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Antioxidant Properties of Crude Methanolic Extract & Partitioned Fractions of *Swietenia mahagoni* (L) Seeds.

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Abstract

The genus Swietenia (Meliaceae) contains various secondary metabolites, such as flavonoids, limonoids, and other antioxidant compounds. In this study, the antioxidant capacity is evaluated together with the phenol and flavonoid contents of the seeds extracts of Swietenia mahagoni. The antioxidant potential of different extracts of S. mahagoni seeds has been determined by the reduction power of DPPH, FRAP, hydrogen peroxide scavenging tests. The methanol extracts of the seeds of S. mahagoni showed the highest phenol and flavonoid content and reduction capacity, while the hexane extracts showed the lowest level of reduction capacity. The antioxidant potential of S. mahagoni seeds extracts is strongly related ($P<0.02$) to the phenol content of methanol extracts. Catechin, which is used as a control, shows the greatest antioxidant strength in this study. The results show significant antioxidant activity and high concentrations of phenol compounds and flavonoids in the S. mahagoni seeds extracts. Some constituents of the active fractions were detected by chromatography, and were characterized by NMR.

Key words: *Swietenia mahagoni*, Antioxidant, Catechin, Flavonoids, Phenols

INTRODUCTION

We must recall certain fundamentals from science class in order to know what an antioxidant is? Protons, electrons, and neutrons make up atoms. Two or more atoms make up molecules. A molecule needs the correct number of electrons to stay stable; otherwise, it will become a "free radical" [1]. These free radicals target healthy molecules that support vital bodily activities. Both internal and external exposures result in the production of these "pro-oxidants" [2]. Free radicals, which can lead to diseases including cancer, diabetes, and heart disease, are combated by antioxidants [3]. Although free radicals are a normal component of human metabolism, issues might arise when the ratio of free radicals to antioxidants is out of balance. We refer to this imbalance as oxidative stress [4]. Free radical scavengers such as antioxidants are measured. The chance of developing autoimmune disorders, diabetes, heart disease, Parkinson's

disease, Alzheimer's disease, and cancer can be raised by exposure to air pollution, heavy metals, and tobacco smoke. Phyto-nutrients are chemical substances that can be found in plants, and they may offer a number of health advantages for the body, such as antioxidant activity. More than 4,000 phyto-nutrients are thought to exist, however only a small portion have undergone in-depth research [5]. These can be found in berries, eggplant, purple potatoes, carrots, and asparagus, as well as other blue and purple fruits and vegetables. These antioxidants support the health of blood vessels. There are many different chemical forms of this vitamin in food, but only alpha-tocopherol is necessary for human vitamin E requirements for healthy blood vessels [6]. Ascorbic acid is a kind of vitamin C that is listed on our meals. This nutrient is found naturally in many plant-based foods, and in addition to acting as an antioxidant, it also helps other antioxidants, like vitamin E, that have been damaged by free radicals, to recover [7].

An antioxidant molecule prevents the oxidation of other molecules. An oxidizing agent gains electrons or hydrogen from the substance being oxidized in a chemical reaction known as oxidation [8]. Free radicals are created during oxidation events, and these radicals have the power to generate other reactions. A cell may sustain damage or perhaps expire when the chain reaction takes place inside of it [9]. By eliminating the free radical intermediates, antioxidants stop these chain reactions and prevent more oxidation processes. As a result, reducing agents such thiols, ascorbic acid, or polyphenols are frequently found in antioxidants [10]. A sophisticated system of antioxidants, including glutathione, vitamin C, and vitamin E, as well as enzymes like catalase, superoxide dismutase, and different peroxidases is maintained by plants and animals because oxidation reactions, while necessary for life, can also be harmful [11]. Cells may be damaged or killed when there are insufficient antioxidant levels present or when antioxidant enzymes are inhibited [12]. Antioxidants are heavily researched as treatments for diseases including stroke and neurological disorders, as oxidative stress appears to play a significant role in many human diseases. Furthermore, oxidative stress both causes and results in disease [12]. Low antioxidant levels or the control of antioxidant enzymes result in oxidative stress, which can harm or kill cells [13]. The aim of this work is to investigate about the antioxidant activity of *S. mahagoni* seeds extracts.

S. mahagoni belongs to the family of Meliaceae, it is also called as West Indian Mahogany [14]. It is extensively used as medicine for several diseases and widely grown plant of Bangladesh [15]. S. mahagani is a large, deciduous and economically important timber tree; it is mainly cultivated in the tropical zone, such as India, Malaysia and Southern China. Mahogany tree is about 75 feet in height, leaves are evergreen or semi-evergreen, and flowers are unisexual [16].

MATERIALS AND METHODS

1. Collection of Plant Materials and Extraction

S. mahagoni seeds were collected from Nator, Bangladesh. The seeds were to shade drying under sunlight for several days and then oven drying for 24 hours at considerably low temperature (not exceeding 40ºC) for better grinding. The dried seeds were then ground to a coarse powder. The powdered material (1200g) was taken in a properly cleaned, amber color reagent bottle (5 liters) and soaked in 2.5L methanol. After 2

weeks, the bottle content was filtered through a fresh cotton plug and finally with a Whatman filter paper No.1 and the resulting filtrate was then concentrated using a rotary evaporator under reduced pressure. The semisolid mass (141g) of methanolic crude extract (MCE) was obtained. A deal (121g) of MCE was fractionated by the modified kupchan partitioning method [17]. We got Petroleum ether fraction (PEF) (32.4060g), Chloroform fraction (CHF) (27.4318g), Ethyl acetate fraction (EAF) (10.9060g), and Diaion resin fraction (DRF) (42.1833g). The residues were then stored in a refrigerator until further use.

1.1 Determination of total antioxidant capacity

Total antioxidant capacity of different extractives of S .*mahogoni seeds* was determined by the method of Prieto *et al.,* (1999) with some modifications [18]. Antioxidants such ascorbic acid, certain phenolics, -tocopherol, and carotenoids are typically detected by the phosphomolybdenum technique. The phosphomolybdenum method was found on the antioxidant compound's reduction of Mo (VI) to Mo (V), followed by the creation of a green phosphate/Mo (V) complex at an acidic pH. A green phosphate/Mo (V) complex with a maximum absorption at 695 nm is thought to arise because molybdenum is easier to reduce in the complex and an electron-transfer process between reluctant and Mo (VI) takes place.

$$Mo\ (VI) + e \rightarrow Mo\ (V)$$

Experimental procedure: 0.3 mL of plant extract or standard of different concentration solution was taken in a test tube. 3 mL of reaction mixture containing 0.8 M sulphuric acid, 14 mM sodium phosphate and 0.4% ammonium molybdate was added into the test tube. The test tube was incubated at 95^{o}C for 10 minutes to complete the reaction. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. A typical blank solution contained 3 mL reaction mixture and the appropriate volume (300µL) of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples solution.

1.2 Determination of total phenolic content

Total phenolic content of the different extractives of S. *mahogoni seeds* was determined employing the method as described by Singleton *et al.,* (1965) involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard. By using the Folin-Ciocalteu Reagent (FCR), the total phenolic compound content of the plant's various fractions was ascertained [19]. The technique measures the reduction capability of a sample. Although the FC reagent's precise chemical composition is unknown, heteropolyphospho-tungstates-molybdates are thought to be present. Blue species, perhaps $(PMoW_{11}O_{40})_4$ are produced via reversible one- or two-electron reduction reaction sequences. In essence, it is thought that molybdenum is simpler to reduce in the complex and that Mo (VI) and reductants undergo an electron-transfer reaction:

$$Mo\ (VI) + e^{-} \rightarrow Mo\ (V)$$

Experimental procedure: 0.3 mL of plant extract or standard of different concentration solution was taken in a test tube. 2.5 mL of Folin–Ciocalteu (Diluted 10 times with water) reagent solution, 2.5 mL of Sodium carbonate (6%) solution were added into the test tube. The test tube was incubated for 90 minutes at 25ºC to complete the reaction. Then the absorbance of the solution was measured at 760 nm using a spectrophotometer against blank. A typical blank solution contained all reagents except plant extract or standard solution. The total content of phenolic compounds in plant methanol extract and in different fractionates in catechin equivalents (CE) was calculate by the following formula, $C = \frac{c \times V}{m}$ Where, C = Total content of phenolic compounds, mg/g plant extract, in CE;

c = The concentration of catechin established from the calibration curve, mg/mL; V = The volume of extract, (mL); m = The weight of different pure plant extracts, (g)

1.3 Determination of total flavonoids content

Total flavonoid content of the different extractives of S. *mahogoni seeds* determined by aluminum chloride colorimetric method. Catechin was used as standard and the flavonoid content of the extractives was expressed as mg of catechin equivalent/g of dried extract [19]. The well-known aluminum chloride colorimetric method was used to evaluate the total flavonoid content of various plant extractives. With the help of the hydroxyl groups of the flavonoids present in the samples, aluminum chloride creates a complex in this approach. At 510 nm blank, this compound has the highest absorption. All reagents—aside from plant extract or standard solution—were present in a normal blank solution. The formula C=(cV)/m was used to calculate the amount of phenolic compounds present overall in plant methanol extract and various fractionates [20].

Experimental procedure: 0.50 mL of plant extract or standard of different concentration solution was taken in a test tube. 3 mL of methanol, 150µL5% $NaNO_2$ solution, 300µL of 10`% aluminum chloride solution and 1 mL of I M NaOH solution were added into the test tube. The test tube was then incubated at room temperature for 10 minutes to complete the reaction. Then the absorbance of the solution was measured at 510 nm using a spectrophotometer against blank. A typical blank solution contained all reagents except plant extract or standard solution. The total content of flavonoid compounds in plant extracts in Catechin equivalents was calculated by the following formula equation, $C = \frac{c \times V}{m}$

Where, C = Total content of flavonoid compounds, mg/g plant extract, in catechin equivalent (CE);

c = The concentration of catechin established from the calibration curve, mg/mL;

V = The volume of extract, mL; m = The weight of different pure plant extracts, g.

1.4 Determination of Reducing Power capacity

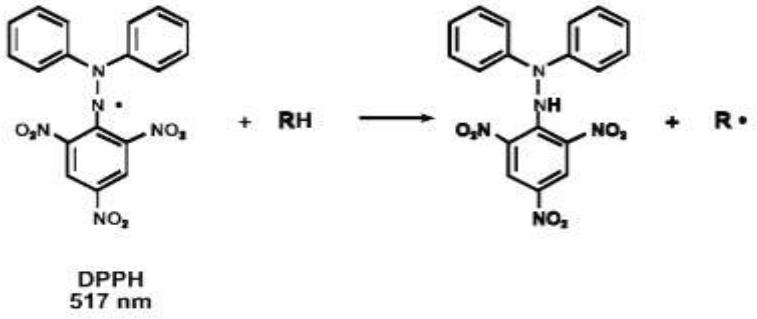
The reducing power of different extractives of S. *mahogoni seeds* was evaluated by the method of Oyaizu (1986) [21]. Depending on the reducing power of the antioxidant samples, the test solution's yellow hue changes to various colors of green and blue in this assay. By accepting an electron, reductants like antioxidants cause the Fe^{3+-} ferricyanide complex to be reduced to the ferrous form in the samples. Then, by monitoring the production of Perl's Prussian blue at 700 nm sowing formula, C=(cV)/m, the amount of Fe^{2+} complex may be observed.

Fe^{3+} + ferricyanide + e $\longrightarrow$ Fe^{2+} + ferrocyanide

Experimental Procedure: 0.250 mL of plant extract or standard of different concentration Solution was taken in a test tube. 0.625 mL of potassium buffer (0.2 M) and 0.625 mL of Potassium ferricyanide [$K_3Fe(CN)_6$], (1%) solution were added into the test tube. The reaction mixture was incubated for 20 minutes at 50°C to complete the reaction. 0.625 mL of trichloro acetic acid, (10%) solution was added into the test tube. The total mixture was centrifuged at 3000 rpm for 10 mins. 1.8 mL supernatant solution was withdrawn from the mixture and mix with 1.8 mL of distilled water. 0.350 mL of ferric chloride (0.1%) solution was added to the reaction mixture. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer against blank. A typical blank solution contained the same solution mixture without plant extract or standard and it was incubated under the same conditions as the rest of the samples solution. Also the absorbance of the blank solution was measured at 700 nm against the solvent used in solution preparation. Increased absorbance of the reaction mixture indicated increase reducing power.

1.5 Determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

DPPH was used to evaluate the free radical scavenging activity of various compounds and medicinal plants (Braca et al., 2001; J. Nat. Prod., 64, 892-895) [22]. The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been frequently used to gauge an antioxidant's ability to scavenge free radicals. When DPPH free radical interacts with hydrogen donors, it is transformed into the equivalent hydrazine. Stable free radicals can be produced using DPPH in aqueous or methanol solutions. The antiradical potency of an antioxidant activity might be assessed using this method by measuring the decline in DPPH absorbance at 517 nm. The absorbance reduced when the DPPH was scavenged by an antioxidant by donating hydrogen to create a stable DPPH molecule, resulting in a color change from purple to yellow. This molecule had an absorbance of 517 nm in its radical form, but it vanished after accepting an electron or hydrogen radical.



Experimental procedure: 1 mL of methanol solution of plant extract or standard at different concentration was taken in a test tube. 3 mL of methanol solution of DPPH was added into the test tube. The test tube was incubated at room temperature for 30 minutes in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against blank. A typical blank solution contained all reagents except plant extract or standard solution. The percentage (%) of scavenging was calculated from the following equation [23].

$I\% = [(A_0 - A_1)/A_0] \times 100,$

Where, I% = percentage of scavenging activity , A_0= absorbance of the control, and

A_1= absorbance of the extract/standard.

Then % inhibitions were plotted against concentration and from the graph IC_{50} was calculated.

2 . RESULTS AND DISCUSSION

2.1 Determination of total antioxidant activity

Table-1: Total antioxidant activity of difference fractions of *S. mahagoni seeds*

Name of the sample	Conc. (µg/mL)	Absorbance (nm)			Absorbance (nm) Mean ± STD
		a	b	c	
+(-)Catechin (standard)	10	0.084	0.091	0.089	0.088±0.0036
	20	0.216	0.216	0.218	0.217±0.0011
	40	0.259	0.261	0.262	0.261±0.0015
	80	0.313	0.314	0.315	0.314±0.0010
	160	0.424	0.423	0.427	0.425±0.0021
	320	0.495	0.493	0.496	0.494±0.0043
Crude methanol extract (CME)	10	0.053	0.066	0.063	0.060±0.0051
	20	0.157	0.151	0.155	0.154±0.0030
	40	0.176	0.174	0.183	0.177±0.0046
	80	0.222	0.219	0.215	0.218±0.0035
	160	0.281	0.265	0.273	0.276±0.0061
	320	0.362	0.355	0.357	0.358±0.0036
Petroleum ether fraction (PEF)	10	0.033	0.037	0.035	0.035±0.0061
	20	0.140	0.141	0.145	0.143±0.0026
	40	0.194	0.197	0.211	0.197±0.0088
	80	0.241	0.253	0.263	0.252±0.0110

	160	0.281	0.303	0.311	0.298±0.0064
	320	0.353	0.355	0.363	0.357±0.0052
Chloroform fraction (CLF)	10	0.067	0.056	0.065	0.063±0.0058
	20	0.135	0.139	0.134	0.136±0.0026
	40	0.235	0.197	0.219	0.221±0.0135
	80	0.282	0.281	0.285	0.286±0.0045
	160	0.327	0.332	0.334	0.331±0.0036
	320	0.381	0.372	0.373	0.375±0.0049
Ethyl acetate fraction` (EAF)	10	0.081	0.073	0.088	0.084±0.0065
	20	0.168	0.179	0.170	0.179±0.0010
	40	0.263	0.270	0.272	0.268±0.0047
	80	0.359	0.366	0.351	0.358±0.007
	160	0.472	0.479	0.473	0.468±0.0055
	320	0.541	0.542	0.545	0.529±0.0073
Dia-ion resin fraction (DRF)	10	0.078	0.088	0.089	0.085±0.0060
	20	0.155	0.153	0.153	0.159±0.0037
	40	0.282	0.294	0.296	0.297±0.0041
	80	0.385	0.372	0.369	0.375±0.0085
	160	0.478	0.481	0.478	0.486±0.0068
	320	0.563	0.575	0.571	0.569±0.0075

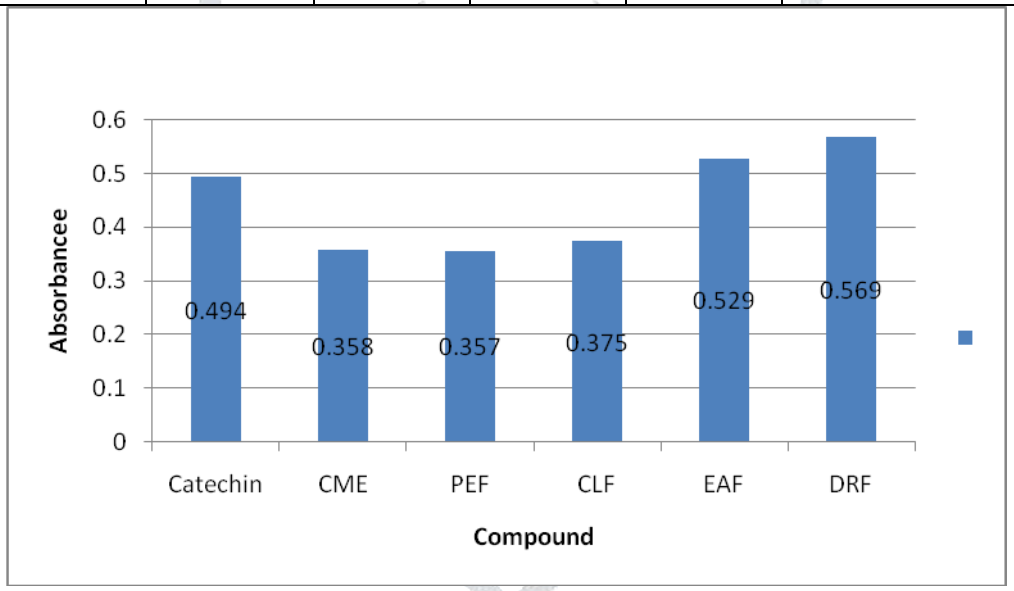


Fig -1: At 320 µg/mL the absorbance of crude methanolic extract (CME) of *S. mahagoni seeds*, its different fractions PEF, CLF, EAF & DRF and (+)-catechin (standard).

The antioxidant activity of the crude methanol extract (CME) and the various components increase as the concentration does. The findings showed that dia-ion resin and ethyl acetate fractions had greater overall antioxidant activity than pure methanolic extract. The activity, meanwhile, was lower than the reference standard catechin. With an increase in concentration, it was discovered that all of the samples had greater overall antioxidant activity. Methanolic extract, petroleum ether fraction, chloroform fraction ,ethyl acetate fraction, dia-ion resin fraction, and reference standard catechin all had absorbance of 0.358, 0.357, 0.375, 0.529,0.569 and0.494 nm, respectively.

2.2 Determination of total phenolic content of crude methanol extract (CME) of *S. Mahagoni* seeds and its different fractions.

Phenolic content of the crude methanolic extract (CME) of ***S. Mahagoni*** **seeds** and its petroleum ether fraction (PEF), chloroform fraction(CLF), ethyl acetate fraction(EAF) and dia-ion resin fraction (DRF) was determined using Folin-Ciocalteu reagent (FCR). Phenolic content of the samples were calculated on the basis of the standard curve for gallic acid as shown in Table: 2 and in figure 2. The results were expressed as mg of gallic acid equivalent (GAE)/g of dried extractives.

Table-2: Absorbance of gallic acid at different concentrations after treatment with Folin-Ciocalteu reagent (FCR).

Concentration (µg/mL)	Absorbance (nm)			Absorbance (nm) Mean ± STD
	a	b	c	
10	0.085	0.076	0.085	0.082 ± 0.0005
20	0.162	0.163	0.173	0.166 ± 0.0006
40	0.274	0.284	0.295	0.284 ± 0.010
80	0.582	0.571	0.562	0.571 ± 0.010
160	1.172	1.183	1.173	1.175 ± 0.0005
320	2.284	2.275	2.283	2.280 ± 0.004

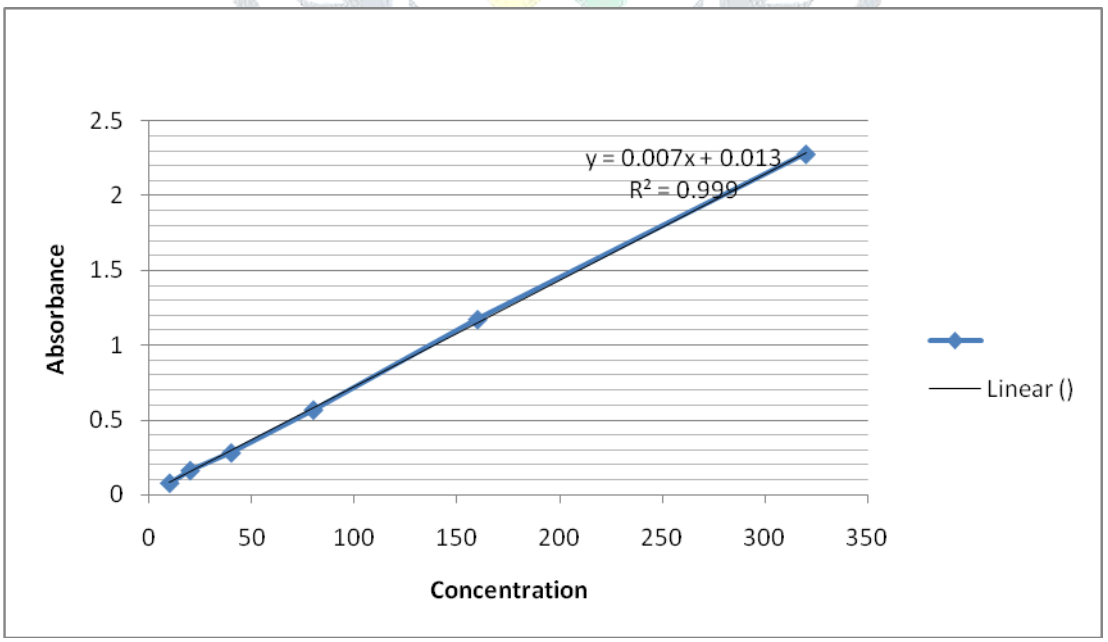


Fig 2 : Absorbance of gallic acid at different concentration after treatment with (FCR).

Table 3: Determination of total phenolic content of difference fractions of *S.mahagoni seeds*

Name of the Sample	No. of the samples	Concentration (µg/mL)	Absorbance (nm)	GAE mg / g of dried sample	GAE mg/ g of dried sample Mean ± STD
Crude methanol extract (CME)	1	250	0.663	92.85	92.9±2.21
	2	250	0.648	90.71	
	3	250	0.679	95.14	
Petroleum ether fraction (PEF)	1	250	0.062	7.00	7.28±0.28
	2	250	0.064	7.28	
	3	250	0.065	7.57	
Chloroform fraction (CLF)	1	250	0.260	61.75	60.88±0.75
	2	250	0.259	60.52	
	3	250	0.258	60.38	
Ethyl acetate fraction (EAF)	1	250	0.576	80.42	80.09±0.83
	2	250	0.567	79.14	
	3	250	0.578	80.71	
Dia-ion resinfraction (DRF)	1	250	0.673	94.28	91.75±2.18
	2	250	0.647	90.57	
	3	250	0.646	90.42	

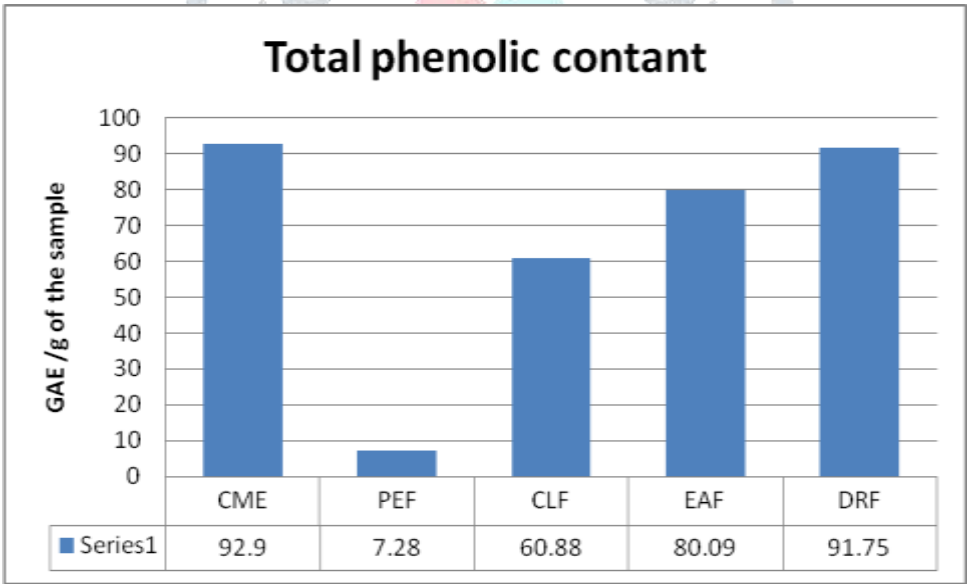


Fig3: Total phenolic content of the crude methanol extract(CME) of *S. mahagoni seeds* and it's different fractions (PEF, CLF, EAF and DRF).

The results of phenolic content of crude methanol extract (CME) and its different fractions were shown in table: 3 and in Fig. 3.

From the result, the phenolic content of crude methanolic extract (CME) was found to be 320 mg of GAE/g of dried extract. Among the fractions of CME, highest phenolic content was found in the ethyl acetate fraction (EAF) (97.28 mg of GAE/ g of dried extract) followed by dia-ion resin fraction (DRF) 61.05 mg of GAE/ g of dried extract, petroleum ether fraction (PEF) and chloroform fraction (CLF)

were7.38 mg and 33.52mg of GAE/ g of dried extract respectively. Comparison of the phenolic content of crude methanol extract with its different fractions, revealed that the ethyl acetate fraction (EAF) contained the highest amount of phenolics.

2.3. Determination of the total flavonoids of crude methanol extract (CME) of *S. mahagoni seeds* and its different fractions.

Table 4: Absorbance of catechin at difference concentrations for quantity determination of total flavonoids.

Concentration (µg/mL)	Absorbance (nm)			Absorbance (nm) Mean ± STD
	a	b	c	
10	0.004	0.004	0.005	0.0043 ± 0.0005
20	0.008	0.009	0.008	0.0080± 0.0012
40	0.017	0.016	0.017	0.0173 ± 0.0005
80	0.035	0.036	0.035	0.0350 ± 0.0015
160	0.072	0.073	0.074	0.0736 ± 0.0058
320	0.150	0.151	0.152	0.1514 ± 0.0018

Total flavonoids content of the crude methanol extract (CME) and its four fractions (PEF, CLF, EAF and DRF) was determined using much known aluminum chloride colorimetric method. Flavonoid content of the samples was calculated on the basis of the standard curve for catechin as shown in Table-4 and in Fig. 4. The results were expressed as mg of catechin equivalent (Cat.E) mg/g of dried sample.

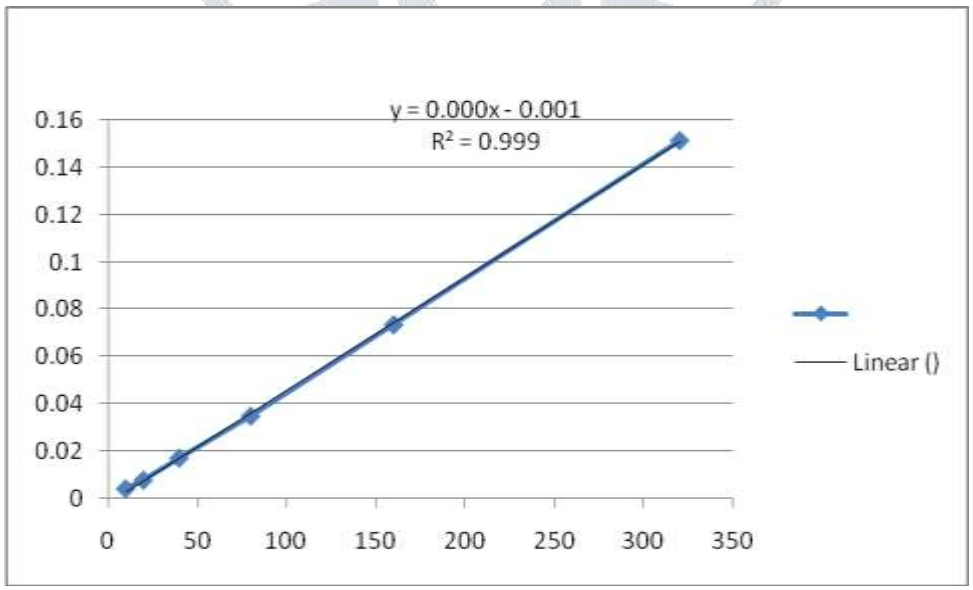


Fig. 4: Standard curve of catechin for the determination of total flavonoids.

Table-5: Determination of the total flavonoid content of different fractions of *S. mahagoni seeds*

Name of the Sample	No. of the samples	Concentration (µg/mL)	Absorbance (nm)	Cat.E mg/ g of dried sample	Cat.E mg/ g of dried sample Mean ± STD
Crude methanol extract (CME)	1	250	0.493	165.46	174.02±5.796
	2	250	0.521	174.80	
	3	250	0.542	181.80	
Petroleum ether fraction (PEF)	1	250	0.005	2.80	2.68±0.2267
	2	250	0.004	2.46	
	3	250	0.005	2.88	
Chloroform fraction (CLF)	1	250	0.098	33.80	33.46±0.3350
	2	250	0.096	33.13	
	3	250	0.097	33.46	
Ethyl acetate fraction (EAF)	1	250	0.430	144.46	144.35±0.5084
	2	250	0.428	143.80	
	3	250	0.431	144.80	
Dia-ion resin fraction (DRF)	1	250	0.523	175.46	175.68±0.6925
	2	250	0.526	176.46	
	3	250	0.522	175.13	

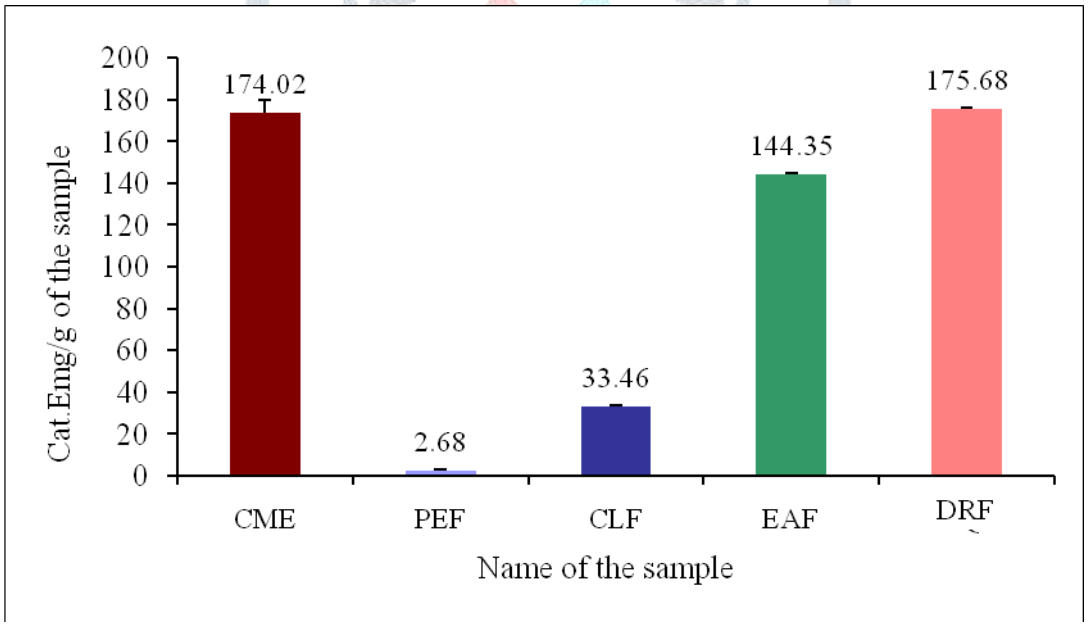


Fig 5: Total flavonoid content of the crude methanol extract(CME) of *S. mahagoni seeds* and it's different fractions (PEF, CLF, EAF and DRF).

The flavonoid content of the crude methanol extract (CME) and its different fractions were shown in Table -5 and Fig. 5.

The flavonoid content of CME was found to be 174.02 mg of Cat.E mg/g of dried extract, whereas the flavonoid content of PEF, CHF, EAF and DRF was 2.68 mg, 33.46 mg, 144.35 mg and 175.68 mg of Cat E mg/g of dried extract respectively. These results demonstrated that both dia-ion resin fraction (DRF) and ethyl acetate fraction (EAF) contained a large amount of flavonoids.

2.4 Determination of Reducing Power capacity

Table-6: Data for Reducing power capacity of the crude methanol extract (CME) of *S. mahagoni* seeds and *its* different fractions.

Name of the sample	Conc. (µg/mL)	Absorbance (nm)			Absorbance (nm) Mean ± STD
		a	b	c	
(+)-Catechin (standard)	10	0.019	0.020	0.021	0.02±0.0015
	20	0.115	0.116	0.115	0.115±0.0010
	40	0.553	0.527	0.531	0.537±0.0140
	80	1.231	1.220	1.219	1.223±0.0066
	160	1.947	1.935	1.827	1.903±0.0661
	320	2.444	2.422	2.420	2.428±0.0130
Crude methanol extract (CME)	10	0.026	0.029	0.027	0.027±0.0020
	20	0.083	0.081	0.081	0.081±0.0052
	40	0.292	0.284	0.272	0.283±0.0100
	80	0.711	0.718	0.695	0.698±0.0117
	160	1.331	1.329	1.355	1.328±0.1446
	320	1.764	1.817	1.798	1.793±0.0268
Petroleum ether fraction (PEF)	10	0.030	0.031	0.031	0.031±0.0052
	20	0.065	0.077	0.081	0.075±0.0083
	40	0.128	0.119	0.117	0.121±0.0058
	80	0.462	0.457	0.455	0.458±0.0036
	160	0.865	0.846	0.848	0.853±0.0104
	320	0.946	0.948	0.962	0.952±0.0087
Chloroform fraction(CLF)	10	0.023	0.024	0.019	0.022±0.0026
	20	0.103	0.089	0.092	0.095±0.0073
	40	0.221	0.211	0.219	0.217±0.0052
	80	0.533	0.557	0.552	0.547±0.0126
	160	0.982	0.981	0.979	0.980±0.1065
	320	1.368	1.372	1.409	1.383±0.0226
Ethyl acetate fraction (EAF)	10	0.009	0.021	0.012	0.014±0.0062
	20	0.176	0.195	0.192	0.187±0.0102
	40	0.506	0.498	0.529	0.511±0.0161
	80	1.003	0.982	0.976	0.987±0.0141
	160	1.612	1.609	1.573	1.598±0.0217
	320	2.202	2.216	2.318	2.245±0.0633
Dia-ion resin fraction (DRF)	10	0.044	0.044	0.038	0.042±0.003
	20	0.221	0.217	0.198	0.215±0.0066
	40	0.594	0.613	0.575	0.597±0.0196
	80	1.337	1.417	1.321	1.358±0.0514
	160	2.197	2.186	2.186	2.189±0.0064
	320	2.598	2.596	2.572	2.588±0.0144

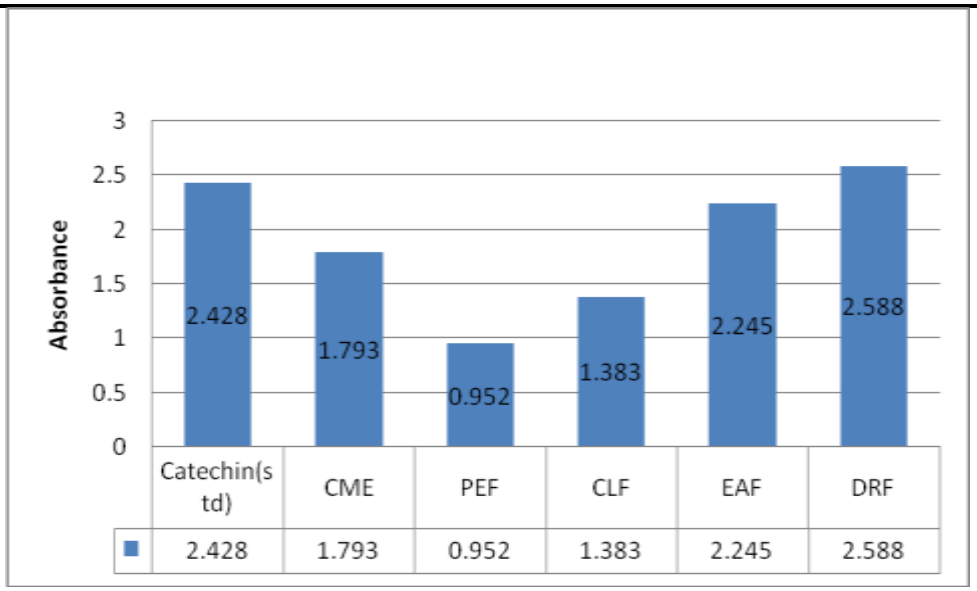


Fig 6: At 320 µg/mL the absorbance of crude methanolic extract (CME) of *S. mahagoni* seeds and, its different fractions PEF, CLF, EAF & DRF and (+)-catechin (standard).

From the results, it was found that crude methanolic extract and it's different fractions tested exhibit reducing activity. Of them ethyl acetate fraction (EAF) and dia-ion resin fraction(DRF) showed the higher reducing power capacity with absorbance of 2.245 and 2.542, respectively at the concentration of 320 µg/mL, whereas the reference standard catechin showed absorbance of 2.452. Petroleum ether fraction (PEF) and chloroform fraction (CLF) showed mild reducing activity.

The activity of the fractions and the reference standard catechin decreases in the following order.

DRF > Catechin > EAF > CME > CLF > PEF.

Table-7: The reducing power capacity of isolated compounds DFC-1, DFC-2, DFC-3, DFC-4, DFC-5 and DFC-6 of *S. mahagoni seeds*

Name of the sample	Conc. (µg/mL)	Absorbance (nm)			Absorbance (nm) Mean ± STD
		a	b	c	
(+)-Catechin (standard)	10	0.023	0.020	0.021	0.021±0.0015
	20	0.124	0.126	0.125	0.115±0.0010
	40	0.523	0.527	0.521	0.527±0.0140
	80	1.221	1.210	1.209	1.213±0.0066
	160	1.937	1.925	1.817	1.893±0.0661
	320	2.554	2.462	2.470	2.495±0.0050
DFC-1	10	0.015	0.020	0.018	0.017±0.0025
	20	0.086	0.108	0.097	0.097±0.0115
	40	0.196	0.202	0.207	0.202±0.0055
	80	0.415	0.428	0.431	0.424±0.0085
	160	0.856	0.861	0.872	0.863±0.0081
	320	1.418	1.428	1.423	1.423±0.0056
	10	0.028	0.027	0.035	0.026±0.0085

DFC-2	20	0.129	0.125	0.131	0.125±0.0063
	40	0.408	0.405	0.423	0.412±0.0096
	80	0.864	0.866	0.883	0.871±0.0104
	160	1.558	1.589	1.581	1.576±0.0161
	320	2.221	2.279	2.286	2.262±0.0356
DFC-3	10	0.032	0.043	0.031	0.035±0.0066
	20	0.215	0.228	0.231	0.224±0.0085
	40	0.842	0.798	0.856	0.832±0.0302
	80	1.512	1.560	1.509	1.527±0.0286
	160	2.176	2.135	2.119	2.143±0.0294
	320	2.732	2.775	2.777	2.761±0.0254
DFC-4	10	0.030	0.041	0.037	0.036±0.0055
	20	0.203	0.219	0.234	0.218±0.0155
	40	0.589	0.593	0.609	0.597±0.0105
	80	1.346	1.407	1.408	1.387±0.0355
	160	1.876	1.886	2.187	1.981±0.1767
	320	2.689	2.671	2.676	2.678±0.0095
DFC-5	10	0.049	0.048	0.056	0.051±0.0043
	20	0.275	0.288	0.283	0.282±0.0065
	40	0.397	0.389	0.394	0.394±0.0040
	80	0.579	0.581	0.595	0.585±0.0087
	160	1.256	1.248	1.281	1.261±0.0172
	320	1.871	1.878	1.882	1.877±0.0055
DFC-6	10	0.050	0.049	0.057	0.052±0.0043
	20	0.276	0.289	0.284	0.283±0.0065
	40	0.398	0.390	0.395	0.394±0.0040
	80	0.580	0.582	0.596	0.586±0.0087
	160	1.257	1.249	1.283	1.262±0.0172
	320	1.872	1.889	1.883	1.881±0.0015

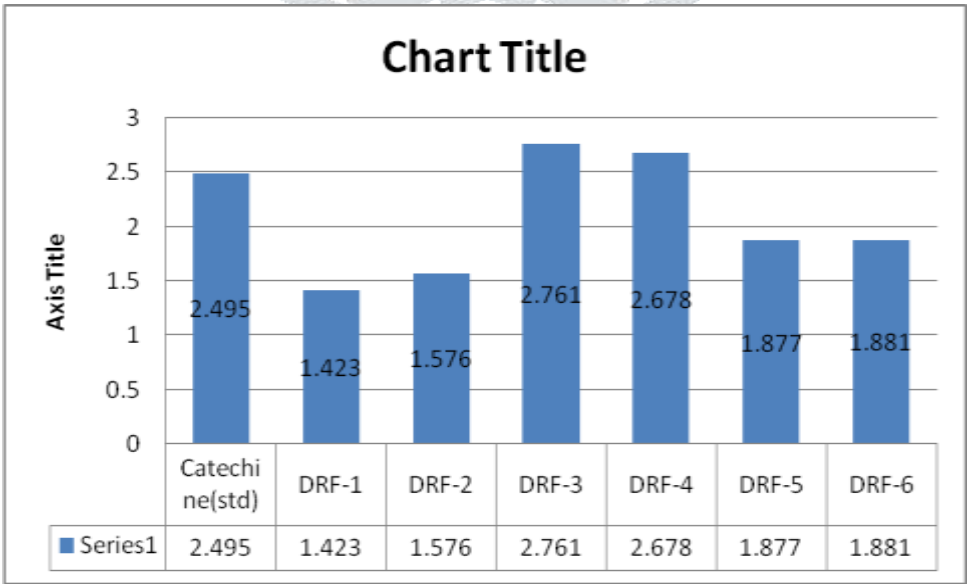


Fig7: At 320 µg/mL the absorbance of compounds DFC-1, DFC-2, DFC-3, DFC-4 & DFC-5 DFC-6 from dia-ion resin fraction and (+)-catechin (standard).

From the results, it was found that the isolated compounds (DFC-1, DFC-2, DFC-3, DFC-4 , DFC-5 and DFC-6), dia-ion resin fraction (DRF) and reference standard catechin tested exhibit reducing activity. Of them compound DFC-1, DFC-2, DFC-3, DFC-4, DFC-5 and DFC-6 showed the higher reducing power capacity which appeared to be very similar to the activity of the reference standard catechin.

The activity of the compounds and the reference standard catechin decreases in the following order.

DFC-3 > DFC-4 > Catechin > DFC-2 >DFC-5 > DFC-6 > DFC-1.

2.5 Determination of DPPH radical scavenging activity

Table-8: Data for DPPH radical scavenging activity of the different fractions of *S. mahagoni seeds*

Name of the sample	Conc. (µg/mL)	% of scavenging			% of scavenging Mean ± STD	IC_{50} (µg/mL)
		a	b	c		
(+)-Catechin (standard)	0.78	4.14	4.26	4.11	4.17 ± 0.0790	16.44
	1.56	24.25	24.37	25.34	24.65±0.597	
	3.125	48.33	47.77	47.75	47.95±0.329	
	6.25	56.41	55.57	55.42	55.83±0.5330	
	12.5	64.27	64.68	64.59	64.51±0.0461	
	25	71.54	72.06	71.45	71.68±0.3292	
	50	83.23	82.51	82.28	82.37±0.4956	
	100	94.12	94.27	93.23	93.87±0.5626	
Crude methanol extract (CME)	0.68	3.46	4.22	4.28	3.98 ± 0.4574	30.17
	1.56	13.85	14.17	14.72	14.25 ± 0.4400	
	3.125	32.59	33.32	33.59	33.16 ± 0.5173	
	6.25	41.27	43.13	42.48	42.29 ± 0.9439	
	12.5	53.43	52.85	54.47	53.58 ± 0.8208	
	25	62.73	65.12	64.38	64.07 ± 1.2235	
	50	76.34	77.26	79.11	77.57 ± 1.4107	
	100	82.76	83.72	82.87	83.11± 0.525	
Petroleum ether fraction (PEF)	0.78	0.035	0.046	0.037	0.039±0.0058	49.30
	1.56	1.16	1.20	1.21	1.19±0.0264	
	3.125	5.65	6.01	5.92	5.86±0.1873	
	6.25	26.02	25.43	25.56	25.67±0.3119	
	12.5	38.12	37.57	37.59	37.76±0.3041	
	25	49.88	49.33	49.38	49.53±0.3041	
	50	61.51	61.64	60.65	61.26±0.5379	
	100	69.34	69.15	68.99	69.16±0.1752	
Chloroform fraction (CLF)	0.78	0.074	0.65	0.086	0.07 ± 0.0124	43.96
	1.56	4.59	4.64	4.67	4.63 ± 0.0404	
	3.125	17.08	16.32	16.35	16.58±0.4303	
	6.25	29.37	30.17	29.47	29.67±0.4358	
	12.5	41.51	41.67	41.12	41.43±0.2829	
	25	52.91	53.15	51.95	52.67±0.6349	
	50	65.62	66.07	65.45	65.71±0.3203	
	100	73.09	72.56	71.70	72.45±0.7016	
Name of the sample	**Conc. (µg/mL)**	**% of scavenging**			**% of scavenging Mean ± STD**	**IC_{50} (µg/mL)**
		a	**b**	**c**		

Ethyl acetate fraction (EAF)	0.78	9.47	9.46	9.51	9.48 ± 0.0264	13.65
	1.56	27.83	27.81	27.87	27.84 ± 0.0305	
	3.125	42.37	42.31	42.40	42.36 ± 0.0458	
	6.25	57.85	57.83	57.89	57.86 ± 0.0305	
	12.5	68.11	68.12	68.19	68.14 ± 0.0435	
	25	78.77	78.80	78.85	78.81 ± 0.0404	
	50	90.07	90.10	90.17	90.11 ± 0.0513	
	100	96.35	96.36	96.32	96.34 ± 0.0207	
Dia-ion resin fraction (DRF)	0.78	4.53	4.52	4.55	4.53 ± 0.0158	26.17
	1.56	15.75	15.77	15.82	15.78 ± 0.0360	
	3.125	34.51	34.52	34.59	34.54 ± 0.0435	
	6.25	45.21	45.23	45.28	45.24 ± 0.0360	
	12.5	56.40	56.45	56.47	56.44 ± 0.0360	
	25	68.73	68.72	68.78	68.74 ± 0.0321	
	50	79.34	79.36	79.41	79.37 ± 0.0360	
	100	87.05	89.24	88.98	88.42±1.1964	

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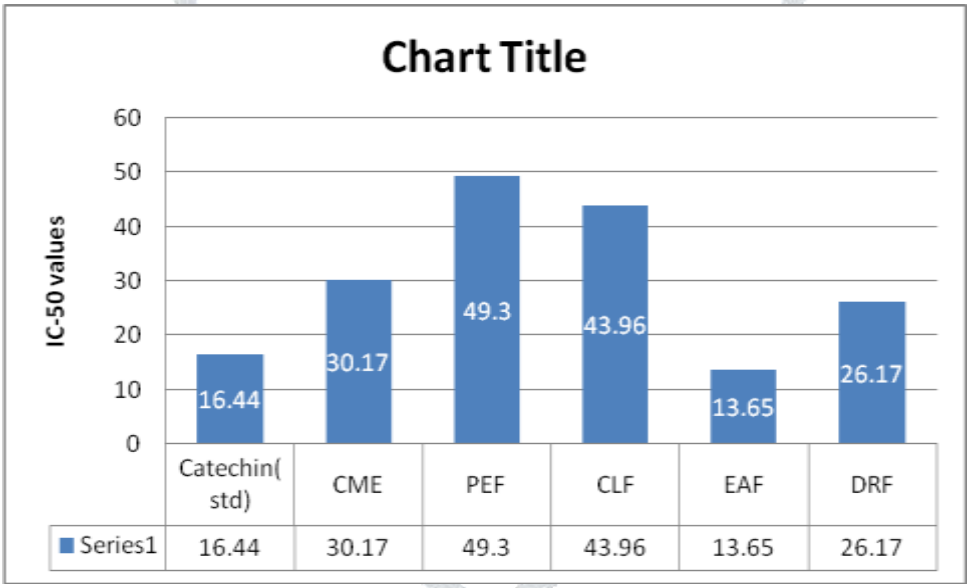


Fig 8: IC_{50} values of DPPH radical scavenging activity for CME, PEF, CLF, EAF and DRF of *S. mahagoni seeds* and(+)-catechin (standard).

Among the fractions of *S. mahagoni* EAF showed the highest scavenging activity with IC_{50} value 13.65 µg/mL. The crude methanol extract (CME) and dia-ion resin fraction (DRF) showed DPPH free radical scavenging with IC_{50} values of 30.17 and 26.17 µg/mL respectively, while the IC_{50} of (+)- catechin was 16.44 µg/mL. The results demonstrated that EAF exhibited strong scavenging activity followed by DRF and CME.

DPPH activity order: EAF > Catachin> DRF > CME > CLF > PEF.

2.6 Determination of DPPH radical scavenging activity of the isolated compounds from dia-ion resin fraction of *S. mahagoni seeds*

Table-9: Data for DPPH radical scavenging activity of isolated compounds from dia-ion resin fraction of *S. mahagoni seeds*

Name of the sample	Conc. (µg/mL)	% of scavenging			% of scavenging Mean ± STD	IC_{50} (µg/mL)
		a	b	c		
(+)-Catechin (standard)	0.78	4.86	4.88	4.91	4.89 ± 0.0360	16.39
	1.56	25.27	25.39	25.36	25.32±0.0624	
	3.125	48.15	47.69	47.77	47.87±0.2457	
	6.25	56.21	55.37	55.32	55.63±0.5000	
	12.5	64.17	63.68	63.59	63.81±0.3121	
	25	71.54	72.06	71.45	71.68±0.3292	
	50	83.23	82.51	82.28	82.67±0.4956	
	100	93.14	92.29	92.25	92.56±0.5026	
DFC-1	0.78	0.55	0.55	0.57	0.54±0.0115	49.80
	1.56	2.21	1.89	1.87	1.99±0.1907	
	3.125	5.68	5.77	5.79	5.73±0.0585	
	6.25	14.63	14.78	14.64	14.68±0.0838	
	12.5	32.51	32.59	32.38	32.49±0.1059	
	25	54.72	54.89	54.73	54.78±0.0953	
	50	67.67	68.08	67.73	67.83±0.2214	
	100	72.45	72.51	72.63	72.53±0.0916	
DFC-2	0.78	0.51	0.58	0.59	0.56±0.0435	45.31
	1.56	4.61	4.54	4.75	4.63±0.1069	
	3.125	9.35	9.74	10.12	9.74±0.3850	
	6.25	20.21	20.23	20.36	20.27±0.0814	
	12.5	42.56	42.72	42.73	42.67±0.0953	
	25	58.57	58.72	58.62	58.64±0.0763	
	50	70.23	69.60	69.69	69.84±0.3407	
	100	74.51	74.60	74.63	74.56±0.0624	
DFC-3	0.78	9.45	9.46	9.56	9.47±0.0608	12.06
	1.56	27.80	27.88	27.91	27.86±0.0568	
	3.125	42.31	42.43	42.33	42.36±0.0642	
	6.25	57.82	57.84	57.92	57.86±0.0529	
	12.5	68.11	68.12	68.19	68.14±0.0435	
	25	78.76	78.88	78.80	78.81±0.0611	
	50	90.16	90.07	90.10	90.11±0.0458	
	100	96.28	96.30	97.31	96.63±0.5889	
DFC-4	0.78	7.65	7.83	7.44	7.64±0.1951	16.39
	1.56	25.78	25.48	25.82	25.69±0.1858	
	3.125	38.45	37.98	38.83	38.42±0.4257	
	6.25	53.53	53.82	52.96	53.44±0.4375	
	12.5	64.89	65.14	64.76	64.93±0.1931	
	25	75.36	75.72	75.86	75.65±0.2579	
	50	87.56	87.91	87.58	87.68±0.1965	
	100	94.54	94.47	93.95	94.32±0.3223	
DFC-5	0.78	5.53	5.51	5.58	5.54±0.0360	26.24
	1.56	14.77	14.69	14.85	14.77±0.0800	
	3.125	34.53	34.54	34.64	34.57±0.0608	
	6.25	45.22	45.24	44.95	45.14±0.1619	
	12.5	56.42	56.44	54.45	55.77±1.1431	
	25	68.73	68.75	68.82	68.77±0.0472	

	50	79.35	79.31	79.43	79.37±0.0611	
	100	88.76	88.61	88.59	88.65±0.0929	

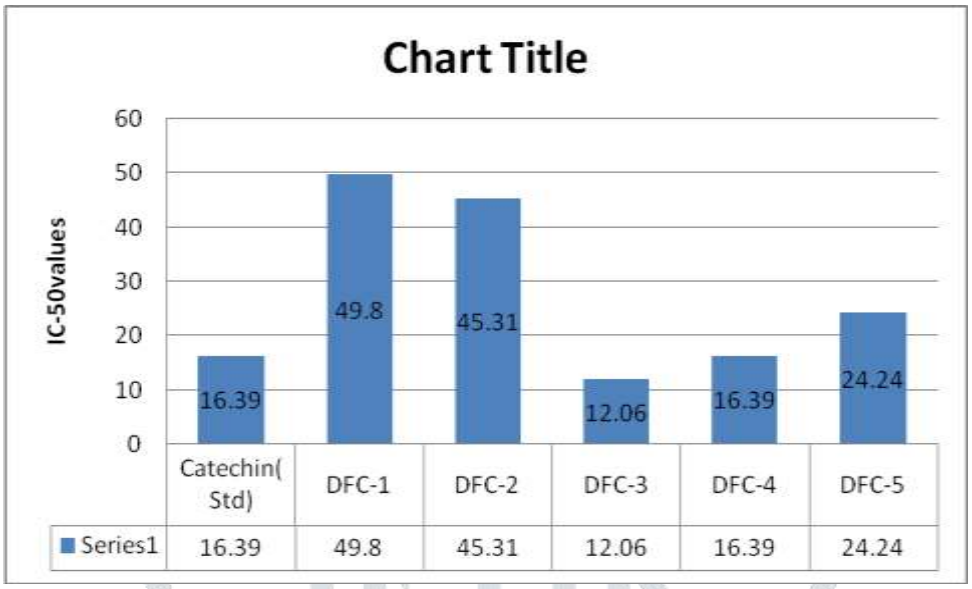


Fig 9: IC_{50} values of DPPH radical scavenging activity for isolated compounds DFC-1, DFC-2, DFC-3, DFC-4 and DFC-5and (+)-catechin (standard).

Among the isolated compounds from the dia-ion resinfraction DFC-3 and DFC-4 showed stronger DPPH radical scavenging activity with IC_{50} values 12.06 and 16.39 µg/mL respectively. While the compounds DFC-1, DFC-2 andDFC-5 showed moderate activity and the IC_{50} values were 49.8, 45.31 and 24.24 µg/mL, respectively.

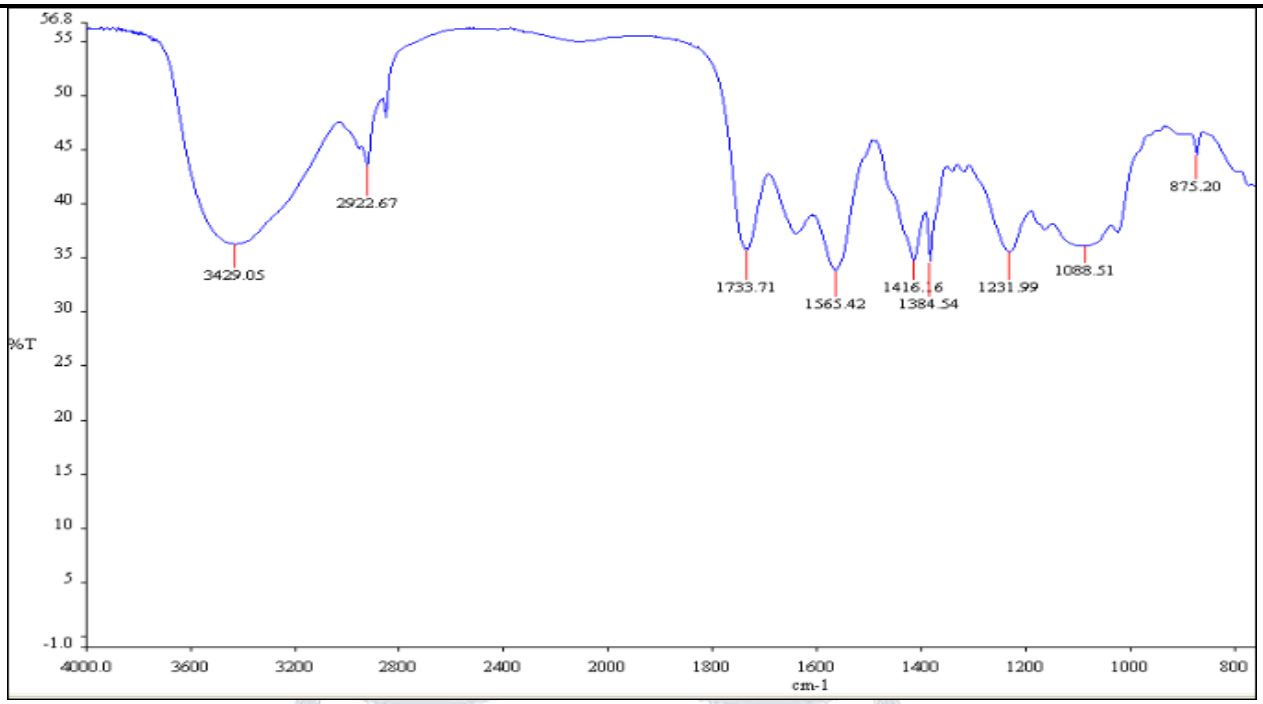
From our result, it is evident that the compounds tested possess DPPH radical scavenging activity and their activity decreases in the following order:

DFC-3>Catechin ≈ DFC-4 > DFC-5>DFC-2 >DFC-1.

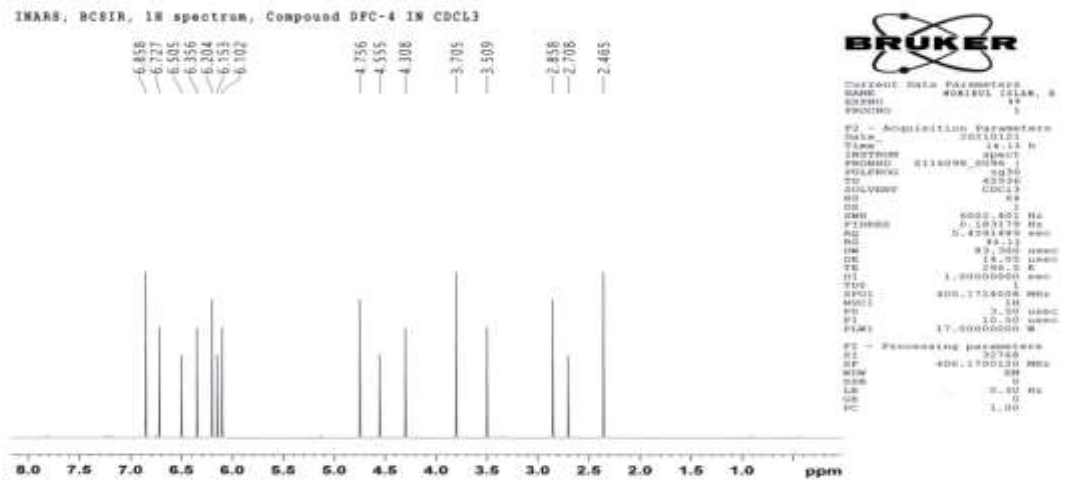
Spectroscopic data of isolated compounds DFC-4 (COM-4)

- **UV λ_{max} (MeOH)** = 212 ,213,242and 290 nm

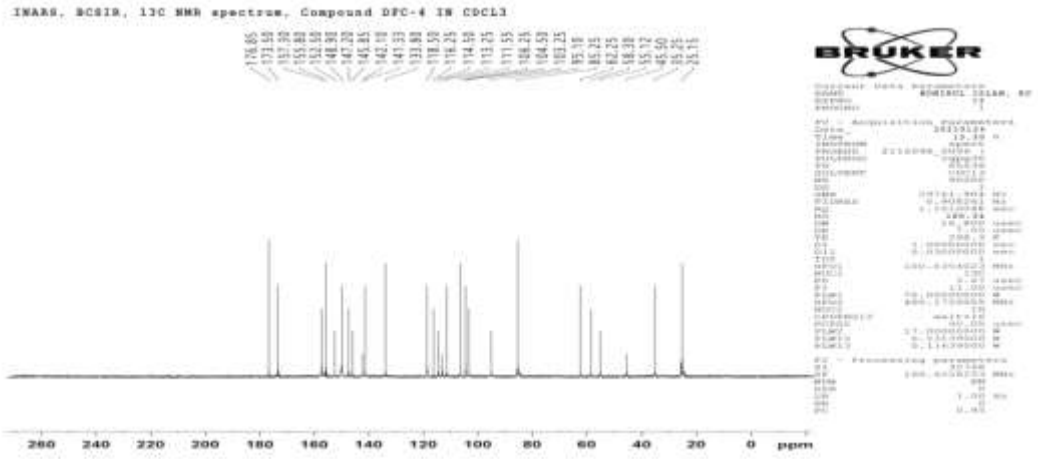
  IR ν_{max} (KBr) = 3429 cm^{-1}(-OH), 2922 cm^{-1} (–CH_3), 1733 cm^{-1}(= CO), 1565cm^{-1}(C=C), 1416cm^{-1}(aromatic C = C) and 1231 cm^{-1} (–$COOCH_3$).



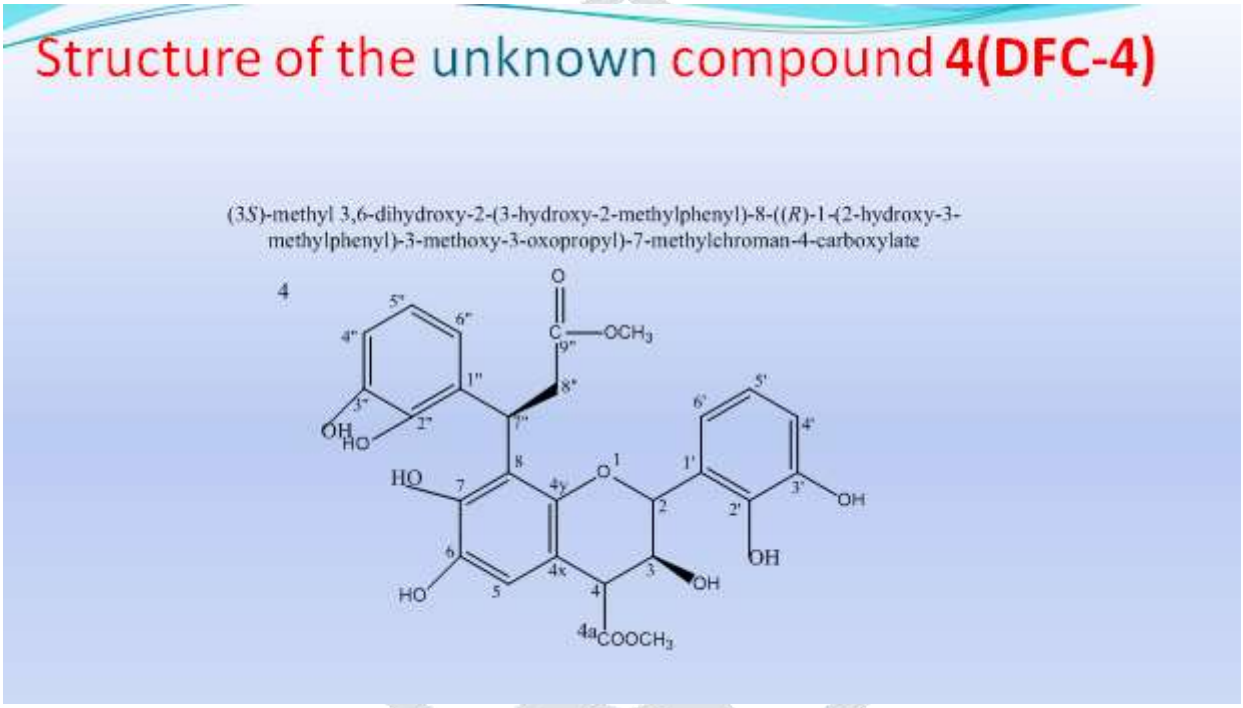
H-NMR (300 MHz) δ_{TMS} ($CDCL_3$) 2.46, 2.70, 2.85, 3.45, 4.45, 4.60, 4.70, 6.00, 6.15, 6.55, 6.60, 6.75, 6.80, and 6.85 ppm.



^{13}C-NMR (300 MHz) δ_{TMS} ($CDCL_3$)
25.15,35.17,45.50,52.34,68.25,82.27,95.50,101.25,102.30,104.05,114.80,115.30,118.80,118.35.119.56,132.60,142.20,143.35,145.20,147.50,149.23,152.75,153.20,156.52,and 176.55 ppm.



EI MS (*m/z*): 497[M]$^{+}$, 466,402,379,290, 272



CONCLUSIONS

The aim of this study was to evaluate the extracts of S. mahagoni seeds. Thus, all fractions specially CME and DRF are as active as the standard antioxidant activity.. The results of this study suggested that MCE and its individual fractions of S. mahagoni seeds are effective of as an anticancer, antioxidant agent. The result of our current research is to isolate phytoconstituents from individual DRF fractions that are responsible for all activities, and also to investigate the exact mechanism of action of isolated individual active compounds against terrible diseases. It seems that this information can be useful for utilization of S.mahagoni seeds, as functional food component as antioxidant supplement.

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