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In Vitro Antioxidant Study of Methanolic Extracts of the Seed Kernel of *Mangifera Indica* & *Artocarpus Heterophyllus*

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Abstract The present study was subjected to investigate antioxidant property of methanolic extracts of the seed kernel of *Mangifera indica & Artocarpus heterophyllus*. For antioxidant properties- Total Phenols, Total flavonoids and DPPH free radical scavenging capacity of the methanolic extracts of the sample were assessed. Total phenolic constituents determination of the plant extract were performed employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard .Moreover, total phenolic concentration equivalents to Gallic acid for *Mangifera indica* was found 433 ± 0.71 mg/gm of extract and for *Artocarpus heterophyllus,* it was 100.75 ± 10.253 mg/gm of extract which is correlated with antioxidant activity. The Total Flavonoid contents of the test sample were calculated using the standard curve of Quercetin, the value of *Mangifera indica* was 129 ± 1.414 mg QUE/gm of extract and the quantity of *Artocarpus heterophyllus* was 52.25 ± 1.06 mg QUE/gm of extract. DPPH free radical scavenging effect of the extract was assessed. The DPPH result of the sample was compared with standard ascorbic acid. IC_{50} values of *Mangifera indica* and *Artocarpus heterophyllus* were 7.3µg/ml and 335.77µg/ml respectively which was comparable to that of the standard ascorbic acid (15.11µg/ml). So, the seed kernel of *Mangifera indica* can be regarded as suitable agent of natural antioxidant having high phenolic and flavonoid contents.
Keywords *Mangifera indica; Artocarpus heterophyllus; DPPH; Antioxidant*

1. Introduction

Since very old times herbal medications have been used for the relief of symptoms of disease (Maqsood *et al.,* 2010). Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts (Vongtau *et al.,* 2005; Oluyemi *et al.,* 2007).

The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human being such as diabetes, atherosclerosis, aging, immunosuppression and neurodegenerator (Harman, 1998).

Some free radicals play a positive role in vivo such as energy production, phagocytosis, regulation of cell growth, intracellular signaling and synthesis of biologically important compounds (Hallowell,

1997). However free radicals are very detrimental in attacking lipid in cell membrane and also DNA, including oxidations that cause membrane damage such as membrane lipid peroxidation, a decrease in membrane fluidity and also cause DNA mutation leading to cancer (Cerutti, 1994).

A potent scavenger of these free radical species may serve as a possible preventive intervention for free radical mediated diseases (Ames *et al.,* 1995). Several studies showed that a number of plant and herb extracts exert potent antioxidant actions (Kiselova *et al.,* 2006; Iqbal *et al.,* 2009).

Recent interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products because they possess multiphase in their multitude and magnitude of activity and provide enormous scope in correcting imbalance (Djeridane *et al.,* 2006; Wannes *et al.,* 2010).

Mangifera indica and *Artocarpus heterophyllus* are commonly used plant in ayurvedic medicine. *Mangifera indica* belongs to the family of Anacardiaceae and *Artocarpus heterophyllus* belongs to the family of Moraceae-Mulberry. Several studies indicate that *M.indica* possesses antidiabetic, antioxidant, anti-inflammatory, antimicrobial, anthelmintic and hepatoprotective properties etc. stem bark of *M. indica* possesses antioxidant, antidiabetic and athelmintic and antiallergic activities (Martinez *et al.,* 2000; Amrita *et al.,* 2009; Garcia *et al.,* 2003). Seed karnel of *M.indica* possesses anti-inflammatory and antimicrobial activities (Das and Das, 1989). Leaf of the *A. heterophyllus* possesses antidiabetic and antioxidant activities (Omar *et al.,* 2011). It also possesses antitumor and cytotoxic activity (Rajendran and Ramakrishnan, 2008; Rajesh *et al.,* 2011)

The objective of the present study was to investigate the antioxidant activity of the seed karnel of both *M. indica* and *A. heterophyllus* by using different method to evaluate a relationship between the antioxidant activity and the phytochemical constituents.

2. Materials and Methods

2.1. Sample Collection and Identification

At first with the help of a comprehensive literature review, ***Mangifera indica & Artocarpus heterophyllus*** from internet websites were selected for this investigation. The seeds of these plants were collected from around Nabinagar, Ashulia, Bangladesh and identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka.

2.1.1. Plant Material Preparation

The kernels are enclosed into seed. At first kernel were separated from seeds and washed clearly. Kernels were sun-dried separately and then, dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50°C) to make suitable for grinding purpose. After that kernels were ground into coarse powders in the Department of Pharmacy using high capacity grinding mill which were then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

2.1.2. Extraction procedure

The powdered plant materials (200 gm seeds kernel) were used for extraction by Soxhlet apparatus at elevated temperature (60°C) using methanol consecutively (500 ml of each solvent). When the powders became exhausted of its chemical constituents as evident from cycles of colorless liquid siphoning in the Soxhlet apparatus, extraction was considered to be complete. After completion of the extraction, the liquid was filtered using a sterilized cotton filter.

2.1.3. Concentration of the plant extract

The seeds kernel of ***Mangifera indica & Artocarpus heterophyllus*** plant extract was concentrated by evaporating the solvent using a water bath at a temperature of 60°C. This procedure was applied similarly for the above mentioned all about dried powdered plant samples.

2.2. Chemicals

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid), ferric chloride, Gallic acid and Quercetin were obtained from Sigma Chemical Co. USA. Ascorbic acid and Aluminium chloride were obtained from SD Fine Chem. Ltd., Biosar, India. Ammonium molybdate, Methanol, Sodium Phosphate, Concentrated H_2SO_4, Folin-ciocalteu reagent, Sodium carbonate, Potassium Acetate, Mono-Sodium phosphate, Bi-sodium phosphate, Potassium ferricyanide and Trichloro acetic acid were purchased from Merck, Germany.

2.3. Phytochemical Screening

The crude plant extracts were subjected to different qualitative tests to find out the presence of chemical constituents such as carbohydrate by Molisch's test, steroids by Liebermann-Burchard's Test, saponins with Frothing test, tannins using Lead acetate test, glycosides and alkaloids. These were identified by characteristic color changes using standard procedure of Harborne, 1988, Sazada et al; 2009.

2.4. Antioxidant Activity Evaluation

2.4.1. Determination of Total Phenolic content

The content of total phenolic compounds in plant methanolic extracts was determined as described previously by Chang *et al.,* 2002 using the Folin-Ciocalteu Reagent (FCR).Total phenol content in the extracts was determined with Folin-Ciocalteu reagent. Extract (200 µg/ml) was mixed with 400 µl of the Folin-Ciocalteu reagent and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 hrs. Then the absorbance at 765 nm was determined. The concentration of total phenol content in the extracts of the seed kernel of M*.indica & A. heterophyllus* was then determined as mg of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph.

2.4.2. Determination of Total Flavonoids Content

Total flavonoid was determined using the Aluminum chloride colorimetric method described by Kumaran and Karunakaran. 1 ml of plant extract in methanol (200 µg/ml) was mixed with 1 ml aluminium trichloride in methanol (20 mg/ ml) and a drop of acetic acid, and then diluted with methanol to 25 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 ml of plant extract and a drop of acetic acid, and then diluted to 25 ml with methanol. The total flavonoid content was determined using a standard curve of quercetin (12.5-100 µg /ml) and expressed as mg of quercetin equivalent (QE/gm of extract).

2.4.3. DPPH Free Radical Scavenging Assay

The free radical scavenging capacity of the extract was determined using stable free radical 1, 1-Diphenyl-2-picrylhydrazyl, (DPPH). Extract was mixed with methanol to prepare the stock solution (5 mg/ml). DPPH solution (0.004% w/v) was prepared in methanol. Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and extract was added followed by serial dilutions to every test

tube so that the final volume was 3 ml and after 10 min, the absorbance was read at 515 nm using a spectrophotometer (HACH 4000 DU UV – visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (5mg/ml). Control sample was prepared containing the same volume without any extract and reference ascorbic acid. Methanol was served as blank. % scavenging of the DPPH free radical was measured by using the following equation:

$$\% \text{ Scavenging Activity or \% inhibition} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}\right) \times 100$$

IC_{50} is the concentration at which 50% of the total DPPH free radical is scavenged/ neutralized and can be determined by linear regression method from plotting % inhibition against corresponding concentration.

3. Results and Discussions

3.1. Preliminary Phytochemical Tests

Preliminary phytochemical screening of the crude methanolic extract of seeds kernel ***Mangifera indica*** and ***Artocarpus heterophyllus*** revealed the presence of various types of phytochemical constituents of which glycoside; carbohydrate, tannin and alkaloids are remarkable and the results of plant chemical tests have been compiled in the Table 1.

Table 1: *Phytochemical tests of the methanolic extract of the seeds kernel of Mangifera indica and Artocarpus heterophyllus*

Name of test	***Reagent used***	***Result***	
		Mangifera indica	***Artocarpus heterophyllus***
Test for carbohydrates	Molisch's reagents	Positive (+)	Positive (+)
Test for Reducing sugar	Fehling's solution	Negative (-)	Negative (-)
Test for Glycoside	General test	Positive (+)	Negative (-)
	Fehling's solution	Negative (-)	Negative (-)
Tests for Alkaloids	Mayer's reagent	Negative (-)	Positive (+)
	Hager's reagent:	Negative (-)	Positive (+)
	Wagner's reagent	Negative (-)	Positive (+)
	Dragendroff's reagent	Negative (-)	Positive (+)
Test for Saponins	Frothing test	Negative (-)	Positive (+)
Test for Steroids	Licbermann-Burchard's test	Positive (+)	Positive (+)
Test for Tannins	Lead acetate test	Positive (+)	Negative (-)

Symbols Positive (+) stands for presence and Negative (-) stands for absent.

3.2 Antioxidant activity

3.2.1 Total Phenol Content Determination

Total phenolic contents of the methanolic fractions of the seeds kernel of the ***Mangifera indica & Artocarpus heterophyllus*** were determined by using the Folin-Ciocalteu reagent and were expressed as gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of gallic acid (y= 0.009x + 0.074; R^2 = 0.93)

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching single and triplet oxygen, or decomposing peroxides (Osawa, 1994). The anti-oxidative effect of the extract may be due to presence of phenolic components. From the results of phenolic contents, it can be said that methanolic fractions of the seeds kernel of the *Mangifera indica & Artocarpus heterophyllus* posses antioxidant activity.

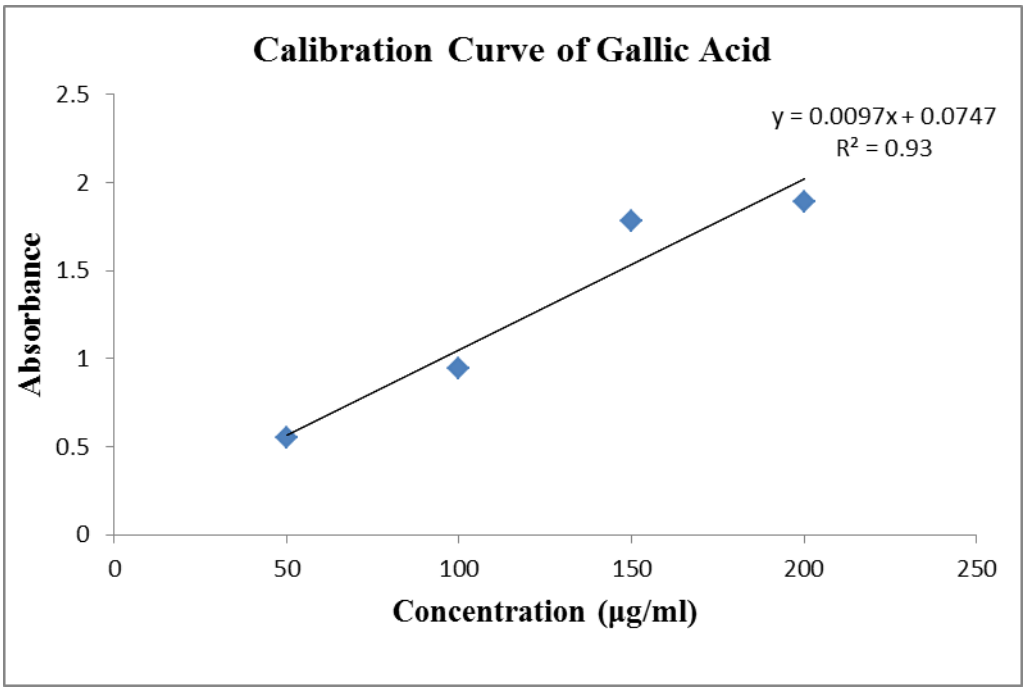

Figure 1: *Calibration curve of gallic acid*

Total phenol contents of the methanolic extract of the seeds kernel of the ***Mangifera indica*** *&* ***Artocarpus heterophyllus*** in mg/g(Gallic acid equivalent) has been shown in the Table 2.

Table 2: *Total phenol contents of the methanolic extract of the seeds kernel of Mangifera indica & Artocarpus heterophyllus*

Name of extract	mg of GAE/g of extract
Methanolic extract (*Mangifera indica)*	433 ± 0.71
Methanolic extract (*Artocarpus heterophyllus)*	100.75 ± 10.253

3.3. Determination of Total Flavonoid Content

Aluminium chloride colorimetric method was used to determine the total flavonoid content**s** of the methanolic extract of the seeds kernel of the *Mangifera indica & Artocarpus heterophyllus*. The total

flavonoid content was calculated using the standard curve of quercetin (y = 0.003x - 0.022; R^2 = 0.985) (Figure 2) and was expressed as quercetin equivalents (QE) per gram of the plant extract.

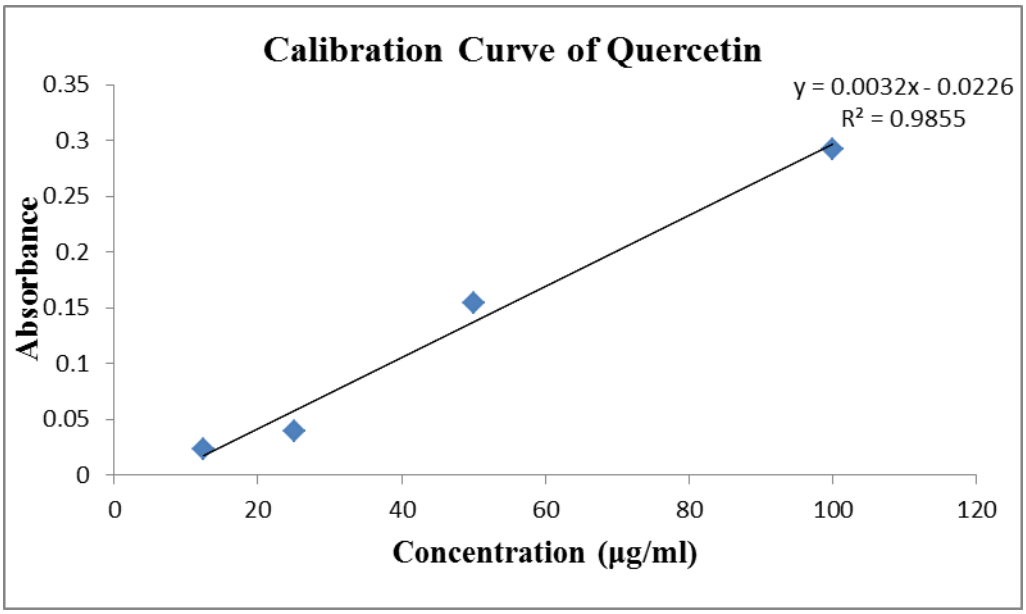

Figure 2: *Calibration curve of Quercetin.*

Total flavonoid contents of the methanolic extract of the seeds kernel of the ***Mangifera indica*** *&* ***Artocarpus heterophyllus*** in mg/g (quercetin equivalent) of extract has been given in the Table 3

Table 3: *Quantity of flavonoids in the plant extracts expressed in terms of quercetin equivalent (mg of QUE/g of extract)*

Fraction	mg of QUE/g of extract
Methanolic extract (***Mangifera indica)***	129 ±1.414
Methanolic extract (***Artocarpus heterophyllus)***	52.25 ±1.06

Flavonoids, a subclass of polyphenols, are the most common polyphenolic compounds found in nature and are further divided into several subclasses including flavones, flavonols, isoflavones, anthocyanins, flavanols, and proanthocyanidins. Flavonoids exerts their antioxidative properties of by several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation (*Benavente-Garcia et al.,* 1994). High flavonoids content in the plant extracts of *Mangifera indica & Artocarpus heterophyllus* indicate that these plant extracts have antioxidant property.

3.4. DPPH Free Radical Scavenging Assay

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. IC_{50} values of the methanolic extract of seeds kernel of ***Mangifera indica*** and ***Artocarpus heterophyllus*** *has been represented in the Table 4 which is comparable with* standard ascorbic acid.

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance. The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damage caused by oxidative stress. Lower

IC_{50} value of the methanolic extract of seeds kernel of *Mangifera indica* denotes the higher antioxidant capacity compared to *Artocarpus heterophyllus* (IC_{50}=335.77 µg/ml)

Table 4: *IC_{50} values of the methanolic extract* ***Mangifera indica*** *and* **Artocarpus heterophyllus**

Sample/Standard	IC_{50} (µg/ml)
Ascorbic acid	15.11
Methanolic extract of ***Mangifera indica***	7.3
Methanolic extract of ***Artocarpus heterophyllus***	335.77

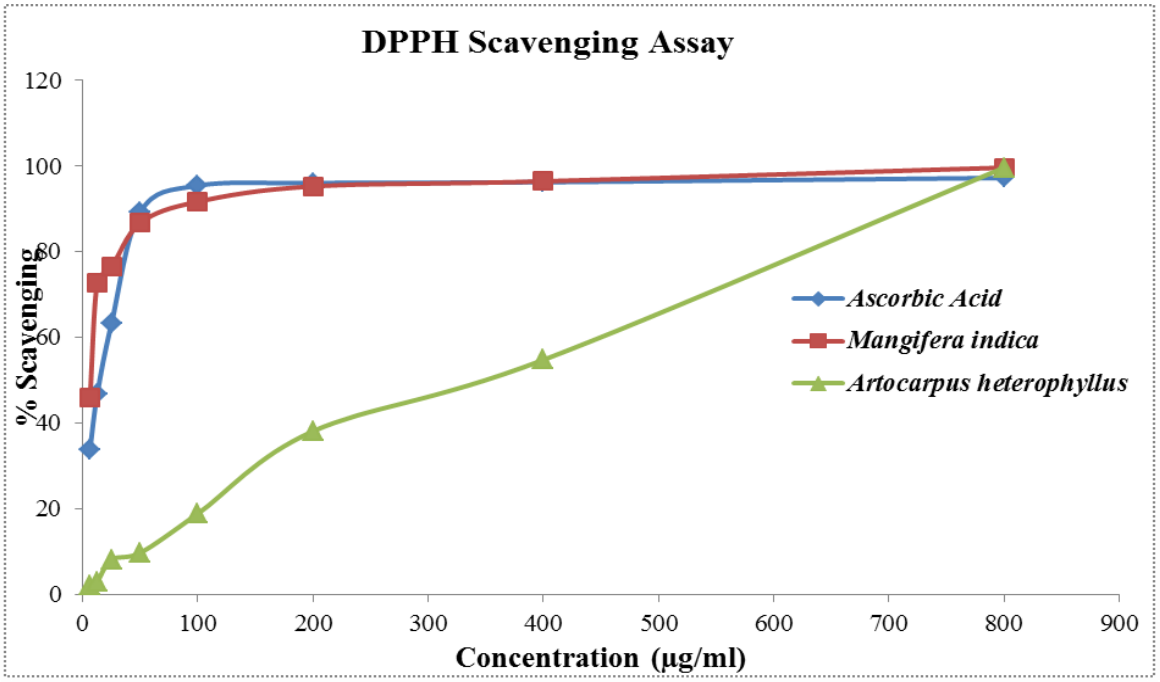

Figure 3: *DPPH radical scavenging activity of the methanolic extract of the Seeds kernel of* ***Mangifera indica*** *and* **Artocarpus heterophyllus** as compared with Ascorbic Acid

4. Conclusion

It was observed from the present study that the Methanolic extract of seed kernel of *Mangifera indica* has the significant total phenol content, total flavonoids and DPPH scavenging ability and *Artocarpus heterophyllus* possesses moderate antioxidant activity. This indicates that the antioxidant activity of the methanol extract was well correlated with the content of its phenolic compounds, flavonoids, reductivity etc.

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to rich a concrete conclusion about the findings of the present study. Elaborate phytochemical investigation must be arranged that might lead to isolation and characterization of chemical constituents present in the crude extracts.

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