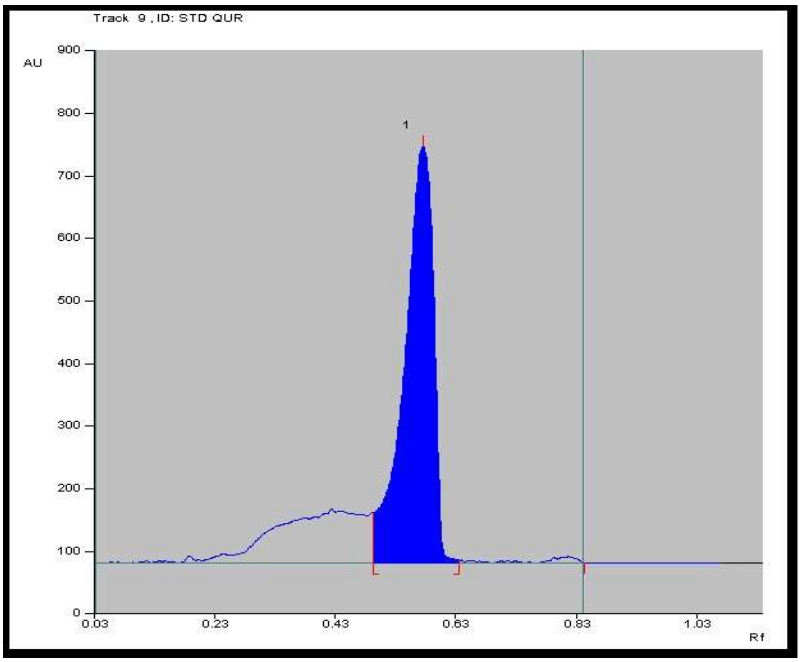


Figure No.14 HPTLC Chromatogram of Std Quercetin

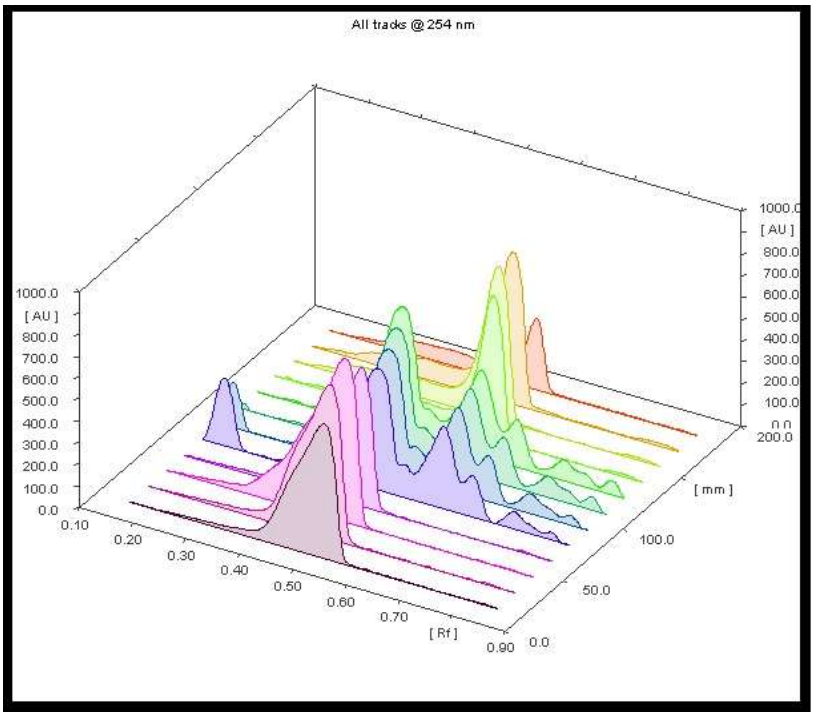


Figure No.15. 3D image of all tracks of, std gallic acid, extract & quercetin at 254 nm.

Seperation of phytoconstituent from ethyl acetate soluble fraction of methanolic extract by column chromatography:

After column chromatography all isolated fraction are evaluated by Thin layer chromatography. All fraction showed two spots on TLC plates.

Data for column eluents of ethyl acetate fraction.

Fractions	No of spots	Color	R_f Values
1-3	No spot	-	-
4-6	Two spots	Blue & yellowish green	0.50 & 0.62
7 – 9	Two spots	Blue & yellowish green	0.52 & 0.63
10 – 15	Two spots	Blue & yellowish green	0.49 & 0.61
16 – 18	Two spots	Blue & yellowish green	0.50 & 0.61
19- 55	No spots	-	-

Table No.20: Details of the fraction eluted by column chromatography.

TLC of isolated fraction.

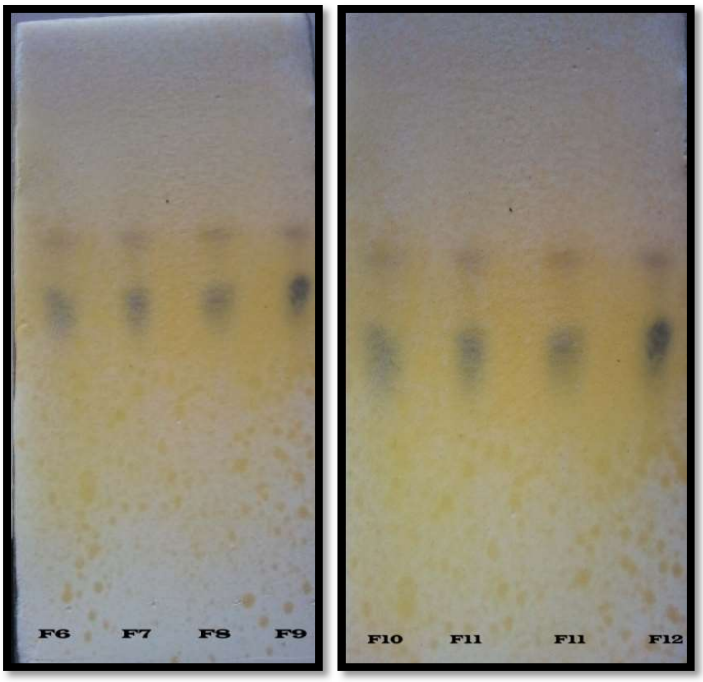


Figure No.16 TLC Of isolated fraction by column chromatography.

Recolumn: All fraction showed two spots on TLC plates hence all fraction are collected and prepared for recolumn.

TLC evaluation of isolated fraction after recolumn:

Stationary phase **:** Silica gel g.
Mobile phase **:** Toluene: Ethyl acetate: Formic acid
Proportion **:** 5: 4: 1
Detection **:** U.V - 254

Data for column eluents of Solvent ether fraction.:

Fractions	No of spots	Colors	R_f Values
1-4	No spots	-	-
6-12	1 spots	Yellowish green	0.61
13-18	2 spots	Yellowish green	0.61

Table No. 21: Details of fraction eluted by recolumn.

Chemical Test of Isolated compound:-

Chemical test	Observation	Inference
Sodium Hydroxide	Decoloration	Flavonoids may be present

Table No.22: Chemical examination of isolated compound – A

Figure No.17 TLC of isolated fraction after recolumn

Yield of isolated compound:-

Isolated compound	Yield from column
Compound-A	60mg

Table No.23: Yield of isolated compound after recolumn.

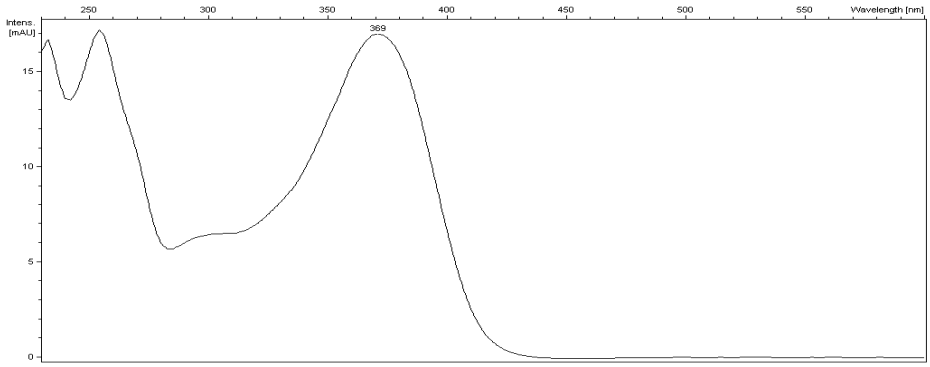
Parameters of isolated compound:-

Parameters	Compound
Physical state	Solid crystalline
Color	Yellowish green
Solubility	Methanol
Melting point	215-218°C

Table No.24: Physical parameters of isolated compound

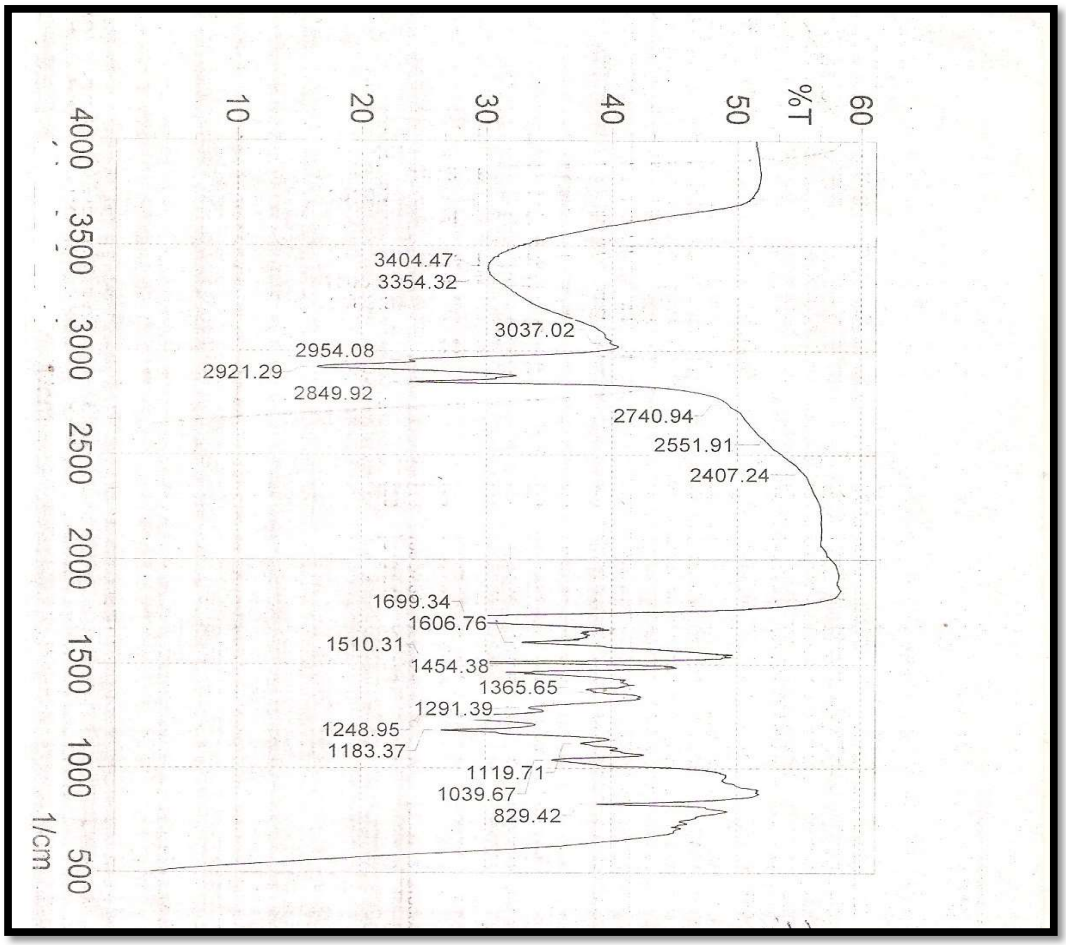
Spectral Analysis:

U.V Spectra:



Graph No. 1. U.V spectra of isolated compound

FT-IR:



Graph No.2: FT-IR Spectra of isolated compound

Charecterization of isolated compound:-

Spectra	Charecters
U.V	Two peak with λ max at 255 & 369nm
FT-IR	Peaks at following wave number are observed Wave number(cm^{-1}) 3037.02 C-H Stretching 1699.34 C=O Stretching 3354.32 O-H Stretching 1606.76 C=C Stretching 1291.39 O-H bending 1119.71 C-O-C Stretching

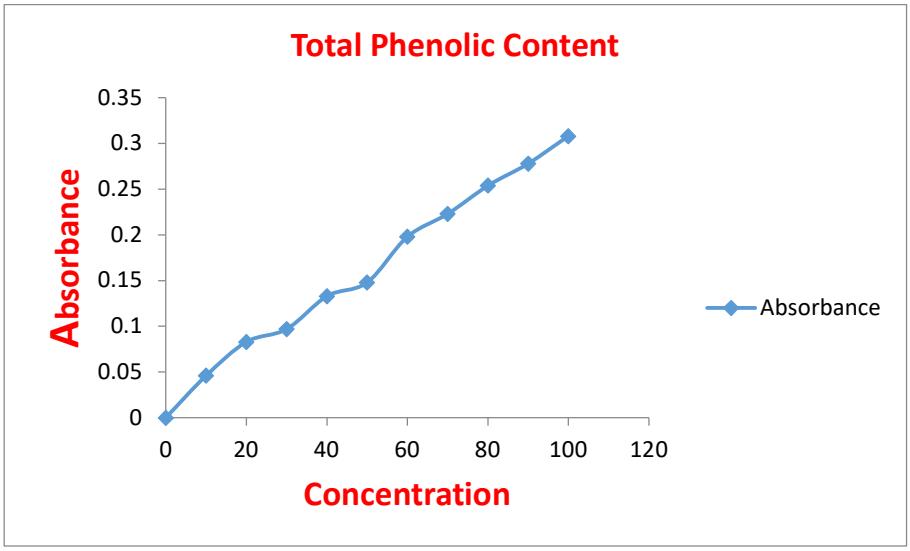
Table No.25: Characterization of Isolated compounds – A

Quantitative estimation of phytoconstituent

Total Phenolic Content:

Sr.no	Concentration	Absorbance
1	10	0.046
2	20	0.083
3	30	0.097
4	40	0.133
5	50	0.148
6	60	0.198
7	70	0.223
8	80	0.254
9	90	0.278
10	100	0.308

Table.No.26: Absorbance of Std Gallic acid at different concentration.



Graph No.3: Concentration response curve for gallic acid at different concentration

Sr.no	Sample	Absorbance	Concentration ug/ml
1	Methanolic extract	0.218	72.66
2	Ethyl aetate extract	0.212	70.66
3	Aqueous extract	0.133	44.33

Table No.27: Result of Total phenolic content of extract

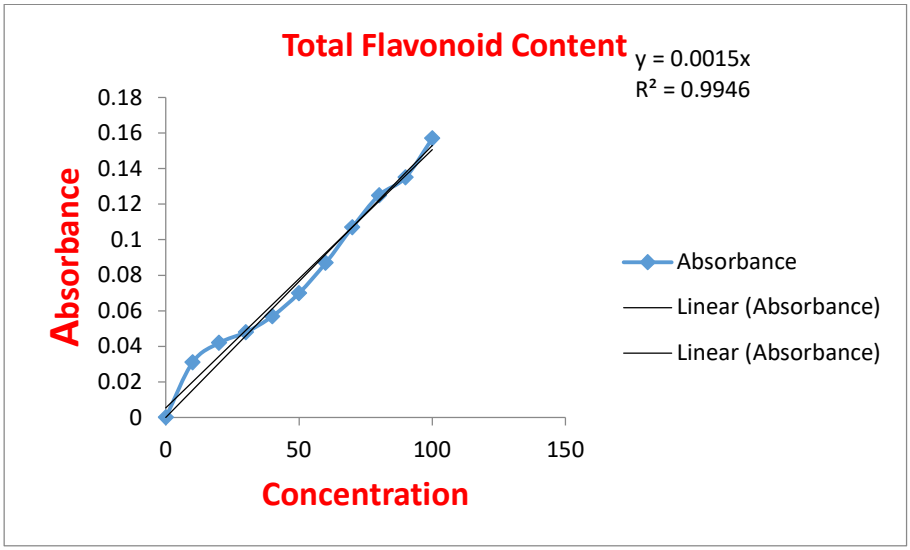
Result

Equation Y=0.003x was obtained from graph From this equation concentration of extract was determine. The Total phenolic content in Methanolic, Ethyl acetate and Aqueous extract of plant *Mimusops elengi was* found to be 72.66, 70.66, &44.33ug/ml respectively.

Total Flavonoid content:

Sr.no	Concentration	Absorbance
1	10	0.031
2	20	0.042
3	30	0.048
4	40	0.057
5	50	0.070
6	60	0.087
7	70	0.107
8	80	0.125
9	90	0.135
10	100	0.157

Table No.28: Absorbance of std quercetin at different concentration.



Graph No4. Concentration response curve for Quercetin at different concentration

Sr.no	Sample	Absorbance	Concentration ug/ml
1	Methanolic exttrac	0.037	24.66
2	Solvent ether extract	0.035	23.33
3	Aqueous extract	0.014	09.33

Table No.29: Result of total flavonoid content.

Result

Equation Y=0.0015x was obtained from graph. From this equation concentration of extract was determine. The Total flavonoid content in Methanolic, Solvent ether and aqueous extract of plant *Mimusops elengi* was found to be 24.66, 23.33&09.33ug/ml respectively.

In-Vitro Antioxidant Activity

Nitric oxide radical scavenging activity

Sr. No.	Conce. (µg/ml)	Methanolic Extract (nm)	Aqueous Extract (nm)	Ascorbic Acid (nm)	Solvent ether Soluble fraction (nm)	Ethyl acetate soluble fraction (nm)
1	25	0.095± 0.0008*	0.105 ± 0.0008*	0.081 ± 0.0012*	0.0101 ± 0.0008*	0.122 ± 0.0008*
2	50	0.080 ± 0.0006*	0.096 ± 0.0015*	0.066 ± 0.0008*	0.088 ± 0.0011*	0.106 ± 0.0012*
3	75	0.068± 0.0005*	0.081 ± 0.0003*	0.057 ± 0.0008*	0.076 ± 0.0008*	0.101 ± 0.0011*
4	100	0.055 ± 0.0003*	0.074 ± 0.0012*	0.046 ± 0.0008*	0.068 ± 0.0014*	0.090 ± 0.0008*
5	125	0.042 ± 0.0008*	0.062 ± 0.0012*	0.039 ± 0.0008*	0.057 ± 0.0014*	0.080 ± 0.0012*
6	150	0.031 ± 0.0003*	0.045 ± 0.0012*	0.024 ± 0.0008*	0.048 ± 0.0005*	0.071 ± 0.0014*
7	175	0.022 ± 0.0008*	0.034 ± 0.0017*	0.013 ± 0.0015*	0.041 ± 0.0012*	0.066 ± 0.0001*

8	200	0.014 ± 0.0008*	0.029 ± 0.0008*	0.006 ± 0.0003*	0.032± 0.0008*	0.063 ± 0.0003*

(* Data and results are expressed as mean ± SEM and mean is representation of three experiments.)

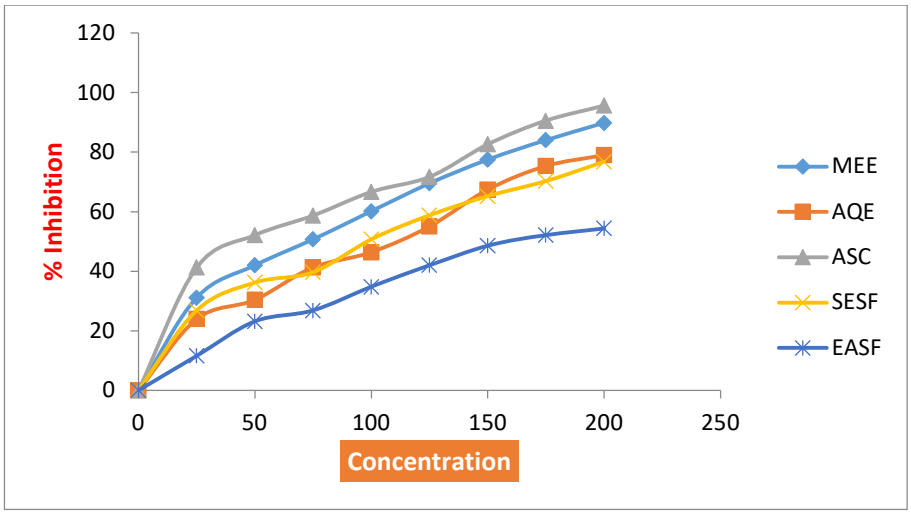
Table No.30:- Nitric oxide radical scavenging activity of various extract of plant *Mimusops elengi* with **standard ascorbic acid (Absorbance)**

Sr. no.	Conce. (µg/ml)	Methanolic Extract (% inhibition)	Aqueous Extract (% inhibition)	Ascorbic acid (% inhibition)	Solvent ether Soluble fraction (% inhibition)	Ethyl acetate soluble fraction (% inhibition)
1	25	31.16 ± 0.62*	23.91± 0.62*	41.30 ±0.85*	26.81 ± 0.62*	11.59 ± 0.62*
2	50	42.02 ± 0.47*	30.43 ± 1.08*	52.17 ±2.25*	36.23 ± 0.85*	23.18 ± 0.81*
3	75	50.72 ± 0.62*	41.30 ± 0.23*	58.69 ±0.62*	39.70± 0.81*	26.81 ± 0.62*
4	100	60.14± 0.23*	46.37 ± 0.85*	66.66± 0.62*	50.72 ± 0.61*	34.78 ± 1.02*
5	125	69.56 ± 0.62*	55.07 ± 0.85*	71.73 ± 0.63*	58.79 ± 0.94*	42.02 ± 1.02*
6	150	77.53 ± 0.23*	67.39 ± 0.85*	82.60 ± 0.62*	65.21 ± 1.03*	48.55 ± 0.40*
7	175	84.05 ± 0.62*	75.36 ± 1.24*	90.57 ± 0.63*	70.28 ± 0.71*	52.17 ± 0.85*

8	200	89.85 ± 0.62*	78.98 ± 0.62*	95.65 ± 0.24*	76.81± 0.40*	54.34 ± 0.62*

(*Data and results are expressed as mean ± SEM and mean is representation of three experiments.)

Table No.31:- Nitric oxide radical scavenging activity of various extract of plant *Mimusops elengi* with **standard ascorbic acid (Percentage inhibition)**



Graph No.5: Nitric oxide radical scavenging activity of various extracts of bark of *Mimusops elengi* with **standard Ascorbic acid**

IC 50 of Nitric oxide scavenging activity:-

Sr. No	Extract	IC_{50}
1	Methanolic	97.08 µg/ml
2	Aqueous	116.00 µg/ml
3	Solvent ether Soluble fraction	115.47 µg/ml
4	Ethyl acetate soluble fraction	161.81 µg/ml
5	Ascorbic Acid	89.76 µg/ml

(Calculated by regression equation)

Table No.32: IC_{50} result Nitric oxide radical scavenging activity of various extracts of bark of *Mimusops elengi* with **standard Ascorbic acid.**

Result: In Nitric oxide radical scavenging activity model, it is observed that methanolic and aqueous extract of **bark of** *Mimusops elengi* have demonstrated dose dependent increase in the nitric oxide anion scavenging property. The methanolic and aqueous extract shows 89.85% and 78.98 % inhibition at 200µg/ml respectively and ascorbic acid has shown 95.65 % inhibition at 200µg/ml. In this case methanolic extract shows patent antioxidant activity than aqueous, solvent ether soluble fraction, Ethyl acetate soluble fraction with reference to standard ascorbic acid. The results are shown in table no and. The IC_{50} value for extract and standard ascorbic acid shown in table no 32.

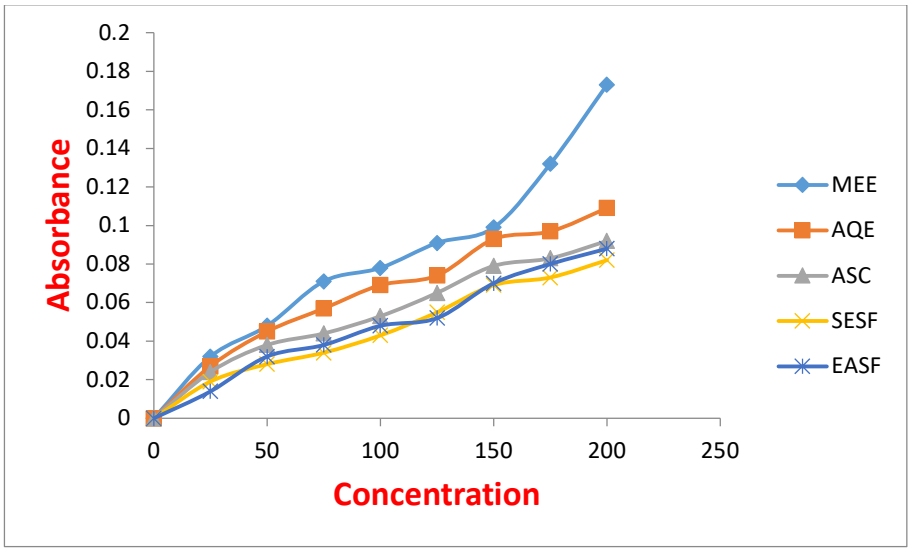
Reducing power determination:

Absorbance of standard Ascorbic acid at different concentration:-

Sr.no	Conc. (µg/ml)	Absorbance (nm)				
		Ascorbic acid	**Methanolic extract**	**Aqueous extract**	Solvent ether Soluble fraction	Ethyl acetate soluble fraction
1	25	0.032 ± 0.0008*	0.027 ± 0.0847*	0.024 ± 0.0010*	0.019 ± 0.0005*	0.014 ± 0.0006*
2	50	0.048 ± 0.0005*	0.045 ± 0.0008*	0.038 ± 0.0011*	0.028 ± 0.0008*	0.032 ± 0.0007*
3	75	0.071 ± 0.0005*	0.057 ± 0.0006*	0.044 ± 0.0003*	0.034 ± 0.0004*	0.038 ± 0.0002*
4	100	0.078 ± 0.0008*	0.069 ± 0.0003*	0.053 ± 0.0003*	0.043 ± 0.0004*	0.048 ± 0.0002*
5	125	0.091 ± 0.0006*	0.074 ± 0.0003*	0.065 ± 0.0008*	0.055 ± 0.0006*	0.052 ± 0.0007*
6	150	0.099 ± 0.0008*	0.093 ± 0.0006*	0.079 ± 0.0003*	0.069 ± 0.0005*	0.070 ± 0.0004*
7	175	0.132 ± 0.0008*	0.097 ± 0.0003*	0.083 ± 0.0008*	0.073 ± 0.0005*	0.080 ± 0.0006*
8	200	0.173 ± 0.0011*	0.109 ± 0.0008*	0.092 ± 0.0011*	0.082 ± 0.0010*	0.088 ± 0.0008*

(*Data and results are expressed as mean ± SEM and mean is representation of three experiments.)

Table No.33: Observation of reducing power determination of Ascorbic acid, Methanolic, Aqueous Solvent ether soluble fraction **and** Ethyl acetate soluble fraction **extract of bark of** *Mimusops elengi*



Graph No.6: Concentration response curve of Reducing Power determination for Ascorbic acid, Methanolic, Aqueous Solvent ether soluble fraction **and** Ethyl acetate soluble fraction **extract of bark of** *Mimusops elengi*

Result: -

It is observed that the **Methanolic, Aqueous** Solvent ether soluble fraction **and** Ethyl acetate soluble fraction of plant *Mimusops elengi* demonstrated dose dependant increase in the reducing property. To find the active species which is capable of donating hydrogen and subsequently its leads to the reducing power activity was determine. The high reducing power is indicative of the hydrogen donating ability of the active species present in extract. The reducing power assay of various extract of plant *Mimusops elengi* was estimated by using potassium ferricyanide reduction method. In the present study the reducing power of the methanolic extract of plant *Mimusops elengi* was found to be excellent and steadily increase in direct proportional to the increasing concentration extract as compare to other extract in comparison with standard ascorbic acid. The reducing power of standard ascorbic acid, **Methanolic, Aqueous** Solvent ether soluble fraction **and** Ethyl acetate soluble fraction at concentration 200µg/ml was found to be 0.173, 0.109, 0.092, 0.082, 0.088 and 0.171 respectively. Table no. and graph no.

Result of Anthelmintic activity:

Anthelmintic activity of extracts of bark of *Mimusops elengi.*

Test Substance	**Concentration (mg/ml)**	**Time taken by *Pheretima posthuma* for Paralysis (P) and death (D) of worms in min**	
		P	**D**
ME	10	56.08 ± 3.50	64.07 ± 3.79

	25	25.77 ± 2.30	50.06 ± 3.80
	50	17.07 ± 1.89	38.40 ± 2.89
CHE	10	62.26 ± 3.49	88.50 ± 3.94
	25	46.05 ± 2.99	68.12 ± 3.43
	50	33.06 ± 2.52	54.09 ± 2.99
PE	10	68.18 ± 3.63	104.37 ±4.13
	25	57.43 ± 3.16	85.20 ± 3.57
	50	35.29 ± 2.68	69.37 ± 3.13
AQE	10	55.04 ± 1.76	77.40 ± 3.0
	25	39.13 ± 2.30	64.15 ± 2.80
	50	26.22 ± 3.10	51.25 ± 3.76
SESF	10	46.18 ± 3.13	67.53 ± 4.0
	25	29.24 ± 2.44	51.45 ± 3.43
	50	19.23 ± 2.0	39.22 ± 3.03
EASF	10	59.18 ± 3.13	73.53 ± 4.0
	25	31.24 ± 2.44	55.45 ± 3.43
	50	22.23 ± 2.0	42.22 ± 3.03
ALB	20	12.06 ± 1.67	32.36 ± 2.56
Control	-	-	-

Table No. 34: Effect of extract of various concentration of methanolic, aqueous, petroleum ether, chloroform, solvent ether and ethyl acetate extracts of *Mimusops elengi* bark on paralysis and death time in min. of *Pheretima posthuma* earthworm for studying in vitro anthelmintic activity.

Graph No: Time of paralysis and Death of all extract and std drug.

Values are expressed as MEAN ± SEM, one way ANNOVA followed by Dunnet's test.

Note:- n=5 in each group. *P<0.05, **P<0.01, ***P<0.001

Where, ME: Methanolic extract

Aqs: Aqueous extract

Pet: Petroleum ether extract

Chl: Chloroform extract

E.A: Ethyl acetate soluble fraction of methanolic extract

STD: Standard Albendazole.

Result: - In vitro Anthelmintic activity of pet. ether, chloroform, methanolic, solvent ether, ethyl acetate soluble part of methanolic extract and aqueous extract of bark of the plant *Mimusops elengi* was carried out and found that the methanolic extract take less time for paralysis and death of the *Pheretima posthuma* earthworm it indicate that methanolic extract has potent anthelmintic activity. In case of solvent ether soluble fraction of methanolic extract shows the time of paralysis and death as near by methanolic extract, but standard albendazole take less time when compared with all extract.

DISCUSSION

The pharmacognostic, phytochemical and antioxidant, antimicrobial, anthelmintic potential of bark of *Mimusops elengi* was evaluated.

In pharmacognostic study, the morphology of bark of plant were shows presence of color-glossy dark green, odour-not charecterstics, Taste-astringent, Size-varying in size, Shape-Oval or elliptical, Apex-monocronate, Venation-dicotyledonous, Margine-entire, Lamina-thick, Petiole-pulvinus. In microscopy of bark were studies & shows presence of epidermis, vascular bundles, covering trichomes, collenchymas & parenchyma etc. In microscopic of powder characteristics were shows presence of starch grains when stained with dilute iodine solution, calcium oxalate crystals when stained with dilute acetic acid and dilute sulphuric acid, Fibres when stained with phloroglucinol & conc HCL (1:1).

The physicochemical properties of powder of bark were examined like as foreign organic matter, total ash, water soluble ash, acid insoluble ash, extractive values (water soluble & alcohol soluble extractives), loss on drying etc.

Determination of bark constant of *Mimusops elengi* such as vein islet number, vein termination number, stomatal number and stomatal index were examined .

Proximate values for the bark of *Mimusops elengi L* are as follows. Foreign organic matter (0.1%), Total ash value (9.05%), acid insoluble ash value (2.0%), water soluble ash value (4.0%), alcohol soluble extractives (26.4%), water soluble extractives (24.0%), and, loss on drying (11.0%). These values are criterion to put the guidelines of identity or purity of drugs.

Preliminary phytochemical investigation of pet.ether, chloroform, methanol and aqueous extract were revealed that presence of tannins, flavonoids, alkaloids, steroids, saponins, triterpinoids, glycosides, where in, the steroids, glycosides may present in petroleum ether extract, the Methanolic extract may contains steroids, flavonoids, alkaloids, tannins & phenolic compounds, the Aqueous extract may contains Alkaloids, flavonoids, tannins, saponins & phenolic compounds and the chloroform extract contains steroids, tannins, flavonoids.

In case of phytochemical investigation the methanolic extract were introduced for chromatography separation by using TLC, HPTLC & column chromatography for separation of important phytochemicals present in methanolic extract which shows potent

The isolated compound A revealed following analytical data.

U.V Spectra: Two peak with λ max 255 & 369nm

IR Spectra: wave number. At 3037.02 for C-H Stretching, 1699.34 for C=O Stretching, 3354.32 for O-H Stretching, 1606.76 for C=C Stretching, 1291.39 for O-H bending, 1119.71 for C-O-C Stretching.

^{1}H NMR Spectra:7.73 for (1H, H-6, S), 4.90 for 1H, H-3, H-4, S), 6.87 for (1H, H-7, S), 6.394 for (1H, H-7, S). 6.186 for (1H, H-5, S), 7.613-7.737 for for (multiplets, all remaining protons of aromatic rings).

MS Spectra : base peak at 303.02.

The isolated compound B revealed following analytical data.

U.V spectra: two peak with λ max 220,& 270nm.

IR Spectra: wave number at 3160.47 for C-H stretching, 1612.54 for C=O stretching, 3420.87 for O-H stretching, 1308.75 for C=C stretching, 1171.79 for O-H bending.

^{1}H NMR: Delta value 8.275 for (1H, H-7, S), 7.60-7.715 for (multiplets, all remaining protons of aromatic rings), 4.93 for (1H, H-3, H-4, H-5, S).

MS Spectra: base peak at 164.12

7.779 µg/ml. The IC_{50} value of methanolic extract was 65.0 µg/ml when compared to their corresponding aqueous petroleum ether and, chloroform extract with IC_{50} value of 92.0, 178.61, and 144.31 µg/ml respectively.

50mg/ml concentration. The methanolic extract caused paralysis at 18.07±1.89 min. and time of death at 34.40±2.89 min. while aqueous extract revealed paralysis at 27.22±3.10 and time of death 50.25±3.76 min. The ethyl acetate soluble fraction revealed paralysis at 20.23±2.0 and time of death at 40.22±3.03, the chloroform extract revealed paralysis at 32.06±2.52 and time of death at 55.09±2.99 and the petroleum ether extract revealed paralysis at 36.29.23±2.68 and time of death at 68.37±3.13 respectively against the earthworm *Pheretima posthuma*. The standard drug Albendazole showed paralysis at 14.06±1.67 min. and the time of death at 33.36±2.56 minutes.

CONCLUSION

It can be concluded that the complete and accurate physicochemical values of the present study will be benificial for identification and authentification of *Mimusops elengi* bark powder. Preliminary phytochemical investigation of petroleum ether, chloroform, methanol and aqueous extracts have revealed the presence of tannins, flavonoids, triterpenoids, steroids, saponins, glycosides, alkaloids and phenolic compound.

The ethyl acetate soluble part of methanolic extract of plant *Mimusops elengi* subjected to TLC, HPTLC and Column chromatography for separation of active principle. And two compounds were isolated.

The isolated compound A and B was characterized by U.V, FT-IR, ^{1}H NMR and MASS spectroscopy. Spectral data reveal that the compound A co-relates with member of flavonoids. While compound B co-relates with member of natural tannins.

The present study demonstrated that methanolic and aqueous extract of bark of Mimusops *elengi* belongs to family Sapotaceae showed significant in-vitro Antioxidant activity by preventing the formation of free radicals due to the presence of phenolic compounds such as flavonoids and tannin.

The study has also shown that methanolic, petroleum ether, chloroform, aqueous extract and ethyl acetate soluble fraction of bark of *Mimusops elengi have* significantly determined anthelmintic activity. But methanolic extract and ethyl acetate soluble fraction of methanolic extract of *Mimusops elengi* shown most significant anthelmintic activity as compare to the other extracts. While all extract shows significant antibacterial and antifungal activity.

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