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SCREENING OF ANTIOXIDANT ACTIVITIES OF BARK OF *CARICA PAPAYA* L BY DIFFERENT METHODS

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Abstract

In this study, we have investigated ethanolic extract of bark of *Carica papaya* L. to identify antioxidant activities by using different methods. The yield of bark extract was found to be 2.81% w/w after extraction. At first, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay was performed where the IC_{50} (inhibitory concentration) value of extract was 23.89 µg/ml and the IC_{50} value of ascorbic acid which is used as standard antioxidant was 12.9 µg/ml. The bark extract showed significant TPC (Total Phenolic Content) of 328.56 mg GAE/gm of dry extract by using Gallic acid calibration curve, TFC (Total Flavonoid Content) of 195.329 mg QE/gm of dry extract by using Quercetin calibration curve and TTC (Total Tannin Content) of 47.239 mg GAE /gm of dry extract by using Gallic acid calibration curve. The above study suggested that *Carica papaya* L. bark extract showed significant antioxidant activities which may play an important role in drug discovery from natural products.

Keywords: Carica papaya L., Antioxidant, Ascorbic acid, DPPH, Gallic acid, Quercetin.

Introduction

Medicinal plants commonly used in traditional medicine which are harmless and natural. In addition, the availability, affordability, reliability and low toxicity of medicinal plants have made them acceptable and popular in health care services all over the world [1]. *Carica papaya* is known as pawpaw which belongs to Caricaceae family. It is primarily native to the tropics of Africa and South America but is now widely cultivated all over the world throughout the year [2]. It generally lives about five to ten years, and grows with a single unbranched stalk. The leaves are lobed, up to 75 cm across, on long, hollow petioles [3].

Carica papaya has been widely reported health and nutritional benefits [4]. The chemicals chymopapain, papain and cell strengthening supplements have been discovered in papaya which are useful in bringing down irritation. *Carica papaya* leaves used for the treatment of bacterial dengue and fungal infections as well as anti-aging products [5]. Thus, the traditional use of *Carica papaya* is well established.

Methods

1.1 Sample collection: Fresh barks of *C. papaya* were collected from Narayanganj, Bangladesh.

1.2 Preparation of *C. papaya* bark extract: The collected barks materials were washed with clean water to avoid any adulterations and then dried for about 30 days. The dried barks were cut into smaller pieces and made them into a fine powder. The dried powder was soaked with ethanol for 7 days and then subjected to solvent extraction using the Soxhlet apparatus. Finally, the extracted sample was stored at 4°C and was ready for further analysis [6].

1.3 DPPH free radical scavenging assay

1.3.1 Qualitative Antioxidant Assay based on TLC

To perform qualitative antioxidant assay by using the technique DPPH free radical scavenging assay, the extracted sample of bark of *C. papaya* was diluted with ethanol and then uniformly spotted on TLC plates. The plates were run in polar solvent, medium polar and non-polar solvent systems to separate polar, medium polar and non-polar components of the bark extract. The TLC plates were then dried at normal temperature and the sprayed with 0.02% DPPH which was mixed ethanol. After spraying DPPH, the plates were placed under UV light to observe different components of the bark extract based on different color changes in shorter and longer wavelengths respectively which specified the presence of UV positive materials in the bark extract and they were marked.

1.3.2 Quantitative DPPH Free Radical Scavenging Assay

At first, stock solution of crude extract and ascorbic acid were prepared separately by using ethanol as a solvent containing the concentration 1024 µg/ml. Then the stock solution was serially diluted to get the concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml for bark extract and ascorbic acid. The prepared solutions were stirred vigorously for minimum 15 seconds to get uniform mixture. Then the solutions were permitted to stand at dark place at room temperature for 30 min. After 30 min, absorbance was measured against a control at 517 nm with Shimadzu UV/ visible spectrophotometer. The percentage of DPPH radical-scavenging activity of bark extract and ascorbic acid was calculated by using the following formula:

$$\text{Percent scavenging activity} = \left(1 - \frac{\text{Absorbance of sample or standard}}{\text{Absorbance of control}}\right) \times 100$$

Finally, the percent DPPH radical-scavenging activity was plotted against the plant extract and ascorbic acid concentration (µg/ml) separately [7].

1.4 Total Phenolic Content Assay

The TPC (total phenolic content) of the bark extract of plants was tested by the modified Folin-Ciocalteu method [8]. 0.5 ml of ethanol solution of bark extract (l mg/ml) was mixed with 5 ml 10% (v/v) Folin-Ciocalteu reagent dissolved in distilled water. Then 4 ml 7.5% w/v aqueous sodium carbonate solution was added to the mixture. Blank was concomitantly prepared by using same procedure. Then final solution was diluted by 10 times. The mixture was properly mixed for 15 seconds by vortexing and allowed to incubate at 40°C for 30 minutes. The absorbance was measured at λ_{max}=765 nm against the blank by using spectrophotometer. All determinations were performed in duplicate. The standard curve was prepared using 0.5,0.4,0.3, 0.2,

0.1 mg/ml solutions of Gallic Acid in methanol :water (50:50, v/v).The following equation was obtained from a standard Gallic acid calibration curve.

$$Y=0.942X+0.016,\ R^2 = 0.991$$

Where Y is the absorbance and X is the concentration of Gallic acid (mg/ml).

Based on the measured absorbance, Gallic Acid Equivalent (GAE) was measured (mg/ml) from the calibration line by using the following equation-

$$\mathrm{GAE} = \frac{\text{Absorbance of sample} - 0.016}{0.942}$$

Then total phenolic content (TPC) in plant ethanol extract in Gallic acid equivalents (GAE) was calculated by using the following equation

$$\mathrm{TPC(mg\ GAE/g)} = \frac{\mathrm{GAE}}{\text{Sample concentration(g/ml)}}$$

1.5 Total Flavonoid Content Assay

The TFC (total flavonoid content of ethanol) extract was tested using aluminum trichloride colorimetric method. 1 ml methanol solution of bark extract (1 mg/ml) was mixed with 4 ml distilled water and 0.3 ml 5% (w/v) sodium nitrate solution. Five minutes later, 0.3 ml 10% (w/v) aluminum chloride was added to the mixture. After one minute, 2 ml of 1 M sodium hydroxide solution was added to the mixture. The final volume of the mixture was adjusted to 10 ml with distilled water. Blank was concomitantly prepared. The mixture for both blank and extract were vortexed for 15 seconds. Then the solution was placed to stand for 30 min for reaction at normal temperature. The absorbance was taken at λ_{max} = 510 nm against the blank by using spectrophotometer (double beam Shimadzu UV/ visible spectrophotometer (Model 1800, Japan). All determinations were performed in duplicate [9].

The calibration curve was prepared using 0;0.1;0.2;0.3;0.4 and 0.5 mg per ml solutions of Quercetin in methanol. The following equation was obtained from a standard Quercetin calibration curve

$$y=1.034x+0.009,\ R^2 = 0.990$$

Based on the measured absorbance total Flavonoids content values are expressed in terms of Quercetin equivalents (QE) per gram of dry extract.

1.6 Total Tannin Content Assay

The tannins of bark extract were determined using the Folin-Ciocalteu phenol reagents. Briefly 0 .1 ml of the sample extract is added with 7.5 ml of distilled water and then added 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and diluted to lo ml with distilled water. The mixture was shaken well kept at normal temperature for 30 min and absorbance was measured at 725 nm with a double beam UV/ Visible spectrophotometer double beam Shimadzu UV/ visible spectrophotometer [10].

The standard curve was prepared using 0.5; 0.4; 0.3; 0.2; 0.1 mg/ml solutions of Gallic acid in methanol. Total tannin content was determined as mg of Gallic acid equivalent per gram of dry extract using the following equation obtained from a standard Gallic acid calibration curve.

$$y = 1.125x + 0.002,\ R^2 = 0.998$$

Results

2.1 DPPH free radical scavenging assay

2. 1.1 Qualitative Antioxidant Assay based on TLC

After applying DPPH on the TLC plate, yellow color on purple background was observed which indicated the presence of antioxidant components in the extract of plants (Figure 1). In the TLC-based qualitative antioxidant assay using DPPH assay, bark of ***C. papaya*** showed the free radical scavenging properties indicated by the presence of yellow spot on a purple background on the TLC plate.

2.1.2 Quantitative DPPH Free Radical Scavenging Assay

In the present study, the IC_{50} value of ascorbic acid (standard antioxidant), was **12.9 µg/ml** where the IC_{50} value of bark of *C. papaya* was **23.89 µg/ml** which is a well-known antioxidant (**Figure 2 & Table 1**).

2.2 Total Phenolic Content Assay

The result of the present study showed that total phenolic contents of ***C. papaya*** bark extract was found ~ 329mg GAE/gm of dry extract respectively (**Figure 3 & Table 2**).

2.3 Total Flavonoid Content Assay

The result of the present study showed that total flavonoid contents of bark of *C. papaya* extract was found ~196mg QE/ gm of dry extract respectively (**Figure 4 & Table 3**).

2.4 Total Tannin Content Assay

The result of the present study showed that total tannin contents of bark of *C. papaya* extracts were found ~ 48mg GAE/gm of dry extract respectively (**Figure 5 & Table 4**).

Discussion

Plants have been traditionally well recognized as an important source of novel bioactive compounds [11]. Health care and nutrition are strongly interrelated and many plants have been consumed both as medicinal purposes and for food. TLC plates with numerous detecting reagent used did not identify all compounds present in bark extract, and latter was seen under UV light at 254 nm and 366 nm [12]. Phenolic compounds can be recognized using at 254 nm in UV spectrophotometers [13]. At 366nm, coumarins and flavonoids are fluorescence and easily detected. Detection and quantification were reported at λ = 254 nm for Gallic acid and 366nm for rutin and quercetin. The present study shows that the qualitative antioxidant presence test was done on TLC plate using ethanolic extract of bark of *C. papaya* with ascorbic acid (standard) after applying 0.02 % DPPH with various types of solvents. From the above discussion it can be determined that ethanol extract of bark of *C. papaya* contains phytochemical compounds that have potent free radical scavenging antioxidant property.

Free radical is one of the common barriers for maintaining a healthy life [14]. DPPH is a stable free radical which is reduced to the DPPH-H when antioxidants react with DPPH as a result the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging power of the antioxidant compounds of bark extract in terms of hydrogen donating ability. The quantitative DPPH antioxidant assay of bark of *C. papaya* was based on the ability of 1, 1-diphenyl-2picrylhydrazyl (DPPH), a stable free radical, to decolorize (visible deep purple color) in the presence of antioxidants [15]. The DPPH radical scavenging activity was increased by increasing the concentration of the sample extract. In the present study, the IC_{50} value of ascorbic acid (standard antioxidant), was **12.9 µg/ml** where the IC_{50} value of bark of *C. papaya* was **23.89 µg/ml** which is a well-known antioxidant.

Phenolic compounds are secondary metabolites that are derivatives of the shikimate, pentose phosphate, and phenylpropanoid pathways in plants. These compounds contain an aromatic ring bearing one or more hydroxyl groups and these hydroxyl groups are responsible for their free radical scavenging ability [16]. Bark of *C. papaya* contains many types of phenolic compounds and these phenolic compounds act as free radical scavenger. In my study, total phenolic content of extract was **328.56 mg GAE/gm** of dry extract by using Gallic acid calibration curve (R^2 = 0.985). Since the ethanol extract was found to contain phenolic compounds, bark of *C. papaya* will protect different organs of the body from free radical damage and free radical causing diseases.

Flavonoids possess a broad spectrum of biological and chemical activity, including radical scavenging properties [17]. They act as efficient scavengers of free radicals and due to their ability to act as hydrogen donors and they interrupt oxidative chain reactions. Flavonoids may help to provide protection against diseases by contributing along with antioxidant vitamins and enzymes. Total flavonoid content of ethanol extract was **195.329 mg QE/gm** dry extract by quercetin calibration curve (R^2 = 0.991).

Tannin are secondary metabolites, which are soluble in polar solution and are distinguished from other polyphenolic compounds by their ability to precipitate proteins. This phenolic compounds react with Folin-Ciocalteu Reagent (FCR) only under basic conditions. Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing FCR. This supports that the reaction occurs through electron transfer mechanism. The blue compounds formed between phenolate and FCR are independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complexes formed between the metal center and the phenolic compounds. Phenolic compounds are very essential for plants due to their quenching ability because of the presence of hydroxyl groups [18]. They belong to a class of antioxidant compounds which act as free

radicals inhibitors [19]. FCR does not only measure total phenols but also react with any reducing substance. The reagent therefore measures the total reducing capacity of a sample. This reagent also reacts with some nitrogen-containing compounds such as hydroxylamine and guanidine [20]. The reagent has also been shown to be reactive towards thiols, many vitamins, the nucleotide base guanine, the trioses glyceraldehyde and dihydroxy acetone, and some inorganic ions. Copper complexation increases the reactivity of phenols towards this reagent [21]. Total tannin content of extract was **47.239 mg GAE/gm** of dry extract by using Gallic acid calibration curve (R^2 = 0.994. Tannins have been reported to possess anti carcinogenic and antimutagenic potentials as well as antimicrobial properties.

Conclusion

Ethanolic extract of bark of ***Carica papaya* L.** showed potential antioxidant activity by different methods which may be an important source of natural products of health benefits.

Conflict of interest

The authors have no conflict of interest to declare.

Funding

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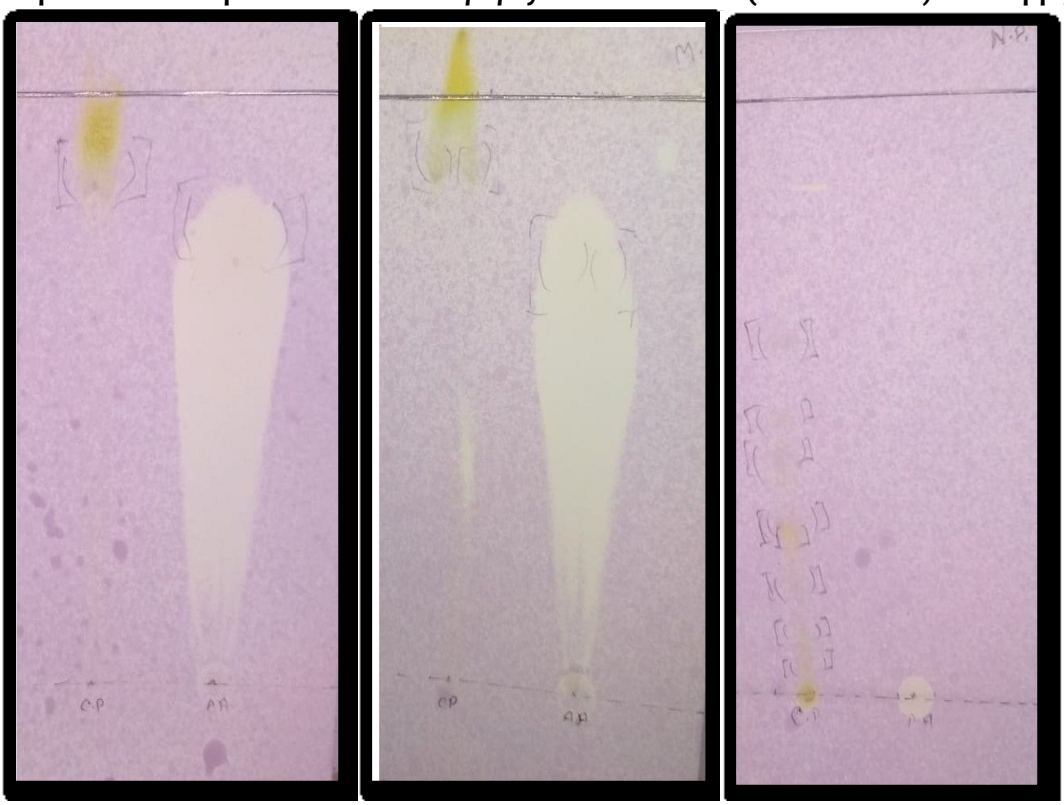
Figure 1: Comparison of TLC plate for bark of C. *papaya* with Standard (Ascorbic acid) after applying DPPH.



Polar		Medium polar		Non polar	
Sample	Standard	Sample	Standard	Sample	Standard
($CHCl_3$:CH_3OH:H_2O) (40:10:1)		($CHCl_3$:CH_3OH) (5:1)		(n-hexane: Acetone) (3:1)	

Figure 2: Comparison of DPPH scavenging activity of bark of C. *papaya* and Ascorbic acid.

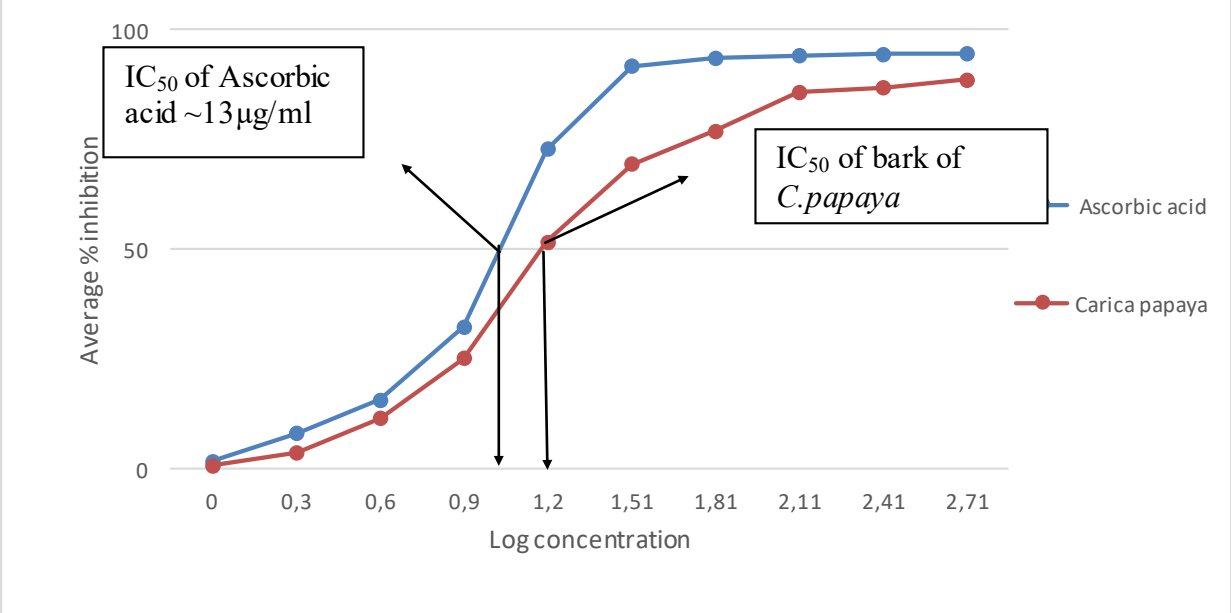


Table 1: IC_{50} values of extracts with ascorbic acid in DPPH assay

Plant Extracts	IC_{50} (ug/ml)
Ascorbic acid	~13
Bark of *C. papaya*	~24

Figure 3: The calibration curve of Gallic acid to determine total phenolic content

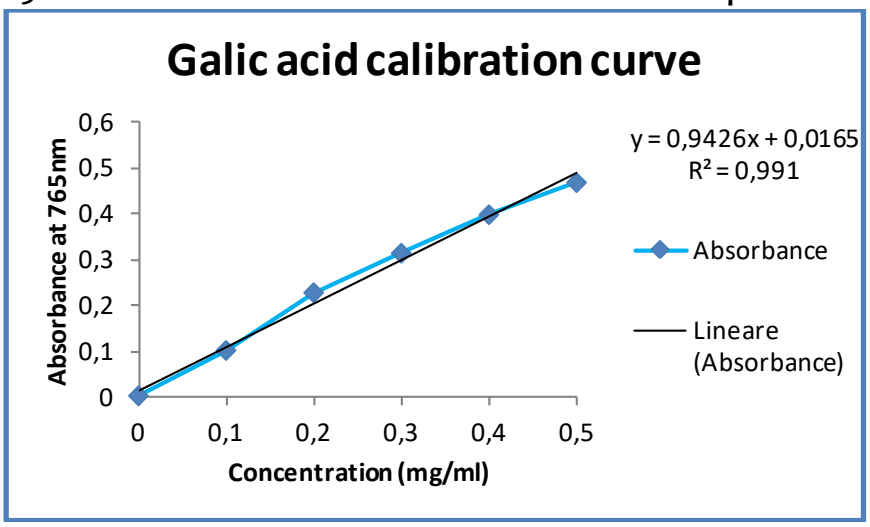


Table 2: Data to determine Total Phenolic Content of bark of *C. papaya bark* extract

No. of observation	Absorbance at 765nm			Average absorbance	Grand Average of absorbance	Total Phenolic Content (mg GAE/gm of dry extract)
	1st	2nd	3rd			
1	0.333	0.323	0.320	0.325	0.3255±0.0005	328.56
2	0.332	0.324	0.322	0.326		

Values are expressed as grand mean ±SD (n=2)

Figure 4: The calibration curve of quercetin to determine total flavonoids content

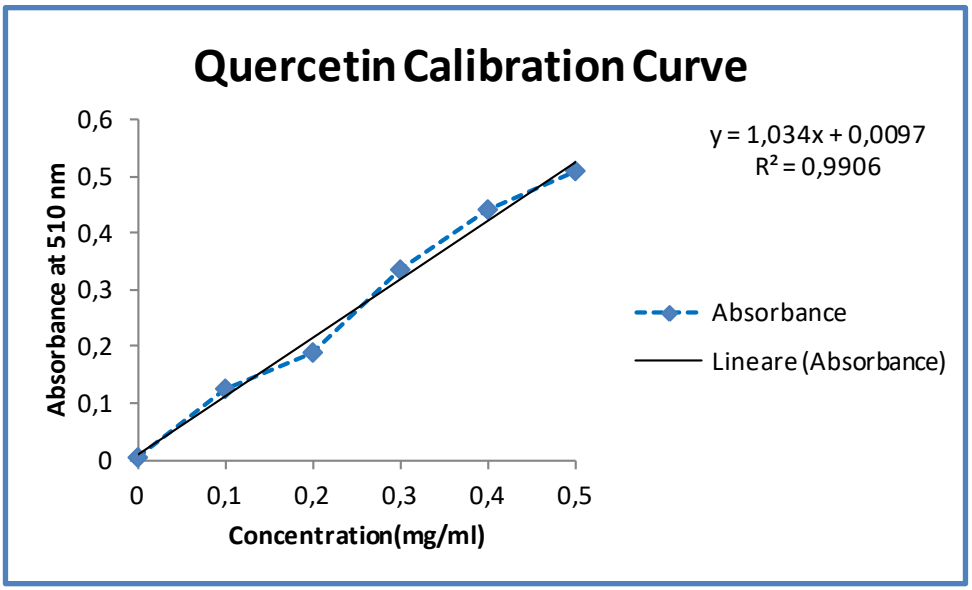


Table 3: Data to determine Total Flavonoids Content of bark of *C. papaya* extract

No. of observation	Absorbance			Average absorbance	Grand Average of absorbance	Total Flavonoid Content(mg QE/gm dry extract)
	1st	2nd	3rd			
1	0.202	0.197	0.197	0.199	0.2±0.001	195.329
2	0.201	0.202	0.201	0.201		

Values are expressed as mean ±SD (n=2)

Figure 5: The calibration curve of Gallic acid to determine total tannin content

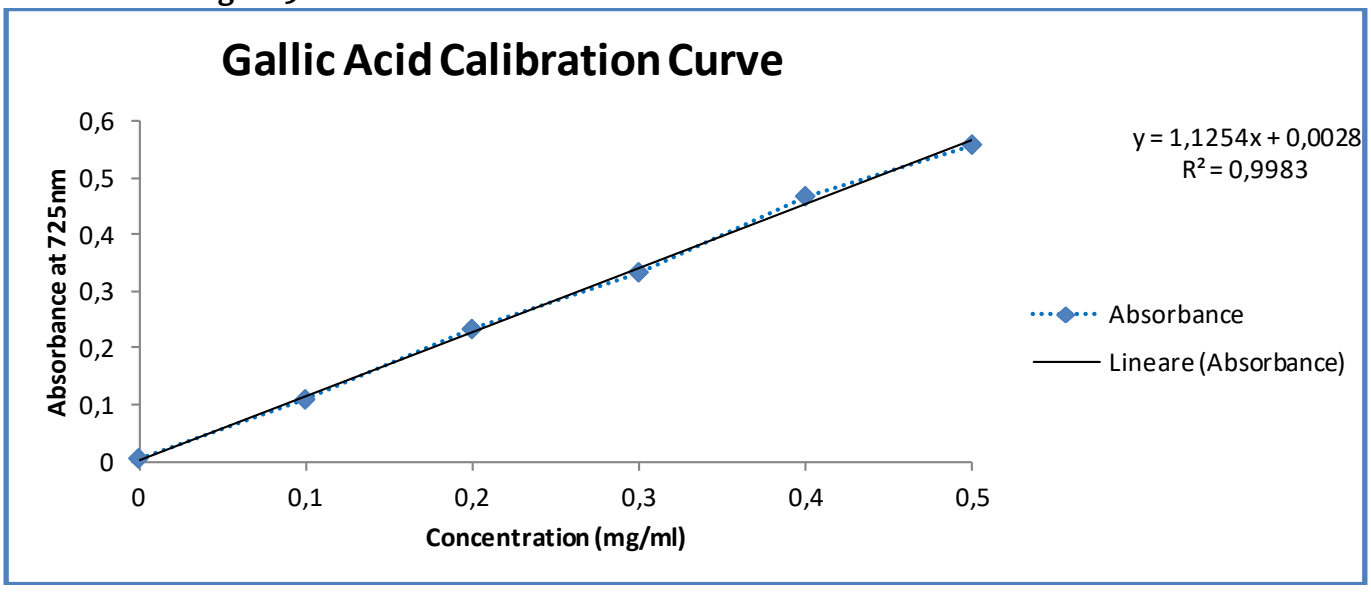


Table 4: Data to determine Total Tannin Content of bark of *C. papaya* extract.

No. of observation	Absorbance			Average absorbance	Grand Average of absorbance	Total Tannin Content (mg GAE/gm dry extract)
	1st	2nd	3rd			
1	0.061	0.062	0.059	0.060	0.0605±0.0005	47.239
2	0.060	0.061	0.062	0.061		

Values are expressed as mean ±SD (n=2)