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Phytochemical and Qualitative Analysis of Leaves of *Melia azedarach* and Seeds of *Piper longum*

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ABSTRACT

Background: India has a strong history of traditional herbal system of treatment. In spite of substantial advances in medicinal plant research and rapid advances in modern medicine, there was increasing problem of liver disorders and demands for more precise, safe and effective treatments of liver disorders. Materials and methods: The aim of the current study was to perform phytochemical and qualitative extracts of Melia azedarach and seeds of Piper longum to identify the presence of active phytochemicals and phytoconstituents by using tests extract and HPTLC finger printing. Quantitative analysis of macronutrients and minerals including protein pattern was done by SDS-PAGE analysis. Results: In the present investigation preliminary phytochemical screening of the MAE, PLE shows the presence of constituents like alkaloid, carbohydrates, phytosterol, tannins, phenol, flavonoids, glycosides, terpenes, and lignin. The presence of heavy metals and minerals of the plant extracts evidence for their antioxidant and radical scavenging activity. Conclusion: The study concludes the presence of active components responsible for antioxidant and hepato protective activity. Further studies are needed with these plants to assess their pharmacological potentials, isolate, characterize and elucidate the structures of the bioactive compounds responsible for their activities and other medicinal values.

Keywords: *Melia azedarach; Piper longum;* Hepatoprotective activity; Antioxidant activity

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INTRODUCTION

M Azedarach is well known for its medicinal uses. Its various parts have antihelmintic, antimalarial, cathartic, emetic and emmenagogic properties and are also used to treat skin diseases. It has also been used as an abortifacient, an antiseptic, a purgative, a diuretic, an insect repellent. It is used for generally healing arthritis, rheumatism, for Pulmonary, stomach troubles, diarrhoea and dysentery. It is also used as vermifuges, to treat, cutaneous, subcutaneous parasitic infection and for menstrual cycle problems.

Many studies have been performed with an antiviral compound isolated from the leaves of *Melia azedarach* L. named meliacine. Meliacine strongly inhibited the replication of HSV-1 and HSV-2 *in vero* cells [1] and exhibits a synergistic antiviral activity when combined with acyclovir [2]. Studies performed by Alché et al. suggested that MA exerts the antiviral action on both synthesis of viral DNA and maturation and progress of HSV-1 on Viral cells [1]. Moreover, meliacine is a weak inducer of tumor necrosis factor alpha (TNF-α) in murine macrophage cultures and causes a synergistic effect on the production of TNF-α induced by LPS Ellerman-Eriksen (1994) [3]. The isolated constituents and n- hexane extracts of piper longum were found to show varying degree of antibacterial activity against all the tested bacteria [4]. Administration of alcoholic extract of Piper longum (10 mg/dose/animal) as well as piperine (1.14 mg/dose/animal) could inhibit the solid tumor development in mice induced with DLA cells and increase the life span of mice bearing Ehrlich ascites carcinoma tumor to 37.3 and 58.8%, respectively. Administration of *Piper longum* extract and piperine increased the total WBC count to 142.8 and 138.9%,

respectively, in mice [5]. Ethanol extract of *Piper longum* fruits and five crude fractions, petroleum ether (40-60), solvent ether, ethyl acetate, butanol and butanone were subjected to preliminary qualitative chemical investigations. The ethanolic extract and all other fractions were screened orally for hepatoprotective activity in adult Wistar rats. The ethanolic extract and butanol fraction have shown significant activity, lowering the serum enzymes glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in rats treated with carbon tetrachloride when compared to control and Liv-52-treated rats [6].

EXPERIMENTAL SECTION

Collection of Plants

The leaves of *Melia azedarach* and seeds of *Piper longum* were collected from the IMPCOPS (Indian Medical practitioners co-operative society, Thiruvanmiyur Chennai, India, and were authenticated by Dr. P.T. Kalaichelvan, Professor, Advanced Studies in Botany, University of Madras, Chennai, India. The voucher specimen is available in the herbarium file of the Indian Medical Practitioners Co-operative Society, Thiruvanmiyur, Chennai, India.

Extraction

Preparation of *Melia azedarach* extracts (MAE) and *Piper longum* extracts (PLE):

The leaves of *Melia azedarach* (1 kg) and the seeds of *Piper longum* (1 kg) were shade-dried and pulverized to a coarse powder. The powder was passed through 40-mesh sieve and exhaustively extracted with ethyl acetate in soxhlet apparatus at 60°C. The residue left after ethyl acetate extraction was dried and extracted successively with chloroform and ethanol. The extracts were evaporated under reduced pressure using rota flash evaporator till all the solvent had been removed and extract was stored in refrigerator for further use. All these extracts were subjected to HPTLC finger printing analysis.

Preliminary Phytochemical Screening

The ethanolic extracts of the MAE, PLE were subjected to preliminary phytochemical screening for identification of its active constituents by the method of Kokate et al. [7] (Table 1).

High Performance Thin Layer Liquid Chromatography (HPTLC) Finger Printing

HPTLC finger printing was performed on CAMAG TLC scanner-3 instrument, equipped with Linomat IV applicator and CATS 3. 2 software. Precoated aluminium silica gel 60 F_{254} (E. Merck) plates, layer thickness of 0.2 mm were used. Fingerprints were obtained by development in CAMAG twin chamber and were scanned at 254 nm.

Estimation of Total Proteins and Macronutrients

The estimation of total proteins present in the leaves of *Melia azedarach* and seeds of *Piper longum* were estimated by using Lowry's et al. method [8]. The samples were analyzed for macronutrients and the results were tabulated (Tables 2 and 3).

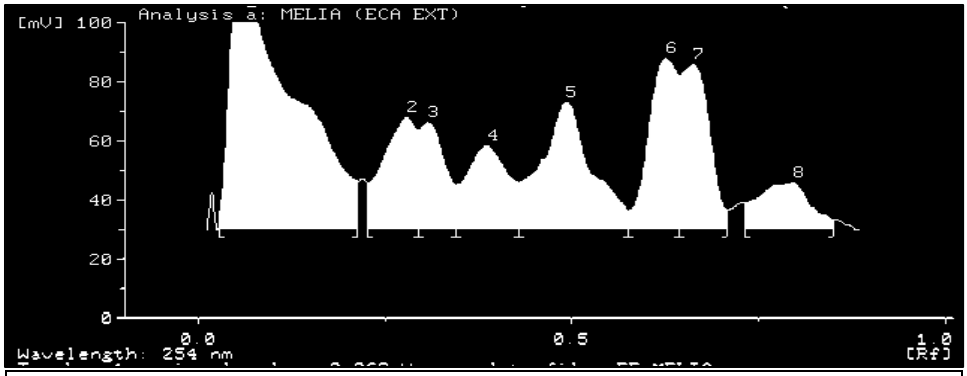
RESULTS AND DISCUSSION

In the present investigation preliminary phytochemical screening of the MAE, PLE shows the presence of constituents like alkaloid, carbohydrates, phytosterol, tannins, phenol, flavonoids, glycosides, terpenes, and lignin. The presence of heavy metals and minerals of the plant extracts evidence for their antioxidant and radical scavenging activity (Figures 1-7).

Table 1: Preliminary phytochemical screening of the test drugs

S No	Phytochemicals	MAE Extract	PLE Extract
1	Alkaloid	+	+
2	Carbohydrate	+	+
3	Protein	-	-
4	Phytosterol	+	+
5	Tannins	+	-
6	Phenolic compounds	+	+
7	Flavanoids	+	+
8	Gums and Mucilage	-	-
9	Glycosides	+	+
10	Saponins	-	-
11	Oils and fats	+	+
12	Terpenes	+	+
13	Lignin	+	+

(+) Indicates the presence of the chemical; (-) Indicates the absence of the chemical

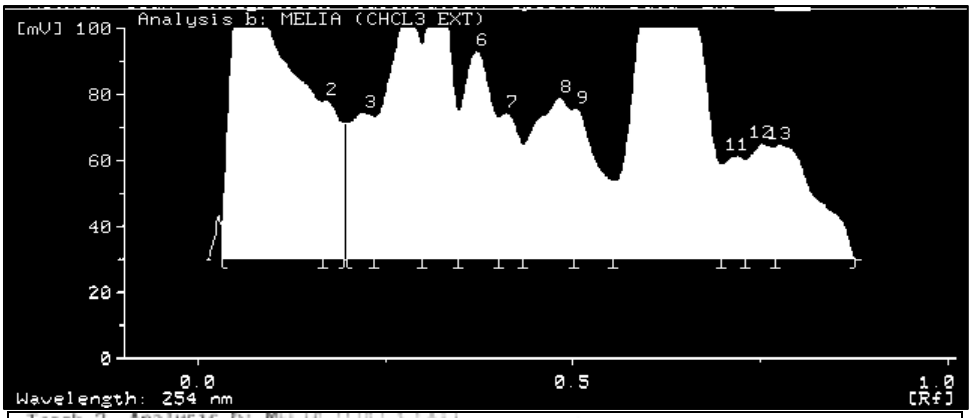


Track 1, Analysis a: MELIA (ECA EXT)

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.02	1.8	0.05	85.7	21.33	0.21	18.1	6246.9	32.83
2	0.22	17.7	0.27	40.2	10.00	0.29	36.1	1484.2	7.80
3	0.29	36.1	0.30	38.6	9.61	0.34	18.0	1134.4	5.96
4	0.34	18.0	0.38	31.8	7.91	0.42	19.6	1496.7	7.87
5	0.42	19.6	0.49	47.3	11.78	0.57	11.3	2881.3	15.14
6	0.57	11.3	0.62	63.2	15.73	0.64	57.7	2107.8	11.08
7	0.64	57.7	0.66	61.5	15.32	0.71	12.8	2001.2	10.52
8	0.73	15.5	0.80	22.8	5.67	0.85	10.7	1543.0	8.11
9	0.89	8.2	0.90	10.7	2.65	0.92	0.2	133.9	0.70

Total height = 401.7 total area = 19029.4

Figure 1: The HPTLC finger print analysis of the ethyl acetate extract of *M. azedarach*

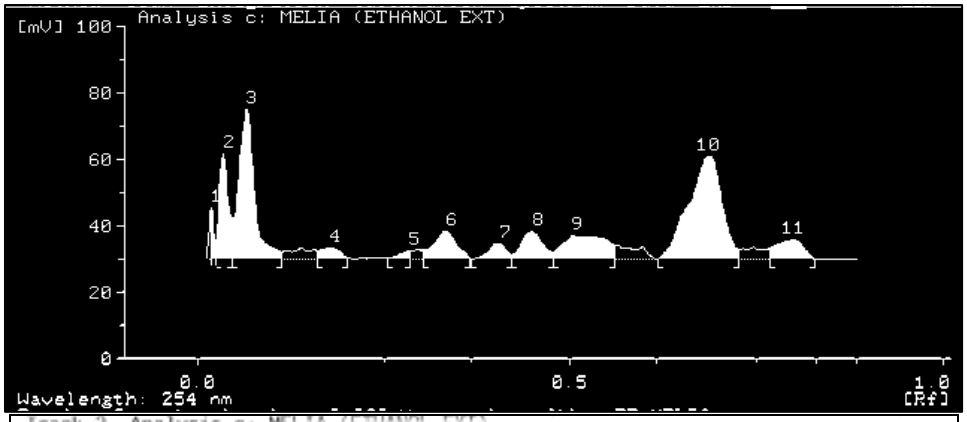


Track 2, Analysis b: MELIA (CHCL3 EXT)

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.02	0.1	0.06	76.3	13.25	0.15	38.6	5080.4	20.94
2	0.19	32.6	0.21	35.8	6.22	0.23	34.9	934.1	3.85
3	0.23	34.9	0.27	71.5	12.41	0.29	57.5	2451.3	10.10
4	0.29	57.5	0.31	78.9	13.70	0.34	38.0	2289.4	9.44
5	0.34	38.0	0.37	56.3	9.77	0.40	36.7	1818.4	7.49
6	0.40	36.7	0.41	38.0	6.59	0.43	28.7	801.0	3.30
7	0.43	28.7	0.48	43.8	7.60	0.49	40.2	1747.7	7.20
8	0.55	19.3	0.62	80.5	13.98	0.70	26.1	6072.2	25.03
9	0.70	26.1	0.72	29.0	5.03	0.73	28.1	640.9	2.64
10	0.73	28.1	0.75	33.1	5.74	0.77	32.2	873.5	3.60
11	0.77	32.2	0.78	33.0	5.72	0.88	0.0	1553.1	6.40

Total height = 576.0 total area = 24262.1

Figure 2: The HPTLC finger print analysis of the chloroform extract of *M. Azedarach*

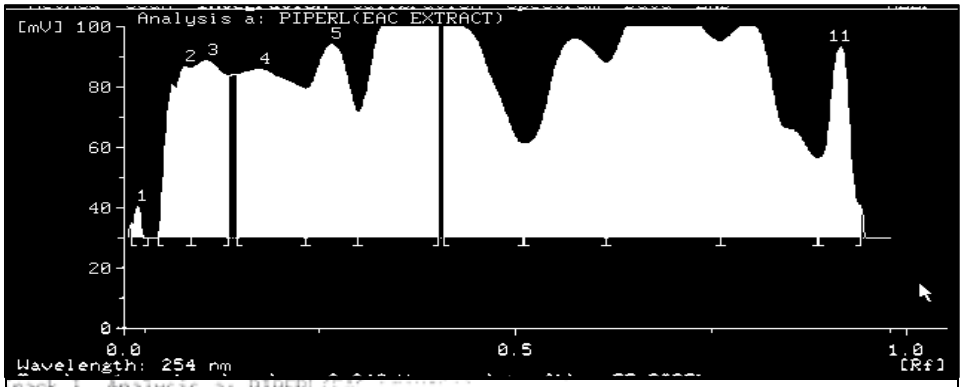


Track 3, Analysis c: MELIA (ETHANOL EXT)

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.01	0.0	0.02	28.0	20.74	0.04	8.7	252.8	8.31
2	0.04	8.7	0.05	42.1	31.14	0.10	0.2	632.3	20.79
3	0.30	2.8	0.33	8.4	6.25	0.36	0.1	209.9	6.90
4	0.36	0.0	0.40	4.6	3.43	0.42	1.2	91.9	3.02
5	0.42	1.2	0.45	8.2	6.06	0.47	2.2	197.1	6.48
6	0.47	2.2	0.50	7.0	5.15	0.56	4.3	339.5	11.16
7	0.62	0.1	0.69	30.9	22.88	0.73	2.9	1140.8	37.51
8	0.77	3.3	0.80	5.9	4.36	0.83	0.0	176.7	5.81

Total height = 135.1 total area = 3041.1

Figure 3: The HPTLC finger print analysis of the ethanolic extract of *M. Azedarach*

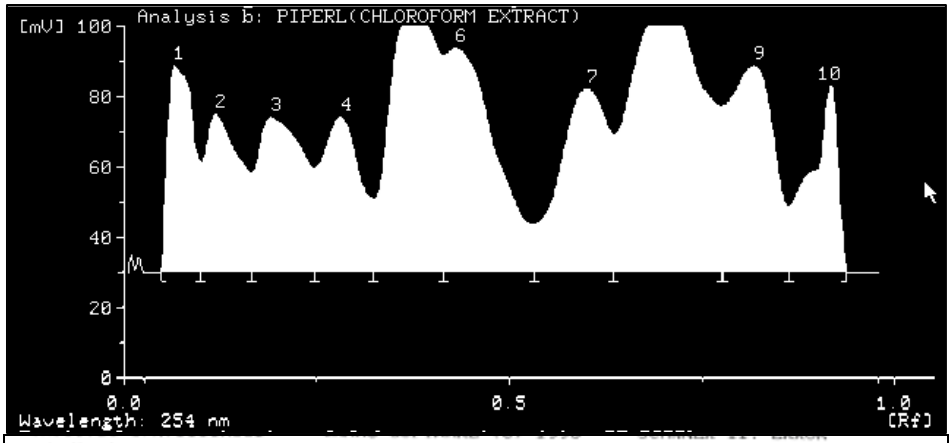


Track 1, Analysis a: PIPERL(EAC EXTRACT)

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.01	4.7	0.01	10.5	1.56	0.03	0.0	72.5	0.19
2	0.04	0.1	0.07	56.9	8.48	0.08	56.2	1335.9	3.51
3	0.08	56.2	0.10	58.7	8.75	0.13	53.7	2099.0	5.52
4	0.14	54.1	0.17	55.8	8.32	0.23	49.2	3526.9	9.27
5	0.23	49.2	0.26	64.1	9.55	0.30	41.4	2828.8	7.43
6	0.30	41.4	0.35	76.3	11.37	0.40	71.8	5369.2	14.11
7	0.41	71.9	0.41	71.9	10.71	0.51	31.1	4246.0	11.16
8	0.51	31.1	0.57	65.9	9.82	0.61	57.8	4249.8	11.17
9	0.61	57.8	0.69	76.9	11.45	0.76	64.9	7935.5	20.85
10	0.76	64.9	0.79	70.9	10.57	0.88	26.3	4857.0	12.76
11	0.89	26.4	0.91	63.1	9.41	0.94	10.7	1533.7	4.03

Total height = 670.9 total area = 38054.2

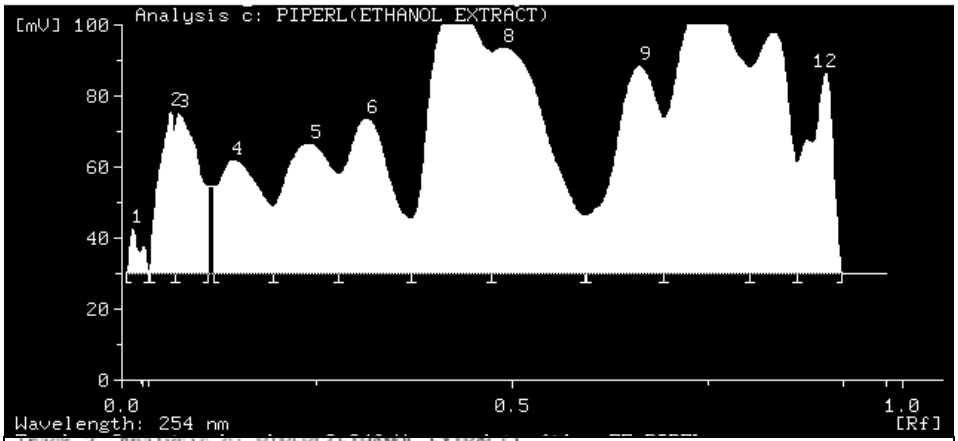
Figure 4: The HPTLC finger print analysis of the ethyl acetate extract of *P. longum*



Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.04	0.3	0.06	58.5	10.34	0.09	31.3	1699.4	5.77
2	0.09	31.3	0.11	44.9	7.93	0.16	28.2	1818.7	6.17
3	0.16	28.2	0.19	44.0	7.76	0.24	29.7	2326.3	7.89
4	0.24	29.7	0.28	44.0	7.77	0.32	20.9	1988.3	6.75
5	0.32	20.9	0.37	73.6	13.00	0.41	61.6	4086.2	13.86
6	0.41	61.6	0.43	63.6	11.23	0.53	13.7	3641.9	12.36
7	0.53	13.7	0.60	52.1	9.21	0.63	39.1	2859.1	9.70
8	0.63	39.1	0.70	74.0	13.07	0.77	47.2	6393.1	21.69
9	0.77	47.3	0.81	58.6	10.35	0.86	18.7	3004.7	10.20
10	0.86	18.7	0.91	52.9	9.35	0.94	0.1	1653.4	5.61

Total height = 566.4 total area = 29471.0

Figure 5: The HPTLC finger print analysis of the chloroform extract of *P. longum*



Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.00	0.2	0.01	12.5	2.05	0.03	0.0	138.9	0.48
2	0.03	1.1	0.06	45.3	7.44	0.06	39.3	729.0	2.52
3	0.06	39.3	0.07	44.9	7.37	0.11	24.7	1147.2	3.96
4	0.11	24.4	0.14	31.7	5.21	0.19	18.8	1537.5	5.31
5	0.19	18.8	0.24	36.4	5.97	0.27	27.9	1945.0	6.71
6	0.27	27.9	0.31	43.5	7.15	0.37	15.2	2217.1	7.65
7	0.37	15.2	0.42	73.4	12.06	0.47	62.2	4544.2	15.68
8	0.47	62.2	0.48	63.5	10.42	0.59	16.1	4045.0	13.96
9	0.59	16.1	0.66	58.1	9.55	0.69	43.6	2955.3	10.20
10	0.69	43.6	0.75	75.7	12.43	0.80	58.0	5493.2	18.96
11	0.80	58.0	0.83	67.7	11.11	0.86	30.9	2652.8	9.15
12	0.86	30.9	0.90	56.2	9.24	0.92	0.0	1572.7	5.43

Total height = 609.0 total area = 28977.9

Figure 6: The HPTLC finger print analysis of the ethanolic extract of *P. longum*

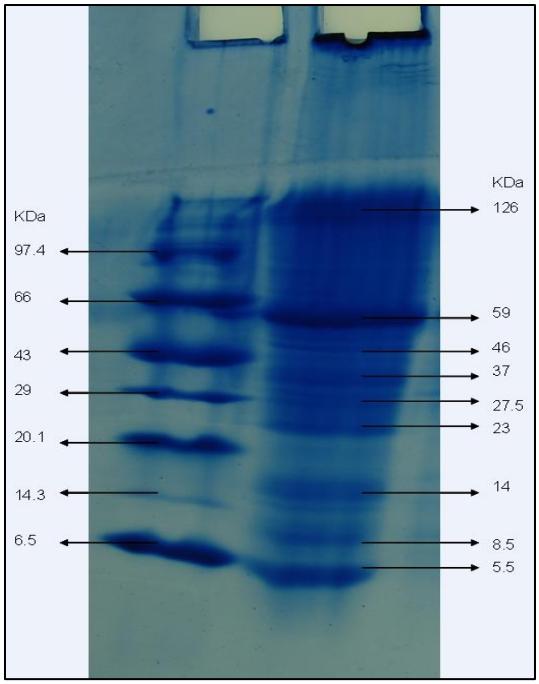


Figure 7: SDS-PAGE electrophoresis results of aqueous extract of *Melia azedarach*

Lane 1 - shows the marker protein bands with their molecular weight ranging 6.50 – 97.4 kDa.
Lane 2 - shows the Melia azedarach leaf protein bands with their molecular weight of ranging 6.50 – 97.4 kDa
Protein used as markers with their molecular weight expressed in kDa: Phosphorylase b-97.4; BSA-66; Ovalbumin 43; Carbonic Anhydrase-29; Soyabean; Trypsin Inhibitor-20.1; Lysozyme-14.3; Aprotinin-6.5.

Table 2: Concentration of macronutrients

S.No.	Macronutrients	Leaves of *Melia azedarach* (Expressed in mg/100gms)	Seeds of *Piper longum* (Expressed in mg/100gms)
1	Total sugars	23.78	13.56
2	Total protein	36.23	7.8
3	Total lipid	27.94	3.78

Table 3: Concentration of minerals present in the plants

S.No.	Macronutrients	Leaves of *Melia azedarach* (Expressed in PPM)	Seeds of *Piper longum* (Expressed in PPM)
1	Aluminum	3.286	2.294
2	Barium	0.299	0.42
3	Calcium	280	340
4	Copper	0.746	0.574
5	Chromium	0.186	0.149
6	Cobalt	0.107	0.081
7	Iron	2.198	2.633
8	Lead	0.311	0.025
9	Magnesium	36.171	26.115
10	Molybdenum	0.81	0.745
11	Mercury	0.371	0.259
12	Manganese	0.136	0.132
13	Nickel	0.429	0.181
14	Potassium	136.5	117
15	Sodium	92	138
16	Silicon	2.343	1.399
17	Selenium	0.0117	0.0094
18	Vanadium	1.2945	2.107
19	Zinc	4.944	4.13

CONCLUSION

Over the past decade, there has been a resurrection of interest in the exploration of medicinal plant as a source of prospective herbal medicine. There is a need to advance research for the development and characterization of new natural drugs with the assist better drugs and its constituents from plants and other natural sources. The study concludes the presence of active components responsible for antioxidant and hepato protective activity. Further studies are needed with these plants to assess their pharmacological potentials, isolate, characterize and elucidate the structures of the bioactive compounds responsible for their activities and other medicinal values.

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