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COMPREHENSIVE EVALUATION INTO THE PHARMACOLOGICAL POTENTIAL AND THERAPEUTIC APPLICATIONS OF ARTOCARPUS HETEROPHYLLUS (JACKFRUIT) SEED COAT [TESTA]

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ABSTRACT Artocarpus heterophyllus (Jack fruit) and its seeds are nutritionally rich packed with proteins, carbohydrates, micronutrients, iron, dietary fibre, vitamin, resistant starch, and antioxidants. They lower the risk of heart diseases, controls blood sugar, promotes weight loss, help in managing stress levels, skin diseases, good eyesight, strengthens immunity of the body, and maintains gut health. The seed coat of jackfruit comprises a thin, waxy, parchment-like testa (husk) and a brown, membranous tegmen. Typically, the white outer membrane of jackfruit seeds is removed and discarded before consumption. In this study, we aimed to assess the nutritional and pharmacological properties of this unexplored slimy, white outer membrane. The methanolic extract of the seed coat membrane [Testa] was obtained using Soxhlet extraction and used for the analysis. Phytochemical analysis of the test sample revealed the presence of flavonoids, saponins, phenols, xanthoprotein, proteins, and phytosterols, with measurable amounts of phenols and proteins. The extract exhibited significant antioxidant activity with an IC50 value of 232 µg/ml, using the DPPH method, and demonstrated cytotoxic properties. GC-MS analysis revealed the presence of several compounds with anticancer and antioxidant properties. The results imply that the extract has the potential to serve as an excellent source for antioxidant and anticancer drug in pharmaceutical medicines.

KEYWORDS : Nutrition, vitamins, antioxidants, dietary, micronutrients.

INTRODUCTION:

The Jackfruit plant (*Artocarpus heterophyllus*), a distinguished member of the Moraceae family, is widely regarded as an indigenous crop with deep-rooted cultural and agricultural significance. It is predominantly found across the Indian states of Kerala, Tamil Nadu, Karnataka, Goa, and Maharashtra, particularly flourishing in the biodiverse regions of the Western Ghats, while also being cultivated in select areas of Africa and South America. The Jackfruit (*Artocarpus heterophyllus*), valued as one of the largest tree-borne fruits globally, is distinguished for its delicious, sweet pulp. Each fruit usually contains 30 to 100 seeds, measuring 2-3 cm in length and 1-2 cm in diameter (Jena & Sinha 2022, Sahu, S., & Mohanty 2023). The seed of the Jackfruit (*Artocarpus heterophyllus*) is enveloped in a complex, multi-layered structure, comprising an outer seed coat and a thin, mucilaginous, white parchment-like outer layer known as the testa, which becomes crinkled and parchment-like upon drying. Beneath the robust testa lies the tegmen, a delicate brownish membrane that forms the inner seed coat, adding further intricacy to the seed's composition. The seed coat, primarily fibrous in nature, serves as a protective covering, while the testa provides an additional layer that shields the seed's nutrient-rich core. Though often discarded, these components, particularly the seed coat and testa, have been shown to hold potential nutritional and functional value. (Haq N. 2006).

The thin, waxy testa is a source of dietary fibre, which is essential for promoting gut health and improving digestion (Hossain M. K. & Nath T. K. 2002). It also contains bioactive compounds such as phenolics, flavonoids, and antioxidants, contributing to its health benefits. Research has revealed that the testa possesses properties that assist in regulating cholesterol and blood sugar levels, rendering it a valuable addition to functional foods aimed at promoting health and wellness. In addition to its nutritional benefits, the jackfruit testa can be used in sustainable food applications. It has potential for incorporation into food products as a natural fibre or as a functional ingredient to enhance the texture and nutritional content of various recipes. Furthermore, utilizing both the seed coat and testa in food systems aligns with the zero-waste approach to reduce food waste, making jackfruit more sustainable and economically viable. The growing interest in jackfruit seed coat not only contributes to waste reduction but also provides a sustainable, nutritious option in the food industry (Reddy, S. S., & Haripriya, S. 2015, Swami et al, 2012). In this study, we aimed to evaluate the pharmacological properties of the methanolic extract of jackfruit seed (Artocarpus heterophyllus) testa through phytochemical analysis, antioxidant activity, cytotoxicity testing, and GC-MS (Gas Chromatography-Mass Spectrometry) techniques.

MATERIALS AND METHODS:

COLLECTION OF MATERIAL AND EXTRACTION:

Jack fruit was collected from local market, Bangalore. The fruit was cut open, and the seeds were carefully extracted and cleansed of any residual pulp. The seeds, along with the testa, were first shade-dried, after which the dried testa was separated from the seeds. The white coloured dried Testa(husk) was peeled and ground into a fine powder. Fifty grams of the powder was placed in the extraction jar, and 500 ml of methanol was added as the solvent. Soxhlet apparatus was set up to allow for continuous solvent circulation until the compounds are fully extracted. Following extraction, the methanol is carefully evaporated, leaving behind a concentrated and purified extract. The resulting extract was subjected to analysis to identify the bioactive compounds and evaluate their potential applications.

PHYTOCHEMICAL ANALYSIS:

The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the extracts following standard protocols (Abdelhak R, Soraya B, 2018).

TOTAL PROTEIN CONTENT:

Total protein content of the methanolic extract was determined by standard Biuret method, using Bovine serum albumin as standard. A range of standard solution measuring from 0.0 to 1ml was pipetted out into series of test tubes. The extract was diluted with distilled water to a concentration of 500 µg/ml and 1000 µg/ml. 3ml of Biuret reagent was added to all the test tubes and the contents in the tubes were mixed and heated at 37 C for 10 mins. The absorbance was read at 540 nms. A standard curve was plotted using the concentration and the concentration of protein in the given extract was calculated. All the experiments were conducted in triplets (Layne, E. 1957).

TOTAL PHENOLIC CONTENT:

Total phenolic content of the methanolic extract was determined by using Folin-Ciocalteu method [12] using gallic acid as a standard phenolic compound. The extracts were diluted with distilled water to a concentration of 500 µg/ml and 1000 µg/ml. A range of 0.0 to 1000 µg of diluted gallic acid solution was mixed with 1 ml of of Folin - Ciocalteu reagent and incubated for 30 mins. The absorbance was precisely measured at a wavelength of 760 nm using a spectrophotometer, ensuring accurate analytical assessment. The experiment was meticulously conducted in triplicate to ensure the reliability and reproducibility of the results. (Di Ciaula et al., 2014, Nolden ES et al, 2023).

ANTIOXIDANT ACTIVITY:

DPPH METHOD

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was used to assess the antioxidant properties of plant extracts. The antioxidant activity was compared to that of ascorbic acid, a natural

antioxidant. The antioxidant activity of each sample was expressed as IC50 values, determined from the graph by plotting the percentage inhibition against the extract concentration [15].

To perform the assay, 1.5 ml of a 0.1 mM DPPH solution was mixed with 1.5 ml of different concentrations of extract, ranging from 10 to 500 µg/ml. The mixture was shaken thoroughly and incubated in the dark at room temperature for 30 minutes. The reduction of DPPH free radicals was quantitatively assessed by measuring the absorbance at 517 nm with the aid of a spectrophotometer. The experiment was repeated in triplicate, and ascorbic acid served as the positive control (Schaich, K. M et al (2015).

ANTICANCER ACTIVITY:

Green grams were purchased from the local market and 10 seeds were individually measured. Initially, the green gram seeds were carefully soaked in distilled water, drug methotrexate and 25mg/ml and 50mg/ml of extract. Soaked seeds were placed on cotton presoaked in water and kept in a petri dish and this treatment was allowed to continue for a total duration of 72hrs. The Petri plates were left at room temperature for 24hrs for imbibition of water. Throughout this experimental period, meticulous readings and observations were diligently recorded at consistent intervals of every 24 hours. After 72 hours all the treated seeds were dried on dry tissue paper and the length of germination of seeds was measured (K. Naga Kumari et., al 2023).

GC-MS ANALYSIS:

The Clarus 680 GC was used in the analysis, employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 2 ml/min. The injector temperature was set at 220°C during the chromatographic run. The 1µL of extract sample injected into the instrument and the oven temperature was set as follows: 50 °C (2 min); followed by 150 °C at the rate of 15 °C min−1; and 150 °C, where it was held for 2min and then followed by 250°C at the rate of 30°C min−1; it was held for 8.00 min. The mass detector conditions were: Inlet line temperature 250 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2017) library 90 (Starlin T, 2019).

RESULTS:

PHYTOCHEMICAL ANALYSIS:

The preliminary phytochemical analysis revealed the presence of several key bioactive compounds in the extract, including flavonoids, saponins, phenols, xanthoproteins, proteins, and phytosterols, all of which tested positive. Notably, glycogen was absent in the analysis, highlighting the selective composition of phytochemicals within the extract.

TOTAL PROTEIN CONTENT

A standard curve was constructed by plotting the absorbance values measured at 540 nm against a series of known protein concentrations, expressed in micrograms (µg). This graph serves as a reference, allowing for the accurate determination of protein content in unknown samples by correlating their absorbance readings with the standard curve. For the specific extract measured, the total protein content was found to be 520 µg in the 0.5 ml extract and 970 µg in the 1 ml extract. These values were extrapolated from the standard curve based on the absorbance data. The higher protein content in the 1 ml sample, compared to the 0.5 ml extract, reflects the increased volume, thereby demonstrating the proportional relationship between extract volume and protein concentration.

TOTAL PHENOL CONTENT

A standard curve was generated by plotting the absorbance values at 720 nm against different concentrations of phenol, measured in micrograms (µg), providing a reliable reference for determining phenol content in unknown samples (Figure 1). By comparing the absorbance of extract 1 (0.5 ml) with the standard curve, the total phenol content was found to be 350 µg and in extract 2 (1 ml) showed 150 µg. The analysis demonstrated a significant amount of phenol content in the extract.

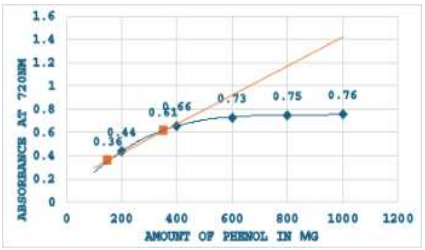

Figure:1. Graph showing Total Phenol content in Jack fruit testa extract.

ANTIOXIDANT ACTIVITY:

The test extract exhibited substantial antioxidant activity using the DPPH method, as reflected by its IC50 value of 232 µg/ml, indicating its potent ability to neutralize free radicals Figure 2. An IC50 value represents the concentration of the extract required to inhibit 50% of the DPPH radicals, and a lower value signifies stronger antioxidant potential.

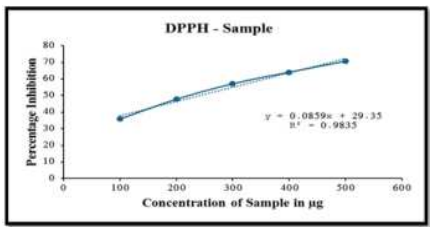

Figure: 2. Graph showing Antioxidant activity in Jack fruit testa extract by DPPH method.

The results indicates that the extract possesses strong free radical-scavenging properties, which can help reduce oxidative stress in biological systems. Further studies could identify the bioactive compounds and determine their specific roles in contributing to free radical-scavenging activity and insight into their mechanisms of action.

ANTICANCER ACTIVITY:

The test extract resulted in reduced germination after 72 hours, suggesting that it may contain compounds that either inhibit or slightly promote growth. The extract was less conducive indicating the presence of inhibitory elements that slow germination without completely halting it. No germination occurred in the methotrexate treatment, as methotrexate is a chemotherapeutic agent known to inhibit cell division. This blockage of cell division during the critical germination phase results in the complete suppression of seed growth.

Table 1. Showing comparative Inhibitory effects of water, extract and methotrexate on Mung Seed Germination.

Sl. No	Time Duration in hours	Growth in Distilled Water cm	Growth in Extract	Growth in methotrexate
1	12	0.1	No Growth	No Growth
		0.2	No Growth	No Growth
2	24	0.4	No Growth	No Growth
		0.5	No Growth	No Growth
3	36	0.7	No Growth	No Growth
		0.7	No Growth	No Growth
4	48	0.9	No Growth	No Growth
		0.8	No Growth	No Growth
5	60	1.0	No Growth	No Growth
		0.9	No Growth	No Growth
6	72	1.3 & 1.2	No Growth	No Growth

Seeds soaked in water exhibited strong germination and healthy growth. Water is the natural medium for seed germination, providing essential moisture needed to activate enzymes that break down stored nutrients in the seed. This results in higher and more consistent germination. Seeds typically thrive in water as it meets the basic needs for hydration and growth without introducing stress factors.

GCMS ANALYSIS:

GC-MS (Gas Chromatography-Mass Spectrometry) analysis revealed a substantial number of compounds within the test sample, many of which are known for their antioxidant and anticancer properties. This

analytical technique allows for the identification and characterization of the chemical components present in the sample. Following were the important compounds identified: 3,4,5-trimethyl-4H-1,2,4-triazole, 7-Oxabicyclo [4.1.0] heptane, Phthalic acid, isobutyl octadecyl ester, Phthalic acid, butyl 2-ethylbutyl ester, Pentadecanal-, E-11(13-Methyl)tetradecen-1-ol, Bicyclo[2.2.1]heptan-2-ol, 2-(2-cyclopenten-1-yl)-, 5-Methyl-1-heptanol, 4-Dodecanol, Phthalic acid, butyl tetradecyl ester, Hexadecanoic acid, methyl ester, Tetradecanoic acid, 12-methyl-, methyl ester, (S)-, Decanoic acid, 2- methyl, 1-Tridecen-1-ol, E-10-Pentadecenol, cis-7-Hexadecenoic acid, Pyridine, 1,2,3,6-tetrahydro-1,2-dimethy, Isoxazole, trimethyl- and 17-Octadecynoic acid.

DISCUSSION:

The presence of compounds in the extract with antioxidant activity is significant because these substances can neutralize free radicals, reducing oxidative stress and protecting cells from damage. This property plays a crucial role in preventing chronic conditions like cardiovascular diseases, diabetes, and neurodegenerative disorders, all of which are linked to oxidative damage.

Additionally, the discovery of anticancer compounds is of great importance. These bioactive molecules can inhibit the growth of cancer cells, induce apoptosis (programmed cell death), and interfere with the proliferation of tumours. The identification of such compounds through GC-MS suggests that the test sample may hold potential in developing natural therapeutic agents or supplements for cancer prevention and treatment.

The combined antioxidant and anticancer activities of the identified compounds make the test sample particularly valuable for further investigation. This could lead to exploring its use in pharmaceuticals, nutraceuticals, or functional foods aimed at promoting health and preventing disease. Further research, including in vitro and in vivo studies, would be essential to validate these biological effects and to determine the efficacy and safety of the identified compounds.

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