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Tahira Foyzun
Department of Pharmacy, Southeast University, Dhaka, Bangladesh

Koly Aktar
Department of Pharmacy, Southeast University, Dhaka, Bangladesh

Correspondence
Tahira Foyzun
Department of Pharmacy, Southeast University, Dhaka, Bangladesh

Comparison and evaluation of total phenolic, flavanoid content and antioxidant activity of crude methanol and ethyl acetate extracts of *Carica papaya* Leaves

Tahira Foyzun and Koly Aktar

Abstract
The present study describes the evaluation and comparison of antioxidant activity of the methanolic and ethyl acetate leaf extract (CME and CEE) of *Carica papaya*. The antioxidant potential was evaluated in terms of total phenolic and flavonoid content, DPPH radical scavenging potential, iron reducing power assay and total antioxidant capacity by standard procedures. Total phenolic and flavonoid contents were estimated as gallic acid and catechin equivalents respectively. Maximum phenolic (42.96 GAE/mg of plant extract) & flavonoid (39.56 CE/mg of plant extract) contents was found in CME. Both CME and CEE showed the potent antioxidant activity with IC_{50} 5.96 µg/ml & 6.79 µg/ ml respectively which are very close to standard ascorbic acid (IC_{50} 2.16 µg/ml). The CME showed potent total antioxidant activity and iron reducing power assay than that of CEE. The results suggested that both the extracts of the leaves of *C. papaya* possess potent antioxidant activities and can be a good source of natural antioxidant.

Keywords: *Carica papaya.* Phenolic content, Flavanoid content, total antioxidant capacity, DPPH

1. Introduction
Natural products from medicinal plants have gained huge interests from researchers around the world for new drugs because of their potential bioactivity effects [1-2]. There are number of advantages associated with using plants and plant phytoconstituents as opposed to pharmaceutical products. The plants extracts and its phytoconstituents are proven for its biological activities such as antidiabetic, antihyperlipidemic, free-radical scavenging, and anti-inflammatory activities. Most of the time, free-radical are playing an important role in the development of metabolic disorders, and it affects the quality of life [3]. Free radicals generated in aerobic metabolism are involved in a series of regulatory processes such as cell proliferation, apoptosis, and gene expression. When generated in excess, free radicals can counteract the defense capability of the antioxidant system, impairing the essential biomolecules in the cell by oxidizing membrane lipids, cell proteins, carbohydrates, DNA, and enzymes. Oxidative stress results in cytotoxic compounds occurrence (malonyl dialdehyde, 4-hydroxynonenal) and alters the oxidant-antioxidant balance (redox homeostasis) that characterizes normal cell functioning [4-6]. Oxidative stress-induced pathology includes cancer [7-8], cardiovascular disease [9], neural disorders [10], Alzheimer's disease [11], mild cognitive impairment [12], Parkinson's disease [13], alcohol induced liver disease [14], ulcerative colitis [15], atherosclerosis [16], and aging [17]. It has been reported that the secondary metabolites from higher plants such as phenolics and flavanoids are very potential to scavenge free radical. It is believed that the antioxidant capacity of the phenolic compounds is due to their redox properties, which allow them to react as a reducing agent [18-19]. So the plant constituents containing greater amount of phenols and flavanoids are able to exert protective effects in biological system against oxidative stress.

However, the study of medicinal plants still remains an area of research interest for unveiling the medicinal value of several plant species that is not studied thoroughly. *Carica papaya* Linn. is one such a plant with potential medicinal value and it is commonly called as paw- paw [20]. *C. papaya* is a member of the Caricaceae family [21]. It originated from Southern Mexico, Central America, and the northern part of South America. It is now cultivated in many tropical countries such as Bangladesh, India, Indonesia, Sri Lanka, the Philippines, and the West Indies including Malaysia [22]. The fruit is the most commonly used part and is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E. In traditional ayurved, the leaves were used in the treatment of asthma, jaundice, gonorrhea, wound healing and fever [22]. *C. papaya* leaves have been used in folk medicine for centuries. Recent studies demonstrated its beneficial effects such as anti-inflammatory [23], wound healing [24], antitumour, immune-modulatory [25], antimicrobial [26] and antioxidant [27] activity.

Extensive evaluation of traditional medicine for various medicinal activities is an obligatory step in the isolation and characterization of the active principle and further leading to drug development. In view of these, this study is therefore designed to evaluate the total phenolic content, total flavanoid content and antioxidant activity in terms of DPPH radical scavenging assay, total antioxidant determination and reducing power capacity assessment of *C. papaya* leaves.

2. Materials and Methods

2.1 Collection and Identification of the Plant Sample

Green leaves of *C. papaya* were collected from the local area of Narsingdi, Bangladesh during the month March 2016. The plant was identified by an expert taxonomist.

2.2 Preparation of Plant Sample

Having collected, the leaves of *C. papaya* were washed thoroughly in tap water and shade dried for several days with occasional sun drying. These were then dried in an oven for 24 hours at considerably low temperature (not more than 45°c) for better grinding. Dry samples of leaves were ground into fine powder in a grinding mill. The coarse powder was then stored in an air tight container and kept in cool and dry place for further use.

2.3 Extraction and Solvent Evaporation

The powdered plant materials were extracted by cold extraction process and taken in an amber colored reagent bottle and soaked in to 500 ml of methanol and ethyl acetate separately. The bottles with their contents were sealed and kept for 7 days with occasional shaking and stirring. The whole mixtures were filtered through cotton and Whatman no.1 filter paper and were concentrated with a rotary evaporator under pressure at 50°c temperature to afford crude extract known as crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya* leaves.

2.4 Determination of Total Phenolics

Total phenolic content of methanolic and ethyl acetate extracts of leaves of *C. papaya* were determined employing the method as described by Singleton *et al.* 1965 [28] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard. Firstly, 0.5 ml of plant extract or standard of different concentration solution was taken in a test tube and 2.5 ml of Folin – ciocalteu (diluted 10 times with water) reagent solution was added into the test tube. Then 2.5 ml of sodium carbonate (7.5%) solution was added and incubated for 20 minutes at 25°c to complete the reaction. Then the absorbance of the solution was measured at 760 nm using a spectrophotometer against blank.

2.5 Determination of Total Flavonoid

Total flavonoid content was determined by following the procedure of Dewanto *et al*. 2002 [29]. Catechin was used as standard and the flavonoid content of the extracts were expressed as mg of catechin equivalent/gm of dried extract. Firstly, 1ml of extract was placed in a volumetric flask, then 5ml of distilled water added followed by 0.3ml of 5% $NaNO_2$. After 5 minutes, 0.6 ml of 10% $AlCl_3$ was added and volume made up with distilled water. The solution was mixed and absorbance was measured at 510 nm.

2.6. Determination of antioxidant activity

2.6.1. DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

The antioxidant activity of both methanol and ethyl acetate extracts were determined in terms of hydrogen donating ability, using the DPPH method with a minor modification [30-31]. Firstly, 2 ml of methanol solution of plant extract or standard at different concentration was taken in a test tube. Then 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 following equation was used to determine the percentage of the radical scavenging activity of each extract.

DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

Where, $Abs_{control}$ = absorbance of DPPH radical + methanol;

Abs_{sample} = absorbance of DPPH radical + sample extract /standard.

The concentrations of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

2.6.2 Total antioxidant activity determination

Total antioxidant capacity of both extracts (CME and CEE) of *C. papaya* was measured spectrophotometrically through phosphomolybdenum method with some modifications by Prieto *et al.* (1999) [31]. An aliquot of 0.5 ml of sample solution was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 ºc for 10 minutes. After the sample had cooled to room temperature, the absorbance of aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions. Ascorbic acid, α-tochopherol can be used as standard.

2.6.3 Iron Reducing power assay

The reducing power of CME and CEE were evaluated by the method of Oyaizu (1986) [32]. 1.0 ml of plant extract or standard of different concentration solution was taken in a test tube. 2.5 ml of potassium buffer (0.2 M) and 2.5 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. 2.5 ml of trichloro acetic acid, 10% solution was added into the test tube. The total mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml supernatant solution was withdrawn from the mixture and mix with 2.5 ml of distilled water. 0.5 ml of ferric chloride ($FeCl_3$), 0.1% solution was added to the diluted 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.

3. Results and Discussions

3.1 Determination of Total Phenolic Content

Total phenolic content of the extracts is expressed as milligrams of Gallic acid equivalent per gram. The yield of

the total phenolic content showed that crude methanolic extract (CME) and crude ethyl acetate extract (CEE) were 42.96±1.420 and 25.20±0.745 mg of GAE/gm of dried sample respectively. The result demonstrated that the total phenolic content of crude ethyl acetate extract (CEE) is lower than that of crude methanolic extract (CME).

Table 1: Absorbance of Gallic acid at different concentrations after treatment with Folin-Ciocaltue reagent.

Concentration (µg/ml)	Absorbance			Absorbance Mean ± STD
	a	b	C	
1	0.078	0.075	0.076	0.076 ± 0.001
2	0.176	0.171	0.181	0.176 ± 0.005
4	0.364	0.368	0.372	0.368 ± 0.004
8	0.722	0.718	0.726	0.722 ± 0.004
16	1.413	1.417	1.423	1.417 ± 0.005
32	2.758	2.752	2.764	2.758 ± 0.006

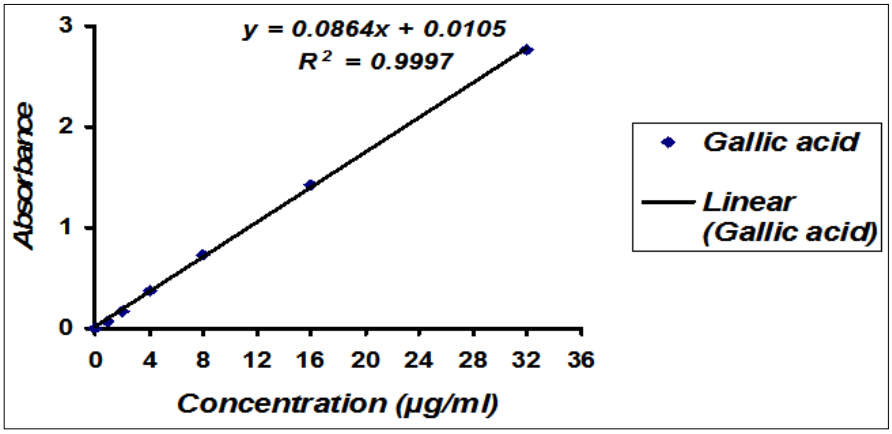

Fig 1: Standard curve of gallic acid for the determination of total phenolic content.

Table 2: Determination of total phenolic content of CME and CEE of *C. papaya.*

Sample	No.of sample	Concentration (µg/ml)	Absorbance	GAE/gm of dried sample	GAE/gm of dried sample Mean ± STD
Crude methanolic Extract(CME)	1	250	0.934	42.97	42.96±1.420
	2	250	0.948	42.69	
	3	250	0.945	43.20	
Crude ethyl acetate extract(CEE)	1	250	0.560	25.302	25.20±0.745
	2	250	0.558	25.209	
	3	250	0.556	25.201	

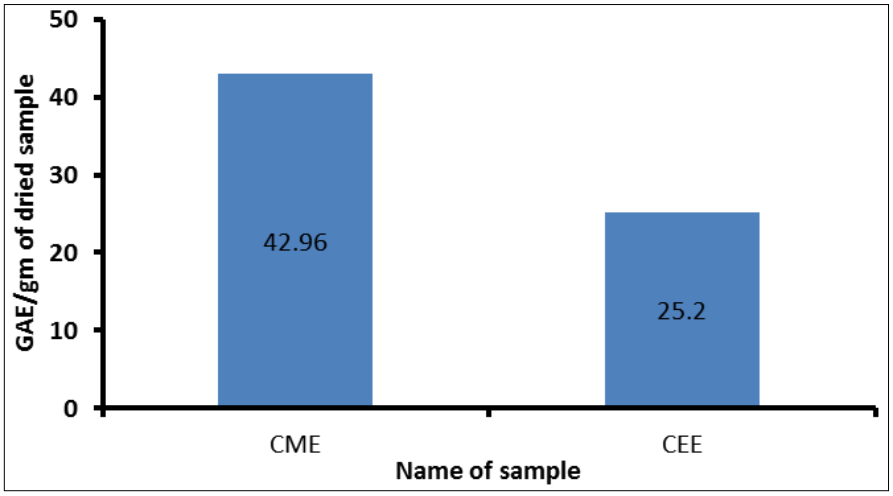

Fig 2: Total phenolic content (mg/gm plant extract of gallic acid equivalent) of the crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya.*

3.2 Determination of Total Flavonoids: The total flavonoid content of crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya*were shown in table 3.3 and figure 3.3. The results were expressed as mg of catechin equivalent per gram of dried sample. The values represented the mean of triplicates ± STD of crude methanolic extract (CME) and crude ethyl acetate extract (CEE).

The results showed that, total flavonoid content (TFC) of crude methanolic extract (CME) and crude ethyl acetate extract (CEE) were 39.564±1.420 and 13.647 ±0.745 mg of CE/gm of dried extract respectively. So the total flavanoid content of the CME is three fold higher than that of CEE.

Table: 3: Absorbance of catechin (standard) at different concentrations for quantitative determination of total flavonoids.

Concentration (µg/ml)	Absorbance			Absorbance Mean ±STD
	a	b	c	
31.25	0.241	0.225	0.260	0.242 ± 0.017
62.5	0.380	0.398	0.362	0.380 ±0.018
125	0.726	0.722	0.731	0.726 ± 0.004
250	1.476	1.481	1.468	1.475 ± 0.006
500	2.667	2.670	2.599	2.645 ± 0.040

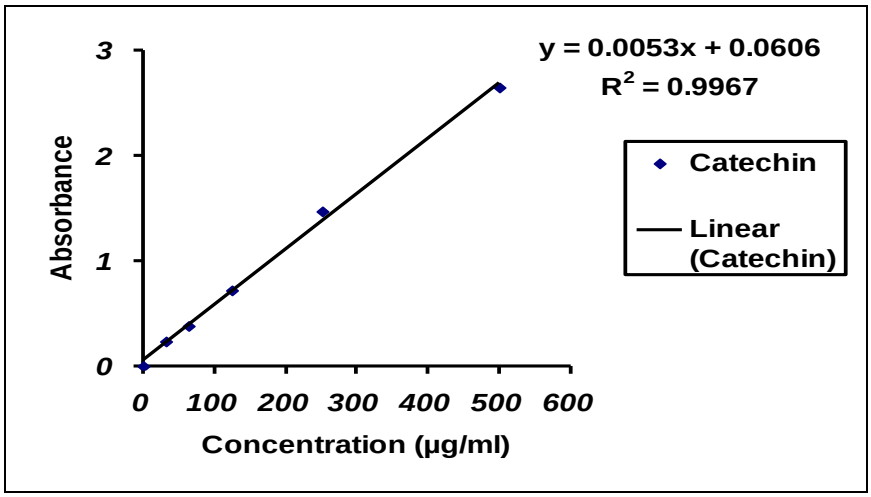

Fig 3: Standard curve of catechin for the determination of total flavonoid content.

Table 4: Determination of total flavonoid content of the crude methanol extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya.*

Sample	No.of sample	Concentration (µg/ml)	Absorbance	CE/gm of dried sample	CE/gm of dried sample Mean ± STD
Crude methanolic Extract (CME)	1	250	0.450	40.37	39.564±1.420
	2	250	0.430	38.51	
	3	250	0.444	39.81	
Crude ethyl acetate extract (CEE)	1	250	0.170	14.33	13.647 ±0.745
	2	250	0.160	13.40	
	3	250	0.158	13.21	

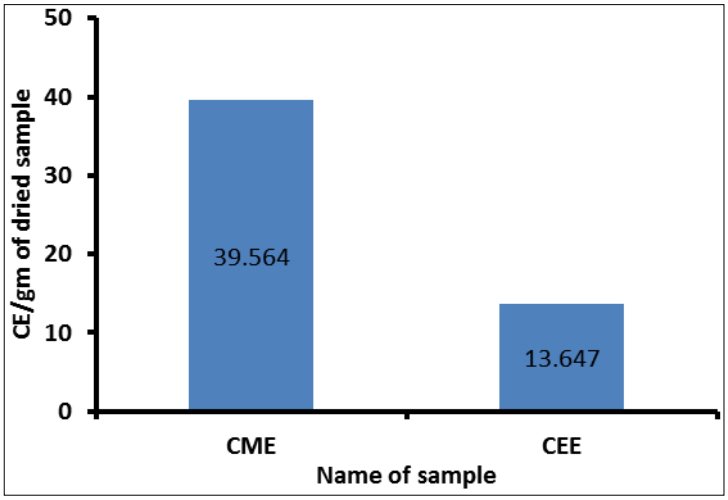

Fig 4: Total flavonoid content (mg/gm plant extract of catechin equivalent) of the crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya* leaves.

3.3 Determination of Antioxidation activity

3.3.1 DPPH Radical Scavenging Activity: The antiradical activity of the crude methanolic extracts (CME) and crude ethyl acetate extract (CEE) of *C. papaya* were evaluated by the ability to scavenge DPPH free radicals and was compared with the standard Ascorbic acid. It was observed that both extract possess potent scavenging activities which were must similar with the standard. Fig 3.5 shows the dose response curve of DPPH radical scavenging activity of CME and CEE with a standard antioxidant Ascorbic acid. At a cocentration of 100 µg/ ml the scavenging activities of CME and CEE were 93.90% and 90.60% respectively while at the same concentration the standard Ascorbic acid scavenges 96.89%. IC_{50} values of crude methanolic extract (CME) and crude ethyl acetate extract (CEE) were 5.96 µg/ml and 6.79 µg/ml respectively compared with the standard Ascorbic acid with a IC_{50} value of 2.16µg/ml.

Table 5: DPPH radical scavenging activity of the crude methanol extract (CME) and crude ethyl acetate extract (CEE) of *C. Papaya* and Ascorbic acid (Standard) at different concentrations.

Name of sample	Concentration(µg/ml)	% of scavenging			% of scavenging Mean ± STD	IC_{50} (µg/ml)
		a	b	c		
Ascorbic acid (standard)	0.8	26.36	27.29	28.81	27.49±1.23	2.16
	1.6	39.4	37.61	39.7	38.90±1.05	
	3.1	73.23	69.86	71.33	71.47±1.69	
	6.25	85.11	81.54	83.18	80.28±1.19	
	12.5	95.23	94.5	95.19	88.37±0.37	
	25	94.15	95.72	96.38	94.38±1.12	
	50	95.18	94.52	93.41	94.43±0.89	
	100	96.54	98.49	95.64	96.89±1.425	
Crude methanolic extract (CME)	0.8	15.23	16.14	16.94	16.20±0.86	5.96
	1.6	23.89	22.53	21.37	22.60±1.26	
	3.1	34.57	33.29	36.25	34.70±1.48	
	6.25	50.53	51.56	52.18	51.42±0.83	
	12.5	62.61	61.51	64.2	62.77±1.35	
	25	72.95	70.42	73.25	75.21±1.42	
	50	87.09	86.95	89.48	87.84±1.27	
	100	93.20	93.41	95.11	93.90±0.96	
Crude ethyl acetate extracts (CEE)	0.8	11.67	13.54	12.38	12.53±0.94	6.79
	1.6	18.67	19.24	17.49	18.47±0.88	
	3.1	28.65	27.55	29.31	28.50±0.88	
	6.25	47.57	47.61	48.16	47.78±0.30	
	12.5	58.49	59.17	56.32	57.97±1.43	
	25	67.22	69.29	65.73	70.01±1.78	
	50	82.37	82.44	82.17	82.33±0.14	
	100	90.23	91.74	89.84	90.60±0.95	

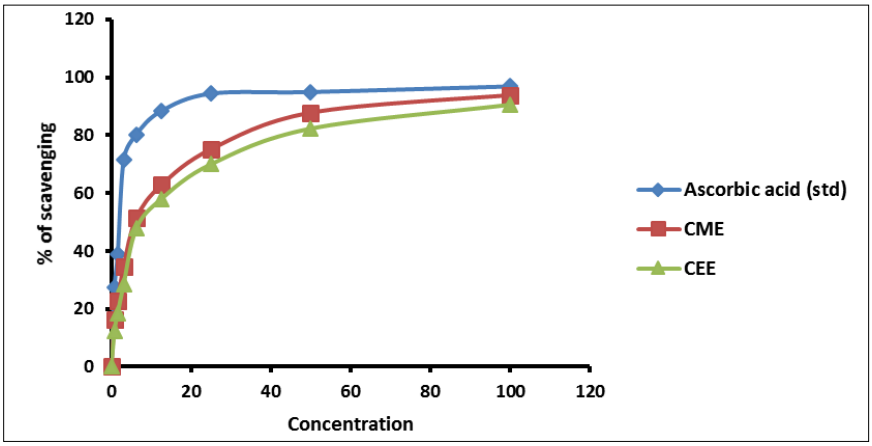

Fig 5: DPPH radical scavenging activity of crude methanol extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya* and Ascorbic acid (Standard).

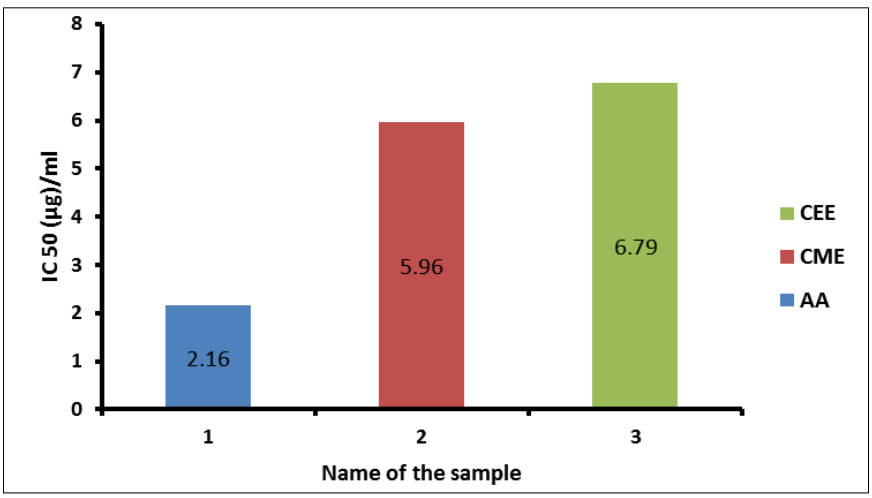

Fig 6: IC_{50} (µg/ml) values of crude methanol extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya* leaves and Ascorbic acid (Standard).

3.3.2 Total antioxidant assay

The total antioxidant activity was measured and compared among crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya* and the reference standard catechin. The high absorbance values indicated that the sample possessed significant antioxidant activity. The results revealed that both extracts had significant antioxidant activities and the effects increased with increasing concentration (table 3.6 and figure.3.7). The absorbance value of Catechin (Standard), crude methanolic extract (CME) and crude ethyl acetate extract (CEE) at 600 µg/ ml were 2.042±0.007, 1.022±0.009 and 0.969±0.011 respectively, which demonstrated that the total antioxidant activity of catechin (standard) is higher than that of crude methanolic extract (CME) crude ethyl acetate extract (CEE). Between the two extracts, the crude methanolic extract is more potent than crude ethyl acetate extract and has moderate antioxidant activity compared to the standard catechin.

Table 6: Total antioxidant activity of crude methanolic extract (CME) andcrude ethyl acetate extract (CEE) of *C. papaya* and catechin (standard) at different concentration.

No. of sample	Concentration (µg/ml)	Absorbance			Absorbance Mean ± STD
		A	B	C	
Catechin (Standard)	200	2.035	2.041	2.049	3.812±0.007
	100	1.575	1.583	1.592	2.291±0.009
	80	0.916	0.932	0.950	1.894±0.017
	40	0.684	0.705	0.721	1.098±0.018
	20	0.518	0.521	0.523	0.64±0.002
	10	0.423	0.419	0.427	0.33±0.004
Crude methanolic Extract (CME)	200	1.013	1.023	1.029	2.45±0.009
	100	0.877	0.892	0.904	1.891±0.012
	80	0.501	0.521	0.539	1.620±0.018
	40	0.434	0.437	0.439	0.937±0.003
	20	0.298	0.305	0.315	0.406±0.010
	10	0.245	0.257	0.279	0.260±0.012
Crude ethyl acetate extract (CEE)	200	0.957	0.969	0.981	1.969±0.011
	100	0.611	0.628	0.644	1.528±0.016
	80	0.421	0.432	0.469	1.341±0.011
	40	0.301	0.303	0.305	0.703±0.002
	20	0.273	0.282	0.294	0.353±0.009
	10	0.174	0.183	0.199	0.185±0.009

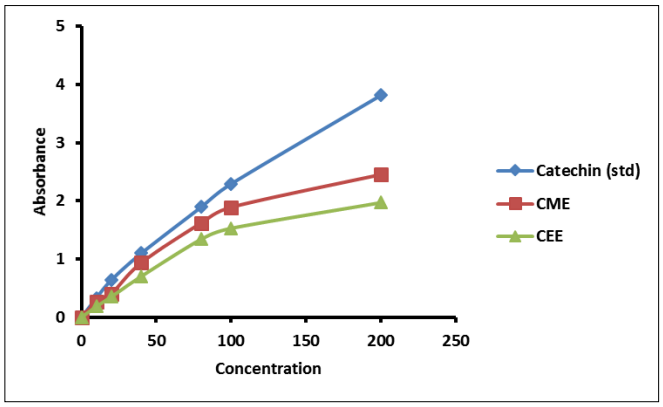

Fig 7: Total antioxidant activity of crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya* leaves and catechin (Standard) at different concentration.

3.3.3 Iron reducing power assay

Reducing power is an important parameter of assessment for the antioxidant activity. The Fe^{3+} reducing power of crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of *C. papaya* and ascorbic acid (standard) was determined. The reducing power activities of the extracts were increased with the concentration of the extract. Both the extracts exhibited the moderate reducing ability compared to the standard ascorbic acid. The methanolic extract (CME) demonstrated the highest power reducing potency than that of CEE. The reductive capabilities of crude methanolic and ethyl acetate extract and standard ascorbic acid were shown in table 3.7. In assays of the reducing power of the crude extract, significant changes in absorbance at 700 nm were observed with increasing concentration.

Table 7: Iron reducing power capacity of crude extract of methanol and ethyl acetate of *C. papaya* and ascorbic acid (standard) at different concentration.

No. of sample	Concentration (µg/ml)	Absorbance			Absorbance Mean ± STD
		A	B	C	
Ascorbic acid (Standard)	5	0.798	0.802	0.807	0.402±0.004
	10	0.914	0.918	0.921	0.921±0.005
	20	1.360	1.363	1.369	1.569±0.006
	40	2.388	2.393	2.397	2.797±0.003
	80	3.624	3.629	3.635	3.629±0.004
	160	4.001	4.003	4.006	4.003±0.009
Crude methanolic extract (CME)	5	0.088	0.092	0.097	0.083±0.005
	10	0.145	0.149	0.154	0.236±0.006
	20	0.274	0.279	0.284	0.523±00004
	40	0.722	0.729	0.736	0.982±0.007
	80	1.525	1.529	1.534	1.753±0.007
	160	2.917	2.919	2.922	2.919±0.006
	5	0.076	0.083	0.091	0.072±00009

Crude ethyl acetate extract (CEE)	10	0.130	0.136	0.141	0.199±0.004
	20	0.219	0.223	0.228	0.379±0.006
	40	0.676	0.681	0.689	0.828±0.005
	80	1.350	1.353	1.355	1.431±0.002
	160	2.816	2.819	2.823	2.592±0.004

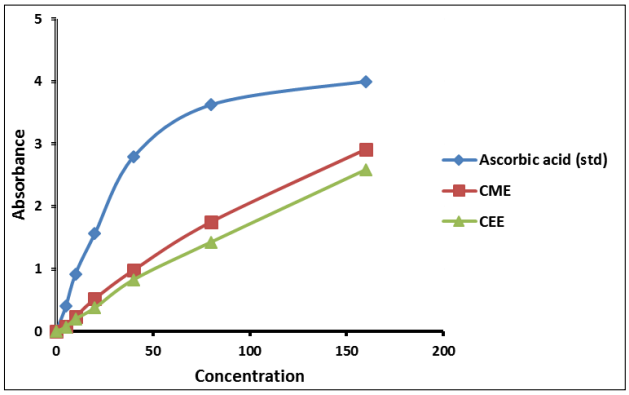

Fig 8: Iron reducing power capacity of crude extract of methanol and ethyl acetate of *C. papaya* leaves and ascorbic acid (standard).

4. Conclusion: In conclusion, the study demonstrates that the crude methanol and ethyl acetate leaves extracts of *C. papaya* are different in their antioxidant effects. The results indicate that the methanolic extract possess the highest antioxidant activity than that of ethyl acetate extract. However, further pharmacological & toxicity studies are necessary to confirm this suggestion. Phytochemicals analysis should be carried out to characterize the compounds in *C. papaya* that act as antioxidant agents. This work suggested that *C. papaya* may be considered as a useful source of human health, as an antioxidant agent.

5. References

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