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RESEARCH ARTICLE

PHARMACOGNOSY

PHYTOCHEMICAL, NUTRITIONAL AND ANTIOXIDANT ACTIVITY EVALUATION OF SEEDS OF JACKFRUIT (*ARTOCARPOUS HETEROLPHYLLUS* LAM.)

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ABSTRACT

In the present work, we have investigated nutritional, phytochemical content and antioxidant activity of seeds of the jackfruit (*Artocarpus heterophyllus* Lam.), one of the most ancient fruit indigenous to Western Ghats of India. The antioxidant properties were evaluated using free radical scavenging, metal chelating, ferric reducing antioxidant power and reducing power assays. Secondary metabolites including alkaloids, saponins, flavanoids and phenolics content were determined in the jackfruit seeds. Nutritional properties including moisture, fats, carbohydrates, proteins, ash content and metal content in the seeds were also estimated. Polyphenolic content and antioxidant properties of dichloromethane: methanol (1:1) extract of jackfruit seeds was found to be high and well correlated. Results indicated jackfruit seeds to be a good source of nutritional and antioxidant components and hold their potential for value addition and nutraceutical development.

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KEYWORDS

Artocarpus heterophyllus, antioxidant activity, mineral analysis, phytochemical analysis, proximal analysis

INTRODUCTION

Plant products are considered to be the most important components of diet for a good health. Fruits and vegetables have been shown to exert a protective effect[1-4]. Fruits and vegetables contains a number of compounds known as phytochemicals which have been found to be responsible for such effects and include caretenoids, alkaloids, vitamins, minerals and polyphenols. Research shows that phenolic compounds such as flavonoids and phenolic acids exhibit antioxidant properties[5,6]. The search for natural antioxidants, especially of plant origin, has notably increased in recent years. In view of this, various plant fruits and vegetables are under investigation for the detection of these bioactive compounds. Not even the fruits but the seeds also are considered to be containing large number of bioactive components which can have therapeutic uses.

The jackfruit (*Artocarpus heterophyllus* Lam.) belonging to family Moraceae is an integral part of common Indian diet and commonly known as “Kathal”. Jackfruit appears in the Indian market in spring and is available till summer. Jackfruit pulp is eaten afresh and used in fruit salads and possesses high nutritional value[7]. Jackfruit also has been reported to contain antioxidant prenylflavones[8]. Recently, antioxidant capacity of fruit pulp has been evaluated[9]. However, jackfruit seeds are less popular as vegetable and are eaten when boiled or roasted. These are believed to be digested with difficulty[10]. The composition of jackfruit seeds has been reported[11,12] and found to contains similar compositions as that of grains. The seeds are also rich source of carbohydrates and proteins and good source of fibre and vitamins. A major protein, Jacalin has been isolated from jackfruit seeds and possessed immunological properties[13,14]. Jackfruit seeds are not much explored in terms of nutrition and antioxidant properties. Chemical composition and mineral content of jackfruit seeds have been studied[15]. Soong and Barlow[16] have evaluated antioxidant properties of jackfruit seeds and found to show more than 70% contribution to the total antioxidant activity and phenolic content. Investigation of various nutritional components, phytochemicals and antioxidant activity present in the seeds of jackfruit was the aim of the present study in order to understand their nutritional and other health benefits.

MATERIALS AND METHODS

Collection of Sample Material and Extraction:

The raw jackfruit was collected from local market and authenticated as *Artocarpus heterophyllus* Lam. under reference number NISCAIR/RHMD/Consult/-2011-12/1703/03 at Raw Materials Herbarium & Museum, NISCAIR, CSIR, New Delhi, India.

The fruits were cleaned and separated into pulp and seeds. The seeds were crushed partially and were then analyzed for various parameters. Crushed jackfruit seeds were extracted using two different solvent systems: dichloromethane: methanol (1:1) and acetone. Extraction was carried out on an orbital shaker for 24 h at room temperature. Solvents were evaporated under vaccum and resulting extracts were stored at 4 ºC.

Determination of Nutritional Attributes:

Proximal Analysis:

Jackfruit seeds were dried in an oven at 105 °C overnight for 17 h to obtain moisture content by weighing the samples before and after drying[17]. The ash content was analyzed by weighing the samples before and after burning at 500 ºC for 24 h[18]. Macro Kjeldhal method was used for estimation of total nitrogen and crude protein content (N x 6.25)[19]. The fat content of the seeds was determined by Soxhlet extraction, using petroleum ether as a solvent[19]. Total carbohydrate was estimated using the formula:

Total carbohydrates (% fresh weight) = {100- moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight)}[18].

Mineral Analysis:

The mineral components of the jackfruit seeds were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). 1 g of crushed jackfruit seeds was digested by 5 ml of concentrated nitric acid in microwave. After digestion, the sample was cooled and volume made up to 25 ml with double distilled water. Set plasma conditions for analysis were: Argon on 151/min, auxiliary 0.21/min, nebulizer flow at 0.851/min, RF power on 1300 W and chiller at 15 ºC. Set of standards were run and then samples were analyzed against the standard.

Phytochemical Analysis:

Total phenolic content of jackfruit seeds in the two solvent systems were determined by Folin Ciocalteu reagent method[20] and expressed in terms of µg Gallic acid equivalents (GAE) /mg of dry extract. Total flavonoids content was also determined using aluminium chloride colorimetric method[21] and expressed in terms of µg rutin equivalents (RE) /mg of dry extract. Concentration of crude alkaloids[22] and saponins were also measured[23].

Determination of Antioxidant activity:

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity:

The principle behind 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical assay holds that the antioxidants react with the stable DPPH radical and convert it into 1,1-diphenyl-2-picryl hydrazine[24]. Scavenging activity on DPPH was assessed according to the method reported by Blois[25] with a slight modification. Briefly, 100 µl of extracts (0.1-0.5 mg/ml) were mixed with 1 ml of methanolic solution of 0.1 mM DPPH. The mixture was shaken well and incubated at room temperature for 30 min and absorbance was measured at 517 nm in a spectrophotometer. Ascorbic acid served as the standard. Experiment was performed in triplicate and the average was taken. Percent inhibition was calculated from control using the following equation:

Scavenging activity (%) = (1-Absorbance $_{sample}$/ Absorbance $_{control}$) x 100

2,2'-Azino-bis(3-ethylbenzthiazoline)-6-sulfonic free radical cation scavenging activity:

Trolox equivalent antioxidant capacity (TEAC) was estimated as 2,2'-Azino-bis(3-ethylbenzthiazoline)-6-sulfonic (ABTS) radical cation scavenging activity according to the method of Re et al.[26] Reagent solution consists of 7 mM ABTS and 2.45 mM potassium persulfate in 100 mM phosphate buffer solution (pH 7.4) and was left to stand for 12 h-16 h at laboratory temperature in the dark to form ABTS radical cation ($ABTS^{\bullet+}$). A working solution was diluted to absorbance values 0.7±0.02 at 734 nm with 100 mM phosphate buffer solution (pH 7.4). 10 µl of standard or seed extracts (2-10 µg/ml) were mixed with the working solution (990 µl) and absorbance was measured at 734 nm after 5 min. Ascorbic acid was used as a standard.

Scavenging activity (%) = (1-Absorbance $_{sample}$/ Absorbance $_{control}$) x 100

Ferric reducing antioxidant power (FRAP):

The assay was based upon the methodology of Benzie and Strain[27]. The FRAP reagent consisted of 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and 250 mM sodium acetate buffer (pH 3.6). FRAP reagent was freshly prepared by mixing 2,4,6-tripyridyl-s-triazine (TPTZ) solution, $FeCl_3$ solution and acetate buffer in a ratio 1:1:10. A 100 µl of extract solution containing 0.1 mg extracts was mixed with 900 µl of FRAP reagent. After the mixture stood at 37 ºC for 4 min, the absorbance at 593 nm was determined against blank. Trolox was used as calibration standard in concentration range, 0.002-0.01 mg/ml (y=0.160x, R^2=0.981). FRAP values were calculated as mg of Trolox equivalents/gm extract from three determinations and are averaged.

Fe^{2+}- chelating activity:

The chelating activity of extract on Fe^{2+} was measured according to the method of Dinis et al.[28] 1 ml of extracts (0.1-0.5 mg/ml) was incubated with 50 µl of 2 mM $FeCl_2$. The reaction was started by the addition of 200 µl ferrozine (5 mM). After 10 min, the absorbance of ferrous ion-ferrozine complex at 562 nm was read. Na_2EDTA served as positive control. Triplicate samples were run for each set and the average was taken. The ability of extracts to chelate ferrous ion was calculated using the following equation:

Chelating activity (%) = (1-Absorbance $_{sample}$/ Absorbance $_{control}$) x 100

Reducing power assay:

The reducing power of extracts was determined as per the method of Oyaizu[29]. 1 ml of extracts (0.25-1 mg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml $K_3Fe(CN)_6$ (1%). After incubating the mixture at 50 ºC for 20 min., 2.5 ml of 10% trichloroacetic acid was added, and then mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm and compared with standard ascorbic acid.

Statistical Analysis:

All data are presented as means ± SD. The mean values were calculated based on the data taken from at least three independent experiments conducted on separate days using freshly prepared reagents. Statistical analyses were performed using student's t-test. The statistical significances were achieved when $p < 0.05$.

RESULTS AND DISCUSSION

Determination of Nutritional Attributes:

Nutritional studies have demonstrated potential benefits of jackfruit seeds. Moisture content and dry matter analysis during nutrition reporting is very important because it directly affects its nutritional content, its stability and storage. Proximal values were calculated and depicted in the Table 1. In our studies, jackfruit seeds were found to be rich in proteins and carbohydrates. Moisture content was also very high. Crude fat and ash content were found to be very low. ICP-OES studies demonstrated jackfruit seeds to be highly rich in K followed by Ca and Na (Table 2). Seeds were also found to be an important source of microelement Zn.

Table 1
Proximate composition of jackfruit seeds

Ash	Moisture	Crude Fat	Total Protein	Total Carbohydrate
0.15±0.01	61.8±0.09	1±0.006	11.85±0.45	26.20±0.56

Data are mean±SD values of triplicate determinations. Concentrations are measured on dry weight basis (g/100g).

Phytochemical Analysis:

The phytochemical content of jackfruit seeds was analyzed and high quantity of saponins (6.32±0.098 g/100 g) was found. Saponins have been known for their medicinal uses, including antispasmodic activity, and toxicity to cancer cells. Some alkaloids function as spasmolytic, anti-cholinergic and anesthetic agents. The alkaloid content in jackfruit seeds was found to be 1.16±0.09 g/100 g. Polyphenolics are known to function as antioxidants through a number of mechanisms including radical scavenging by H-donation, prevention of chain initiation by donating electrons or by binding of transition metal ion catalysts. Flavonoids prevent platelet stickiness and hence platelet aggregation. Colorimetric study of the two extracts of jackfruit seeds showed that dichloromethane: methanol (1:1) solvent system was able to extract more phytochemicals in comparison to acetone (Table 3).

Table 2
Mineral compositions of jackfruit seeds

Analyte	Concentration
K	786.6±1.23
Ca	29.47±0.51
Na	28.39±0.36
Ba	0.275±0.32
Zn	2.280±0.12
Ar	0.047±0.38
Sn	0.031±0.05
Cr	0.018±0.02
Cd	0.010±0.002

Data are mean±SD values of triplicate determinations. Concentrations are measured on dry weight basis (ppm).

Table 3
Total phenolic and total flavonoid content of extracts of jackfruit seeds

Jackfruit seed extracts	Total phenolic content (µg GAE/ mg extract)	Total flavonoid content (µg RE/ mg extract)
Acetone	1.45±0.007[a]	290.6±3.414[a]
Dichloromethane: methanol (1:1)	2.12±0.009[b]	457.1±5.82[b]

Data are mean±SD values of triplicate determinations. GAE, Gallic acid equivalent; RE, Rutin equivalent.
[a,b]Mean values within each column followed by different letters are significantly different at p< 0.05.

Determination of Antioxidant activity:

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity:

DPPH is a free-radical generating compound and has been widely used to evaluate the free-radical scavenging ability of various antioxidant compounds.[30] Fig 1 shows the percentage inhibition of DPPH radicals by jackfruit seed extracts. The dichloromethane: methanol (1:1) extract showed higher radical scavenging activity (IC_{50}= 0.6433±0.0029 mg/ml) than acetone extract (IC_{50}= 0.7867±0.0104 mg/ml). However, standard ascorbic acid (IC_{50}= 0.0065±0.0001 mg/ml) was more active in scavenging as compared to both seed extracts. Both the extracts were significantly different (p< 0.05) in their IC_{50} values.

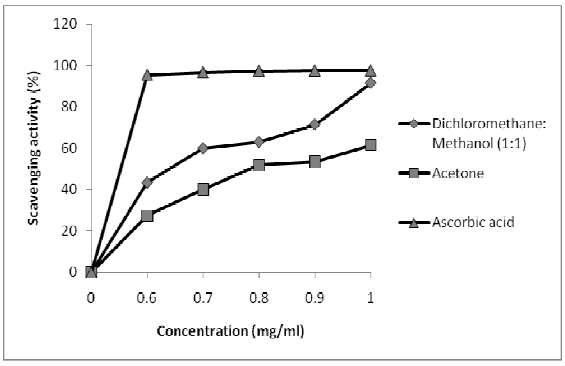

Figure 1
Concentration–response (scavenging activity of DPPH radicals) of the jackfruit seed extracts and the standard, ascorbic acid.

2,2'-Azino-bis(3-ethylbenzthiazoline)-6-sulfonic free radical cation scavenging activity:

ABTS scavenging assay is applicable for screening both lipophillic and hydrophilic antioxidants. Fig 2 shows the percentage inhibition of ABTS radical cations by jackfruit seed extracts and standard ascorbic acid.

Acetone extract (IC_{50}= 0.0491±0.0005 mg/ml) and dichloromethane: methanol (1:1) (IC_{50}= 0.0556±0.0002 mg/ml) showed less scavenging than that of standard ascorbic acid (IC_{50}= 0.0027±0.0003 mg/ml). There was significant difference (p< 0.05) in ABTS scavenging activity of both the extracts.

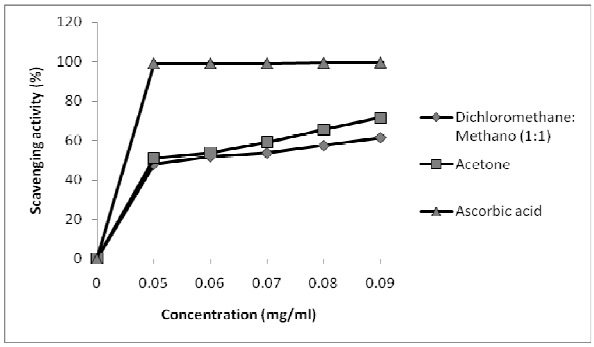

Figure 2
Concentration–response (scavenging activity of $ABTS^{\bullet+}$ radicals) of the jackfruit seed extracts and the standard, ascorbic acid.

Ferric reducing antioxidant power (FRAP):

In this assay, reduction of the ferric-TPTZ to the ferrous complex forms an intense blue colour which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the samples. The ferric reducing activity of the acetone and dichloromethane: methanol (1:1) extracts were found to be 2.195±0.02 and 3.215±0.007 µg TE/mg extract respectively. Thus, dichloromethane: methanol (1:1) extract is more potent antioxidant than acetone extract. Both the extracts of jackfruit seeds were significantly different (p< 0.05), in their FRAP values.

Fe^{2+}- chelating activity:

The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions. Thus, minimizing Fe^{+2} concentrations in Fenton reactions by metal chelation affords protection against oxidative damage. In this assay, both extracts interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture Fe^{+2} ion before ferrozine (Fig 3). IC_{50} values for the dichloromethane: methanol (1:1) and acetone extracts are 0.0646±0.0042 mg/ml and 0.08±0.00 mg/ml respectively and found to be significantly different (p<0.05) by paired t-test.

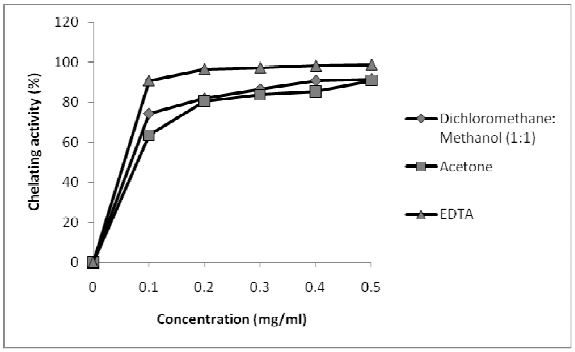

Figure 3
Concentration–response [chelaing activity of Fe^{+2} ions] of the seed extracts and the standard, Na_2EDTA.

Reducing power assay:

In the reducing power assay, the antioxidant compounds convert the oxidation form of iron (Fe^{+3}) to ferrous (Fe^{+2}). Reducing power of dichloromethane: methanol (1:1) extract was found to be 16.678±0.335 µg GAE/mg, which was higher than that of acetone (14.029±0.339 µg GAE/mg) extract. Thus, dichloromethane: methanol (1:1) extract is richer in antioxidant compounds. The two extracts showed significant difference in reducing power activity ($p<0.05$).

CONCLUSION

Jackfruit seeds were found to be rich in proteins, carbohydrates and minerals with moderate amount of phytochemicals and strong antioxidant properties. The fat content of the seeds was negligible making it a good constituent of fat free diet. The results of different *in vitro* antioxidant activity assays indicated that these seed extracts possessed appreciable DPPH, ABTS scavenging effects and metal ion chelating activity in a concentration-dependent manner. The antioxidant activity of the seed extracts may be attributed to their phenolic contents.

Therefore, jackfruit seeds could be used in balanced diets and functional foods which can be consumed safely without any concern of health risk. In countries with high population where the food requirements are not being fulfilled by seasonal vegetables, jackfruit seeds can be used as a good substitute. As jackfruit seeds have shorter shelf life, they go waste during the seasonal glut. So, the seed flour can be an alternative product, which can be stored and utilized, for value addition. This study helps in promoting increased consumption of jackfruit seeds by general public and offers opportunity to develop value added products from them.

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