J. Sci. Trans. Environ. Technov. 2022, 16 (1) : 17 - 22

Evaluation of Antioxidant activity of Jackfruit Leaves (*Artocarpus heterophyllus*, Lam.)

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Article History
Received: 06.07.2022
Revised and Accepted: 08.08.2022
Published: 10.09.2022

https://doi.org/10.56343/STET.116.016.001.004
www.stetjournals.com

Abstract

Oxidative stress has been recognized as a key driver of many ailments affecting humankind. Plants have been used for many ages across time to manage human diseases and have a host of antioxidant phytocompounds. *Artocarpus hetero phyllus* is traditionally used for the management of inflammation, malaria fever, rheumatism, and insanity, among other diseases caused by a disturbed redox state in the body. In this study, *in vitro* antioxidant activities of the methanolic leaf extracts of *Artocarpus heterophyllus* were evaluated by *in vitro* antilipid peroxidation, the 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging, and the ferric reducing antioxidant power assay methods. The obtained results revealed remarkable antioxidant activities of the studied plant extracts as evidenced by the low IC_{50} and EC_{50} values. These antioxidant activities could be due to the presence of antioxidant phytochemicals like flavonoids, carotenoids, tannins, and phenols, among others. Therefore, the therapeutic potency of this plant could be due to its antioxidant properties. This study recommends *in vivo* antioxidant efficacy testing of the studied plant extracts, as well as isolation and characterization of bioactive antioxidant compounds that are potent against oxidative stress.

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INTRODUCTION

Antioxidants accompany the history of life's emergence on earth. In this way, due to the inappropriate environment with extreme conditions responsible for the free radical formation, the antioxidants were fundamental for the complex molecules constitution (Ndhlala *et al*., 2010) It could capture and stabilize free radicals in the oxidation process, and according to their source, they can be classified as synthetic, petroleum-derived, and natural from biomass (Knothe., 2007). Synthetic antioxidants are used as preservatives to prolong the products' shelf life, highlighting the butylated hydroxyl anisole (BHA), the butylated hydroxyl toluene (BHT), the tertiary butyl hydroquinone (TBHQ), and the propyl gallate (PG), which can exhibit, besides the antioxidant potential by capturing free radicals *via* hydrogen transfer, the chelating effect of metals. Although widely used by the food industry in Brazil, the application of synthetic antioxidants is argued due to the evidence that these substances' continued consumption can pose health risks (De Souza *et al*.,2019)

Besides, natural antioxidants incorporated in the food we consume, are thought to inhibit free radical chain reactions in the body by preventing initiation or propagation steps causing chain termination reactions, and thereby delaying the oxidation process. In view of the ethno medical information and uses of *Artocarpus heterophyllus*, the aim of this study was to investigate *in vitro* antioxidant activities of methanolic leaf extracts of this plant, as a preliminary step towards validation of its use and potential development of arsenal molecules against the reported and associated maladies.

P-ISSN 0973-9157
E-ISSN 2393-9249

MATERIALS AND METHODS

Collection of Sample

The Jackfruit leaves samples were collected from N.Vallundanpattu. Thanjavur (DT). Tamil nadu, India

Extraction procedure (Handa and Fermeglia 2008)

In the laboratory, the leaves of *Artocarpus heterophyllus* sample were washed with freshwater and shadow drying. Cleaned plant material was transferred to oven (ECOCELL) at 50 ℃ to stay there for 96 h for drying. Then they were powdered by electric blender. Approximately 100 g of different parts of *Artocarpus heterophyllus* powder was added to 400 ml methanol and soaked for 4 days. Removal of the plant material from solvents was done by filtration through cheese cloth, and the filtrate was concentrated using a rotary evaporator.

Antioxidant activity

1. Reducing power assay:

The reducing power of the *A.heterophyllus* extract was determined by the method of Oyaizu(1996) Various concentrations of *A. heterophyllus* extracts (2.5mL) phosphate buffer (2.5mL., 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5mL) were mixed and incubated at 50ºc for 20min. Ten % TCA (2.5mL) was added to the mixture. The mixture was centrifuged at 3,750×g for 10 min. A portion (2.5mL) of the supernatant was mixed with 2.5mL of deionised water and 0.5mL of 0.1% ferric chloride. After 10 min of incubation the absorbance was measured at 700 nm against a blank.

2. DPPH Radical- Scavenging Activity:

The scavenging effect of sample for DPPH radical were monifored according to the method of Yen and Chen (1995).

Briefly a 2.0ml of aliquot of test sample was added 0.2ml of 0.16mm DPPH methanolic solution. The mixture was vortexed for 1min and then left to stand at room temperature for 30min in the dark and its absorbance was read at 517nm. Synthetic DPPH radical was calculated using the formula Radical Scavenging effect (%) = Ab-As/ Ab×100 where, Ab=Absorbance of blank As= Absorbance of sample.

3. Hydrogen peroxide Scavenging:

Solution of 0.2M potassium dihydrogen phosphate and 0.2M sodium hydroxide solution were prepared as per the Indian pharmacopoeia 1996 standards Agarwal *et al* (2021). 50 ml potassium dihydrogen phosphate solution was placed in a
200 ml volumetric flask and 39.1 ml of 0.2M sodium hydroxide solution was added and finally volume was made up to 200 ml with distilled water to prepare phosphate buffer (pH 7.4). 50 ml of phosphate buffer solution was added to equal amount of hydrogen peroxide and generate the free radicals and solution was kept aside at room temperature for 5min to complete the reaction. Extracts (1ml) in distilled water were added to 0.6 ml hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H_2O_2 of extract was measured. The ability to scavenging the H_2O_2 radical was calculated using the following equation.

H_2O_2 scavenging activity (%)=(A0-A1) / A0×100 Where,

A0 is the absorbance of the control and A1 is the absorbance in the presence of extract sample. A standard of ascorbic acid was run using same concentrations as that of extract.

RESULTS AND DISCUSSION

Plants are an interesting source of bioactive natural compounds such as flavonoids, ascorbic acid, and phenolic acids. Thanks to their beneficial effects on human health and their several

biological activities including antioxidant and antihemolytic capacities, many scientific researchers have been focusing on the study of phenolic acids (Potìf *et al*., 2019 and Sun , Miao. 2019).

Antioxidant activity

The methanol extract of leaves of *A.heterophyllus* showed good radical scavenging activities and reducing power activities which were found to increasing concentration of the extract. The study indicated that the presence of the major phytochemicals *viz* flavonoids and phenols in the methanol extract of leaves of *A. heterophyllus* were 86.75 mg/g and 524.86 mg/g, respectively.

Jackfruit contains many carotenoids including all-trans-Beta-carotene with an important antioxidant activity for human health. Jackfruit exhibited not only free radical scavenging activities but also acted as a significant protective agent against H_2O_2 + UV and Y-irradiation induced DNA damage. Zhu, *et al.,*(2017)found polysaccaride from jackfruit pulp as a dietary source of antioxidant phytochemicals that survive the gastrointestinal digestion process. The DPPH method consists of measuring the capacity of an antioxidant substance to reduce the DPPH• radicals to the corresponding hydrazine, and simultaneously changing its color from violet to yellow, with concomitant decrease in absorbance at 517 nm (Zadra 2012).

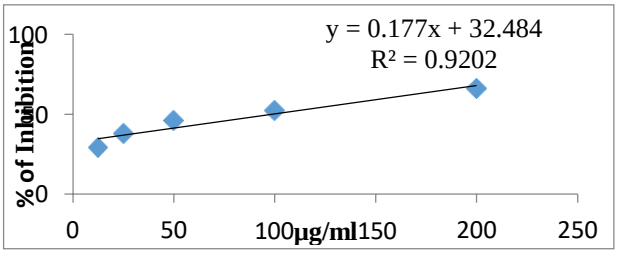
The ABTS method is largely accepted to evaluate the antioxidant capacity of food extracts, natural products, and pure compounds Ilyasov *et al*., (2020). This assay measures the potential of the antioxidant to scavenge the radical (ABTS+•) produced in the aqueous phase at 734 nm Sasikumar *et al*., (2010). The antioxidant potential using the ORAC method proved a 4 % superiority of J1 (13,369 µmolTrolox/ 100g dry weight) in comparison to J2 (14,728.81 µmolTrolox/ 100g dry weight); however, there were no significant statistical difference. Report value of 2,117 µmolTrolox/ 100g dried jackfruit, approximately 15% of the concentration determined in this study.

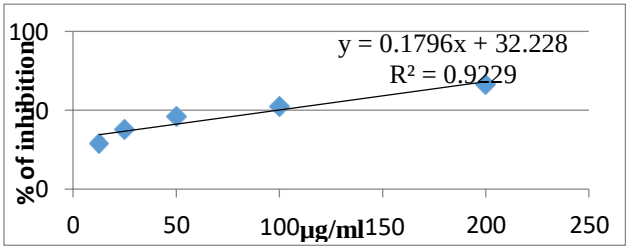
Phytochemical studies on jackfruit have reported the content of polyphenolic compounds and their antioxidant activity. The difference in antioxidant capacity are directly related to the phyto chemicals contained in the fruit, mainly those having functional groups such as hydroxyl (-OH) and that abound in compounds of the polyphenolic type such as: Phenolic acids (gallic acid, ferulic acid and tannins acid), flavonoids (catechin, rutin and myricetin) and/ or tannins (condensed/hydrolysable) mainly.

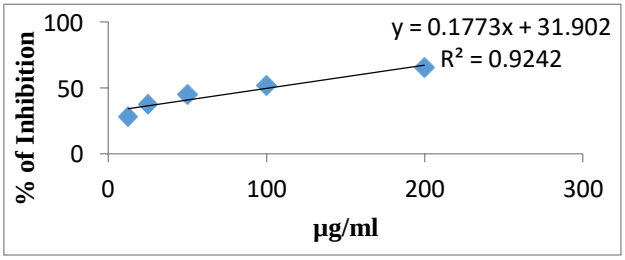
By far, this is a pioneer study on shell of *A.heterophyllus.* No published data on shell portion of *A.heterophyllusLamk* is available till date. Epidemiological evidence supports the hypothesis that food rich in natural antioxidant plays an important role in the prevention of several chronic diseases. On the basis of results presented here, a majority of the compounds identified in *A.heterophyllus*shell powder extract are beneficial for health, as they show strong antioxidant activity. Plant based antioxidant compounds have various biological potential as they serve the purpose without any side effect that is often associated with the synthetic compounds. The presented data might be helpful to ascertain *A.heterophyllus* shell as a potential source of natural antioxidants and nutraceutical/ functional food applications. However, there is scope for further clinical studies which can be carried out to explore the utility and efficiency in the treatment of chronic diseases.This study reveals that tested plant materials have moderate to significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work. In addition, more research is needed to identify and characterize the bioactive compounds responsible for these observed biological activities.

Fig: 1 ABTS

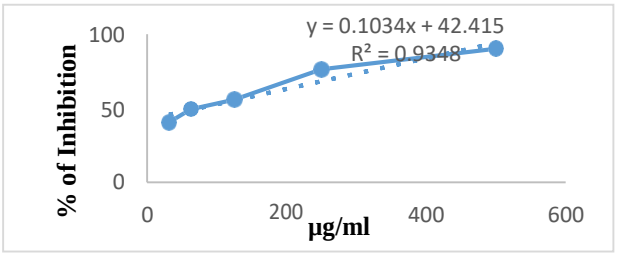
% of Inhibition

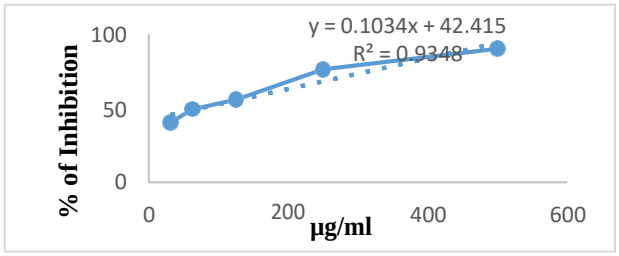




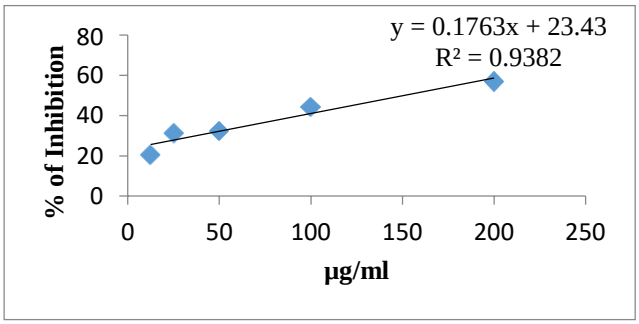


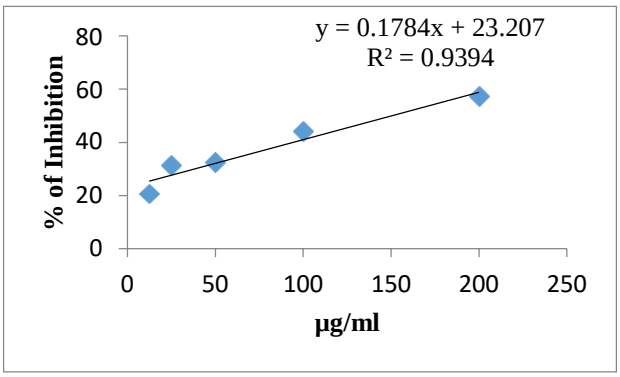
ABTS standard BHT

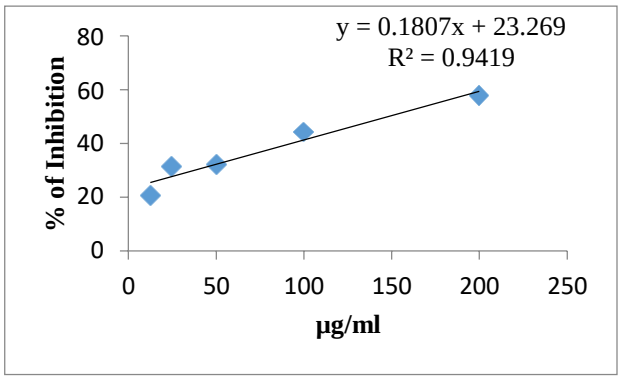




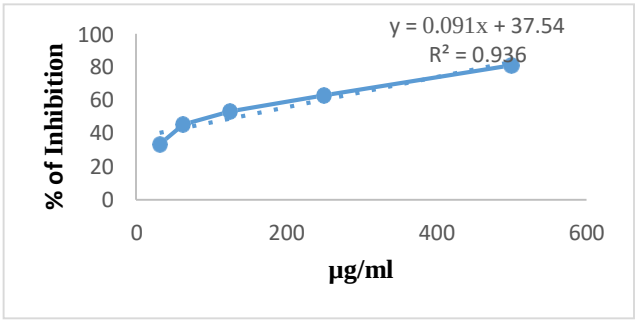
DPPH

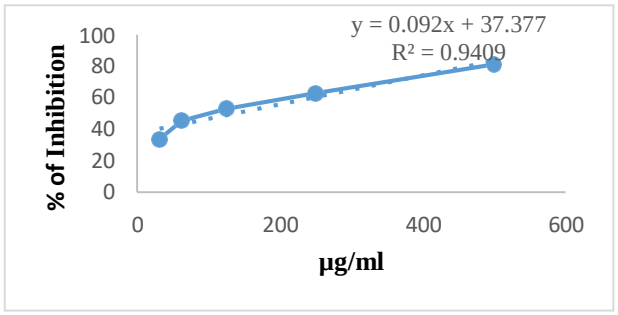


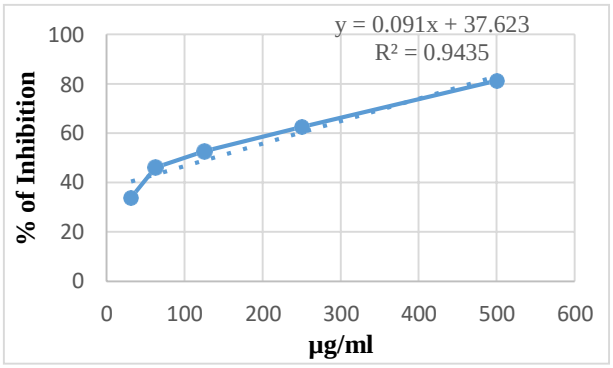




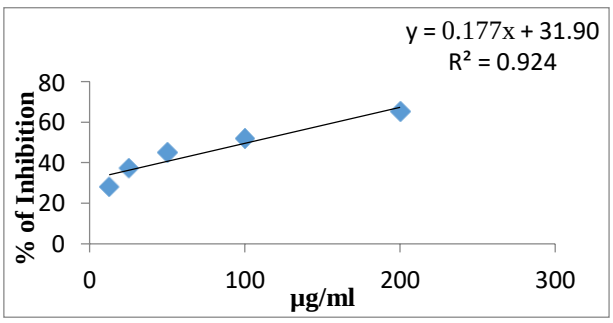
DPPH standard BHT

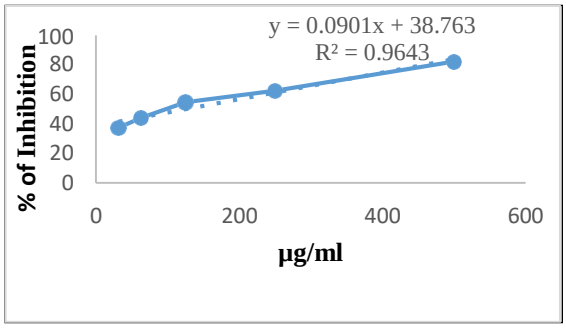


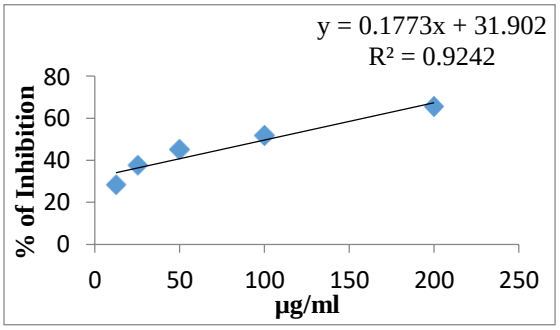




Superoxide standard BHT







Acknowledgement

The authors extend heartfelt thanks to the management authorities, principal A.V.V.M.Sripushpam College (Autonomous), Poondi- 613 503, Thanjavur(DT), TamilNadu State Council Science and Technology for financial assistance and for successful completion of M.Sc project.

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