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Evaluation of *invitro* Antioxidant Activity of *Artocarpus altilis* (Parkinson) Fosberg

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Abstract

Introduction: Antioxidants play a crucial role to protect the damage caused by oxidative stress (OS) in living cells. Plants having different metabolites were reported to possess antioxidant as well as anticancer properties. The present investigation dealt with the analysis of antioxidant properties of crude solvent extracts of matured unripe seedless fruits of *Artocarpus altilis* (Parkinson) Fosberg (commonly known as breadfruit) under *in vitro* condition.

Methods: The antioxidant activities of fruit extracts were evaluated *in vitro* using standard method such as DPPH (1,1-diphenyl-2picrylhydrazine) radical scavenging assay, hydroxyl radical scavenging assay, ferrous reducing antioxidant capacity, phosphomolybdnum assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, hydrogen peroxide radical scavenging assay using ascorbic acid as standard.

Results: All the assays conducted with methanolic breadfruit extracts revealed the free radical scavenging capacity with nitric oxide radical scavenging assay as the better one.

Conclusion: The results revealed the efficiency of breadfruit as a potent biological antioxidant agent.

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1. Introduction

In living systems, oxidation is a basic part of the normal metabolic process, in which reactive oxygen species (hydrogen peroxide and hypochlorous acid) and many free radicals (hydroxyl radical and superoxide anion) are generated[1]. ROS cause the damage of numerous cellular factors such as lipids, proteins and nucleic acids, especially, DNA leading to subsequent cellular death by modes of necrosis or apoptosis [2]. The damage can become more wide due to weakened cellular antioxidant defense systems. All natural systems hold antioxidant defense mechanism that protects against oxidative damages and repairs enzymes to remove damaged molecules. Yet, this natural antioxidant mechanism can be hardly efficient. Hence, dietary intake of antioxidant compounds is much necessary. Consumption of plant products such as fruits and vegetables is known to lower the threat of several diseases, such as cancer, cardiovascular diseases and stroke caused by OS [3] and these health benefits are substantially assessed due to the presence of phytochemicals, such as polyphenols, carotenoids and vitamin E and C [4]. Although the occurrence of phenolic compounds are generally found in both edible and non edible herbs, cereals, fruits, vegetables, oils, spices and other plant materials [5,6] scientific information on radical scavenging capability of endemic plants limited to certain regions is still rather scarce. Therefore, the assessment of similar properties remains an enthiusiastic and useful task, specifically to find new promising sources of natural antioxidants for functional foods and/or nutraceuticals [6 & 7]. *Artocarpus altilis* is one of the highest food yielding plants, with a single tree producing up to 200 or more grape fruit-sized fruits from March to June and from July to September[8] per season and require limited care.Breadfruit is also known as a traditional starch rich crop. Synonyms of *Artocarpus altilis* are *Artocarpus* communis and *Artocarpus incises [9*]. Several studies had emphasized the antioxidant and antimicrobial potentiality of *Artocarpus altilis* along with its therapeutic properties[10]. However, there are only a few reports on antioxidant properties of *Artocarpus altilis* (Parkinson) Fosberg. Therefore, in this study, we evaluated the antioxidant activity of methanolic extracts of breadfruit.

2.Materials and methods

2.1 Plant Material and sample preparation

Matured unripe seedless fruits of *Artocarpus altilis* (Parkinson) Fosberg were collected from Kanyakumari district, Tamil Nadu, India and were made clear of latex, dust particles and sand. Then the skin was peeled off and the pulp part was sliced off, demoistured and dried in shade. Dried material was then powdered and extracted with polar solvent methanol in a ratio of 1: 10 by cold percolation technique. The extract was then filtered, evaporated and stored at 4^{o}C for further experimental analysis.

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2.2 Evaluation of *in vitro* antioxidant activity of *Artocarpus altilis* (Parkinson) Fosberg

2.2.1 DPPH (2,2- diphenyl-1-1-picrylhydrazyl) free radical scavenging assay) [11]

The methanol DPPH solution (0.15%) was mixed with serial dilutions (50-250ug/ml)of fractions and after 10 minutes, the absorbance was read at 515nm. The antiradical activity was expressed as IC50 (ug/ml). Vitamin C was used as standard. All the samples were analysed in triplicates and the ability to scavenge the DPPH radical was calculated using the following equation

DPPH scavenging effect (%)=

$$\frac{\text{Absorbance of control- (Sample with DPPH- sample without DPPH)}}{\text{Absorbance of control}} \times 100$$

2.2.2 Nitric oxide radical scavenging assay [12]

The reaction mixture (3ml) containing sodium nitroprusside (10mM, 2ml), phosphate buffer saline 0.5ml and different concentration of major fractions from unripe pericarp ethanol extracts (20 to 100ug/ml) and standarad solution 0.5ml were incubated at 25^{O}C for 150 minutes.After incubation 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for completing diazoation. Then 1ml of napthylethylenediaminedihydrochloride (1% was added, mixed and allowed to stand for 5 minutes. A pink colouredchromophore was formed in diffused light and the asorbanceofchromophore formed was measured at 540nm against the corresponding blank. Ascorbic acid was used as positive control. The experiment was repeated in triplicates and the radical scavenging activity was calculated using the following formula

Nitric oxide radical scavenging activity (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.2.3 Superoxide radical scavenging assay [13]

Superoxide radicals were generated with in PMS NADH systems by the oxidation of

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NADH and were assayed by the reduction of nitrobluetetrazolium (NBT). TrisHCl buffer (3ml, 16Mm, pH 8.0) containing 1ml NBT(50Um)solution, 1ml NADH (78uM) solution and a sample solution of extract (50-250ug/ml) in water were mixed. The reaction was started when 1ml of phenazinemethosulfate (PMS) solution (10Um was added to the mixture. The reactiom mixture was incubated at 25oC for 5 minutes. Blue chromogen formed due to NBT reduction was read at 560nm against the corresponding blank samples. Quercetin was used as the reference compound. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging property. The scavenging effect was calculated using the formula

Superoxide free radical scavenging activity(%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.2.4 Hydrogen peroxide radical scavenging assay [14]

Scavenging of H2O2 by the extract was used to determine the antioxidant ability of extract according to the method of Gulcin. A solution of H_2O_2(0.2) was prepared in phosphate buffer (Ph 7.4). 1 ml extract of different concentrations (50-250ug/ml)was added to 0.6ml solution of 40Mm H2OS solution. The absorbance of the mixture was measured at 230nm using UV visible spectrophotometer against a blank solution containing phosphate buffer solution without H_2O_2. A known antioxidant such as ascorbic acid was used as positive control.The percentage of H_2O_2 scavenging was calculated following formula

Hydrogen peroxide scavenging activity (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.2.5 Hydroxyl radical scavenging assay[15]

Stock solution of EDTA (1Mm) were prepared in DMSO and and FeCl3 (10mm), ascorbic acid

(1mm), hydrogen peroxide (10mm) and deoxyribose(10mm) were prepared in distilled water. The method was carried out by adding 100ul of EDTA, 10ul FeCl3, 100ul H2O2, 360ul deoxyribose, 1000ul of the extract dissolved in distilled water, 330ul of phosphate buffer(50mm, ph 7.4) and 100ul of ascorbic acid. The mixture was then incubated at 37^{o}C for 1hr. About 1ml of incubated mixture was mixed with 1ml of 10% TCA and 1ml of 0.5% TBA for the development of a pink chromogen which could be then measured spectrophotometrically at 532nm.Vitamin C was used as positive control. The hydroxyl radical scavenging activities of the

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major fractions were reported as percentage inhibition of deoxyribose degradation and were calculated by using the formula

Hydroxyl radical scavenging activity (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.2.6 Ferrous ion chelating assay[16]

The reaction mixture containing 1.0 ml of various concentrations of the extracts (50250ug /ml), 0.05 ml of 2 mM $FeCl_3$ and 0.2 ml of 5 mMferrozine was shaken vigorously and left standing at room temperature for 10 minutes and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A lower absorbance of the reaction mixture indicated a higher ferrous ion chelating ability. The control contained all the reagents except sample. Ascorbic acid was used as standard for comparison and the radical scavenging capacity was assessed using the following formula.

Percentage of inhibition = [(OD of the control- OD of the test)/OD of the control]×100

2.2.7 Phosphomolybdenum assay [17]

Radical scavenging activity of the test sample through phosphomolybdenum assay was done as per the following procedure. 0.3 mL of the different solvent fractions and standard drugs

(25 µg/mL to 400 µg/mL) were taken in test tubes and dissolved in 3 mL of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). Following this, the test tubes were covered and incubated at 95°C in a water bath for 95 min. Then, the mixtures were allowed to cool at room temperature and the absorbance was measured at 695 nm. A mixture containing distilled water instead of the samples served as control. Ascorbic acid was used as standard. Higher absorbance indicates higher antioxidant potential. The percentage of inhibition was calculated using the formulae.

Percentage of inhibition= [(absorbance of sample– absorbance of control)/ (absorbance of sample) x100

3.Results and Discussion

Antioxidant activity can be defined as a limitation or inhibition of nutrient oxidation by restraining oxidative chain reactions. Antioxidants are substances that protect cells from the

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damage caused by free radicals. Antioxidant properties of plants have been demonstrated to play a protective role in the body against diseases, as their consumption lowers the risk of cancer, heart disease, hypertension, dementia and stroke. The search for better alternatives to synthetic antioxidants has triggered a significant research interest on dietary and medicinal plants that can inhibit oxidative stress related diseases. In this quest, we investigated the antioxidant and anticancer activities of breadfruit. Free radical Scavenging activity of the methanolic breadfruit extract was ascertained through different methods such as DPPH (2,2- diphenyl-1-1-picrylhydrazyl) free radical scavenging assay, Nitric oxide radical scavenging assay, Superoxide radical scavenging assay, Hydrogen peroxide radical scavenging assay, Hydroxyl radical scavenging assay, Phosphomolybdnum assay and ferrous ion chelating assay in comparision to standarad ascorbic acid. On analysis of results, methanolic breadfruit extract was found as an effective free radical scavenger with IC 50 values ranging from 32.1 to 346.74 against the standarad IC 50 values ranging from 77.7 to 284.25 (Table: 1 and Fig: 2).Our detailed study results also showed that the increase in sample concentration lead to the increase in absorbance too. Change in colour of extract corresponding to each assay gave us evidence that methanolic extract of breadfruit possess antioxidant properties. The present results were strongly supported by various authors from different regions. The methanolic extract of breadfruit pulp has the characteristics of antioxidant using DPPH assay[18]. The antioxidant capacity of methanolic breadfruit pulp by using the stable 2,2- diphenyl-1- picrylhydrazyl radical scavenging test with an IC50 value of 55+/-5.89ug/ml was also reported [19].Similar reports were also produced by pertaining to free radical scavenging capacity of breadfruit [20]. In contrast, highest antioxidant capability of breadfruit leaf herbal tea infusion was also reported [21]. Besides these informative findings, [22 & 23] too reported the antioxidant potential of breadfruit plant parts.

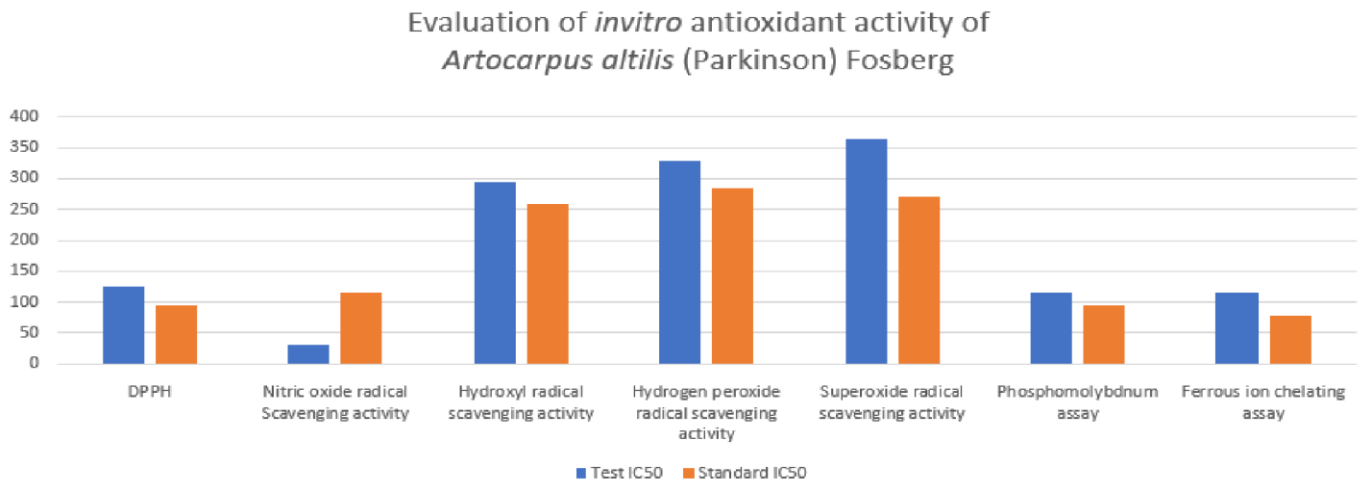

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Fig 1: Evaluation of *in vitro* antioxidant activity of *Artocarpus altilis* (Parkinson) Fosberg

Table 1: Evaluation of *in vitro* antioxidant activity of *Artocarpus altilis* (Parkinson) Fosberg

Concentration (µg/ml)	Methanol Extract						
	DPPH	**Nitric oxide radical Scavenging activity**	**Hydroxyl radical scavenging activity**	**Hydrogen peroxide radical scavenging activity**	**Superoxide radical scavenging activity**	**Phosphomolybdnum assay**	**Ferrous ion chelating assay**
50	16.5 ± 0.35	27.5 ± 0.73	9.37±0.27	47.05±0.02	16.51±0.58	15.1 ± 0.88	21.5 ± 0.35
100	31.3 ± 0.48	45.3 ± 0.57	18.75±0.32	55.22±0.6	26.26±0.84	25.5 ± 0.36	30.5 ± 0.28
150	53.4 ± 0.35	53.2 ± 0.48	28.12±0.61	59.69±0.29	39.25±0.58	36.7 ± 1.17	42.1 ± 0.20
200	66.6 ± 0.40	56.3 ± 0.43	35.21±0.18	63.65±0.64	45.3±0.64	49.5 ± 0.35	67.8 ± 0.54
250	82.8 ± 0.54	60.7 ± 0.82	46.87±0.58	69.54±0.67	53.84±0.8	59.5 ± 0.36	74.4 ± 0.36
IC 50	124	32.1	293.94	329.26	346.74	116.7	114.8
Stanadarad IC 50	94	116.3	258.79	284.25	269.97	94.4	77.7

Conclusion

The results of this study indicate that the pulp of *Artocarpus altilis* encompasses promising antioxidant activity which may be attributed to the presence of various bioactive compounds and hence can prevent the initiation of free radicals by stabilizing them to participate in any deleterious reactions. Yet, future investigations are required to isolate and analyze the chemical constituent responsible for its biological activity. Purification of important secondary metabolites and subsequent structural studies can aid in isolation of active compounds from this medicinally important plant . Therefore, the present study provides scope for scientific studies to fully exploit the medicinal properties of breadfruit to support the traditional claims as well as exploring some new and promising leads .

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