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Research Article

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *ARTOCARPUS HETEROPHYLLUS* AND *MANILKARA ZAPOTA* SEEDS AND ITS REDUCTION POTENTIAL

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ABSTRACT

The four different extracts of *Artocarpus heterophyllus* and *Manilkara zapota* seeds were undertaken and studied for their total phenolics and flavonoids contents, reducing power and antioxidant activity. Total phenolic contents were determined spectrometrically by using Folin-Coicalteu method and were calculated as Gallic acid equivalents. Flavonoids and reducing power were also determined spectrometrically using standards methods. The antioxidant capacity was studied using DPPH radical scavenging assay and ABTS radical scavenging assay methods**.** Plants containing phenols and flavonoids have been reported to possess strong antioxidant properties. The results of antioxidant activity showed high effect in both extracts. From the present study, it can be concluded that *Artocarpus heterophyllus* and *Manilkara zapota* seed extract has effective antioxidant activity due to the presence of high amounts of phenolic compounds. The results revealed that the extracts showed high flavonoids content and reducing potential. Both extracts may therefore be a good functional medicine as well as pharmaceuticals plant-based products.

Keywords: Antioxidant, Total Phenolic, Flavonoids, Reducing potential, DPPH, ABTS

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Free radicals are the main culprit in lipid peroxidation, highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources[1]. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism[2,4]. Free radical oxidative stress caused a wide variety of clinical disorders[3]. A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress[5]. Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements [6].

Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and ROS; any may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers[7, 8]. Natural antioxidants, particularly in fruits, vegetables and beverages have gained interest among consumers[9]. Antioxidants are dietary substance that protects body cells from the oxidative damage to a target molecules caused by oxidation from free radicals by reactive oxygen species (ROS) [10].

During the last two decades there has been in search for new plant derived drugs containing the medically useful alkaloids, glycosides, polyphenolics, steroids and terpenoids derivatives, which contributes to the antioxidant property[11]. Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health have only recently been recognized. Flavonoids are known to possess antioxidant and anticarcinogenic activities[12]. Therefore search for natural antioxidants of plant origin gained momentum in recent years.

The *Artocarpus heterophyllus* (Jackfruit) is a species of tree of the mulberry family Moraceae. It is native to Western Ghats of India, Malaysia and also found in central and eastern Africa, south-eastern Asia, Florida, Brazil, Australia and many Pacific Islands[13]. *Artocarpus heterophyllus* possesses known anti-bacterial, anti-fungal, anti-diabetic, anti-inflammatory, antioxidant and anti-helmintic activities[14].

Manilkara zapota L. (Sapodilla) belongs to the family *Sapotaceae.* It is an evergreen, glabrous tree, 8-15 m in height. It is cultivated throughout India, though it is native to Mexico and Central America. The seeds are aperients, diuretic tonic and febrifuge. Bark is antibiotic, astringent and febrifuge.

The main objectives of the study are to analysis the total phenolic and flavonoid content, reducing power, antioxidant activity.

METHODS AND MATERIALS

Chemicals used

All chemicals were purchased from Sigma Chemical, Merck Chemical Supplies, SD fine and Himedia. All other chemicals used were obtained commercially and were of analytical grade.

Collection of plant material

Artocarpus heterophyllus (Jackfruit) and *Manilkara zapota* (Sapodilla) were collected from Pallamudir, Coimbatore, Tamil Nadu, India.

Preparation of Extracts

The seeds were shade dried for one month and coarsely powdered. Extraction of the active ingredient from the seed powder was carried out using specific method[15]. 25g of the powdered seeds were extracted by soxhlet apparatus using 250ml of four different solvent (ethanol, acetone, ethylacetate and water) in a separate flask. The extraction lasted for six hours. The extract obtained was concentrated by evaporation using water bath at 100°C and stored at 4°C in cold room.

Determination of Total Phenolic content

Total phenolic content was determined by Folin-Ciocalteu reagent[16]. 10ml of the samples were taken in test tubes and made up to the volume of 1ml with distilled water. Then 0.5ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube in triplicate manner. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was recorded at 725nm against a reagent blank. All tests were carried out in triplicate. Total content of phenolic compounds in extracts was expressed as Gallic Acid Equivalent (GAE) in milligram per gram extract.

Determination of Total Flavonoid content

Flavonoid content was determined according to the aluminum chloride colorimetric method with some modifications[17]. The sample solution (0.5ml) was mixed with 1.5ml of 95% ethanol, 0.1ml of 10% aluminum chloride hexahydrate, 0.1ml of 1M potassium acetate, and

2.8ml of distilled water. After incubation at room temperature for 40 minutes the absorbance of the reaction mixture was measured at 415nm. The same amount (0.1ml) of distilled water substituted for the amount of 10% aluminum chloride as the blank. All tests were performed in independent triplicates. The total flavonoid content was expressed in milligram per gram extract of Quercetin Equivalent (QE).

Determination of Reduction potential

The reducing antioxidant power was determined by specific method[18]. 1.0ml of the different concentration of various extracts of the sample was mixed with 2.5ml potassium ferric cyanide and 2.5ml phosphate buffer (pH 6.6). The mixture was incubated at 50ºC for 20 minutes. After the incubation, 2.5ml of Trichloroacetic acid (10%) was added to it and centrifuged at 3000rpm for 10 minutes. 2.5ml of the supernatant was taken and mixed with 2.5ml distilled water and 0.5ml of ferric chloride (0.1%) were added to it. The absorbance of the colour was measured spectrophotometrically at 700nm. Increased absorbance of the reaction mixture indicates increase in reducing power. All tests were carried out in triplicate.

Determination of antioxidant Activity

DPPH (2, 2-diphenyl, 1-picryl hydrazyl) radical scavenging assay

The antioxidant effect of extract on DPPH radical was assayed using specific method[19]. 3.0ml of 1.0mM DPPH in ethanolic solution was added to 2.5µl and 5µl of the plant extract. DPPH solution with ethanol was used as positive control and ethanol alone acted as blank. After 30 minutes, the discoloration from deep violet to yellow colour was measured at 518nm in a spectrophotometer.

The percentage inhibition was calculated by the following formula:

$$\text{Scavenging activity (\% of inhibition)} = \frac{Ac - Ae}{Ac} \times 100$$

Where,

Ac – Absorbance of DPPH radical as control.

Ae – Absorbance of DPPH radical in the presence of plant extract.

ABTS (2, 2'-azinobis (3-ethylbenzthiazoline)-6-sulphonic acid) radical scavenging assay

The effect of extract on ABTS radical was assayed using specific method[20]. $ABTS^{+}$ radical was prepared by adding 2.45mM ammonium per sulphate with 7mM ABTS solution and kept in dark for 12-16 hours at room temperature. 0.5ml of various plant extracts were added to 0.3ml of ABTS solution and the final volume was made up to 1.0ml with ethanol. After 6 minutes, the absorbance was read at 745nm and the percentage inhibition scavenging activity was calculated using the scavenging activity formula as presented earlier.

RESULTS AND DISCUSSION

Plants containing phenols and flavonoids have been reported to possess strong antioxidant properties.

Determination of Total Phenolic content

Phenolic compounds may contribute directly to antioxidative action. The four different solvents at 100 mg/g of seed extract of *Artocarpus heterophyllus* contains total phenolic content was found to be 4.16±0.01mg/g (ethanolic), 2.30±0.02mg/g (acetone), 2.77±0.02mg/g (ethyl acetate) and 1.18±0.01mg/g (aqueous) of Gallic acid equivalent (GAE). The total phenolic content was 4.00±0.01mg/g for ethanolic extract, 2.26±0.02mg/g for acetone extract, 2.54±0.02mg/g for ethyl acetate extract and 1.21±0.01mg/g for aqueous extract of Gallic acid equivalent per 100mg seed extract of *Manilkara zapota* respectively (Table 1). The ethanolic extract of *Artocarpus heterophyllus* and *Manilkara zapota* seeds showed high phenol content than other different solvents.

Phenolic compounds are known as powerful chain breaking antioxidants and act as free radical terminators[21], may contribute directly to antioxidative action[22]. These compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups present in it[23]. The mechanisms of action of flavonoids are through scavenging or chelating process[24, 25]. In general, extracts with high antioxidant activity show high phenolics content. Polyphenols are the major plant compounds with antioxidants activity due to their redox properties; it plays an important role in absorbing and neutralizing free radicals, quenching singlet and triplet O_2 or decomposing peroxides[9]. Phenols contain plants has good antioxidant, anti-mutagenic and anti-cancer properties[26].

Determination of Total Flavonoid content

The total flavonoid content of the four different seed extract of *Artocarpus heterophyllus* was found to be 4.05±0.01mg/g for ethanolic fraction, 2.21±0.02mg/g for acetone fraction, 2.67 ±0.01mg/g for ethyl acetate fraction and 0.86±0.01mg/g for aqueous fraction of quercetin equivalent per 100mg seed extract, whereas in *Manilkara zapota,* it was found to be 3.98± 0.01mg/g (ethanolic), 2.19±0.01mg/g (acetone), 1.01±0.02mg/g (ethyl acetate)and 0.74± 0.01mg/g (aqueous) of Quercetin equivalent per 100mg (Table 1). The ethanolic extract of *Artocarpus heterophyllus* and *Manilkara zapota* seeds showed high flavonoid content than other three different solvents.

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. Flavonoids are a large class of phytochemicals which are omnipresent in human diets. Flavonoids are important for human health because of their high pharmacological activities as radical scavengers[27]. The flavonoids are probably the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties[28]. Flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also as potential therapeutic agents against a wide variety of diseases[29, 30].

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activity[31].The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables[32, 33].

Determination of Reduction potential

Reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts[34]. The results showed that reducing power of *Artocarpus heterophyllus* seed extracts was found to be 13.12±0.01µg/ml, 10.52±0.02µg/ml, 10.13±0.01µg/ml and 09.56±0.02µg/ml for ethanolic, acetone, ethyl acetate and aqueous extracts respectively, whereas reducing power of *Manilkara zapota* seed extracts was found to be 13.96±0.01µg/ml (ethanolic), 10.43±0.01µg/ml (acetone), 10.48±0.02µg/ml (ethyl acetate) and 09.41±0.02µg/ml (aqueous) and were showed in Table 1. The ethanolic extracts of *Artocarpus heterophyllus* and *Manilkara zapota* showed the highest reducing power and the values were comparable to that of phenolic content.

The reducing ability of a compound greatly depends on the presence of reductones, which have exhibit antioxidative potential by breaking the free radical chain by donating a hydrogen atom[35]. The ability to reduce Fe (III) may be attributed from hydrogen donation from phenolic compounds[36], which is also related to presence of reductant agent. In addition, the number and position of hydroxyl group of phenolic compounds also rule their antioxidant activity[37].

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging[38]. Generally the reducing properties

are associated with the presence of compounds, which exert their action by breaking the free radicals chain through donating a hydrogen atom[39, 40].

DPPH radical scavenging assay

The DPPH system is a stable radical generating procedure. The DPPH assay is a simple method to measure the ability of antioxidants to trap free radicals. It is well known that the DPPH has ability to capture free radicals is due to the delocalization of the unpaired electron all over the molecule[41].

The seed extracts of *Artocarpus heterophyllus* and *Manilkara zapota* showed promising free radical scavenging effect of DPPH in concentration dependent upto 500µg/ml. The free radicals scavenging activity of the *Artocarpus heterophyllus* seed extracts on DPPH radicals was found to have IC_{50} of 50.09% at 300µg/ml for ethanolic, 51.90% at 400µg/ml for acetone, 52.28% at 500µg/ml for ethyl acetate and 51.80% at 500µg/ml for aqueous fraction (Table 2), whereas the *Manilkara zapota* seed extracts on DPPH radicals was found to have IC_{50} of 51.08% at 300µg/ml for ethanolic, 50.28% at 400µg/ml for acetone, 51.99% at 500µg/ml for ethyl acetate and 50.46% at 500µg/ml for aqueous fraction (Table 2). Comparatively, ethanol extract is found to exhibit significant antioxidant activity in both seed extracts.

The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods[42, 43]. The DPPH scavenging activity was found to be dose dependent. This radical scavenging activity of plants extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability.

ABTS radical scavenging assay

ABTS radical, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals[44]. The ABTS radical cation scavenging activity of the *Artocarpus heterophyllus* seed extracts at 200 µg/ml was found to be IC_{50} at 51.89% for ethanolic fraction, 52.09% at 300µg/ml for acetone, 50.36% at 400µg/ml for ethyl acetate and 52.04% at 500µg/ml for aqueous fraction (Table 3), whereas the *Manilkara zapota* seed extracts at 200µg/ml was found to be IC_{50} at 50.32% for ethanolic fraction, 51.45% at 300µg/ml for acetone, 50.67% at 400µg/ml for ethyl acetate and 51.75% at 500µg/ml for aqueous fraction.

The methanolic extracts were fast and effective scavengers of the ABTS radicals[45]. The high molecular weight phenolics have more ability to quench free radicals and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group substitution than the specific functional groups was reported by[46]. Higher concentrations of the extracts were more effective in quenching free radicals in the system.

Phenols and flavonoids contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavour. The phenolic compounds act as antioxidant agents. As a whole the antioxidants are vital substances which possess the ability to protect body from damage by free radical induced oxidative stress.

Table 1: Total phenolic and flavonoid content, Reducing potential of different seeds extracts of *Artocarpus heterophyllus* and *Manilkara zapota*

Seed extracts	Different fractions	Total phenolic content, GAE (mg/g)	Total flavonoid content, QE (mg/g)	Reducing potential (µg/ml)
Artocarpus heterophyllus	Ethanolic	4.16±0.01	4.05±0.01	13.12±0.01
	Acetone	2.30±0.02	2.21±0.02	10.52±0.02
	Ethyl acetate	2.77±0.02	2.67±0.01	10.13±0.01
	Aqueous	1.18±0.01	0.86±0.01	09.56±0.02
Manilkara zapota	Ethanolic	4.00±0.01	3.98±0.01	13.96±0.01
	Acetone	2.26±0.01	2.19±0.01	10.43±0.01
	Ethyl acetate	2.54±0.02	1.01±0.02	10.48±0.02
	Aqueous	1.21±0.01	0.74±0.01	09.41±0.02

All values are expressed as Mean ± SD for three determinations; GAE- Gallic acid equivalent; QE -Quercetin equivalent

Table 2: DPPH radical scavenging assay of different seeds extracts of *Artocarpus heterophyllus* and *Manilkara zapota*

Seed extracts	Different fractions	Antioxidant activity - DPPH radical scavenging assay					IC_{50} Values (µg/ml)
		Different concentrations					
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	
Artocarpus heterophyllus	Ethanolic	37.18±0.01	41.56±0.01	50.09±0.01	55.22±0.01	67.77±0.01	300
	Acetone	22.08±0.02	39.11±0.02	44.23±0.01	51.90±0.02	56.78±0.02	390
	Ethyl acetate	20.55±0.01	25.04±0.01	40.39±0.01	48.67±0.01	52.28±0.01	490
	Aqueous	31.12±0.02	33.45±0.02	42.75±0.02	45.33±0.04	51.80±0.02	480
Manilkara zapota	Ethanolic	36.07±0.01	49.52±0.01	51.08±0.01	56.13±0.01	64.49±0.01	290
	Acetone	22.37±0.01	37.91±0.01	46.83±0.01	50.28±0.01	52.83±0.01	400
	Ethyl acetate	19.14±0.02	28.27±0.02	33.38±0.02	44.93±0.02	51.99±0.02	490
	Aqueous	26.86±0.01	30.23±0.01	39.65±0.01	48.92±0.01	50.46±0.01	500

All values are expressed as mean ± SD for three determinations

Table 3: ABTS radical scavenging assay of different seeds extracts of *Artocarpus heterophyllus* and *Manilkara zapota*

Seed extracts	Different fractions	Antioxidant activity- ABTS radical scavenging assay					IC_{50} Values (µg/ml)
		Different concentrations					
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	
Artocarpus heterophyllus	Ethanolic	31.26±0.02	39.93±0.01	51.89±0.02	57.20±0.02	62.01±0.01	290
	Acetone	19.98±0.02	27.54±0.02	41.78±0.01	52.09±0.01	52.78±0.01	380
	Ethyl acetate	21.02±0.01	27.37±0.01	39.23±0.02	46.23±0.01	50.36±0.01	500
	Aqueous	29.87±0.02	35.04±0.01	43.45±0.02	48.32±0.02	52.04±0.02	480
Manilkara zapota	Ethanolic	32.23±0.01	44.91±0.01	50.32±0.02	53.67±0.01	60.66±0.01	300
	Acetone	21.65±0.02	33.98±0.02	43.08±0.01	51.45±0.02	56.05±0.01	390
	Ethyl acetate	20.01±0.02	30.05±0.01	36.34±0.02	40.29±0.01	50.67±0.01	500
	Aqueous	28.46±0.01	29.78±0.01	40.39±0.01	45.67±0.02	51.75±0.01	490

All values are expressed as mean ± SD for three determinations

CONCLUSION

The results obtained in the present study, it was concluded that the seed extracts of both plants contains large amounts of flavonoids and phenolic compounds, exhibits high antioxidant and free radical scavenging activities in addition to having their medicinal properties. It also chelates iron and has reducing power. Both plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, studies on these medicinal plants would be of great importance. Both plants are widely used in number of pharmacological actions with high content of flavonoids and phenol seems to have a high potential for antioxidant activity.

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