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COMPARATIVE EVALUATION OF BIOACTIVE COMPOUNDS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF PULP AND PEEL EXTRACTS OF BREADFRUIT (*ARTOCARPUS ALTILIS*) - AN UNDERUTILIZED FRUIT

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Breadfruit (*Artocarpus altilis)* has been categorized as an underutilized fruit. Different parts of this underused fruit contain essential nutrients that are beneficial for human health. Scientific studies indicate that fruit peels also contribute to several pharmacological properties. The objective of the study was to compare the antioxidant and antibacterial activities of pulp and peel extracts of breadfruit. Antioxidant activity was evaluated using DPPH$^{\bullet}$ radical scavenging activity, Fe^{3+} reduction and phosphomolybdenum reduction assay. Antibacterial activity was determined using agar well diffusion method against six bacterial strains *viz Escherichia coli*, *Bacillus subtilis, Staphylococcus aureus*, *Shigella flexneri*, *Proteus vulgaris* and *Klebsiella pneumonia.* Volatile functional compounds were identified using Gas chromatography-mass spectrometry. Results indicate that the peel extract exhibited greater potential to scavenge DPPH$^{\bullet}$ free radicals along with strong reducing capacity. IC_{50} value of pulp and peel extract was found to be 246.54µg/mL and 98.21µg/mL. With reference to antibacterial activity, the peel extract possessed greater ability to inhibit the growth of pathogenic bacteria. Maximum zone of inhibition was observed for *Staphylococcus aureus* (23 mm at a concentration of 625µg/mL). GC-MS analysis showed the presence of seven volatile functional compounds in pulp extract and thirteen functional compounds in peel extract. Findings of the study highlight the use of pulp and peel of *Artocarpus altilis* as natural antoxidant and antimicrobial sources

Keywords: *Artocarpus altilis,* antioxidant, antibacterial, functional compounds, underutilized fruit

Underutilized fruits refer to fruits that are cultivated and traded either regionally or locally. These fruits can easily grow in adverse soil and agro-climatic conditions (Urvashi and Raju 2014). Some of the under exploited fruits cultivated in India include aonla, bael, karonda, passion fruit, wild apple and wood apple (Gajanna *et al*. 2010). These fruits being rich in essential micronutrients together with the ability to grow in adverse climatic conditions are a boon to our national economy. Effective commercialization and domestication of such neglected fruits can generate employment to rural people, provide food security and can also protect biodiversity.

Artocarpus altilis commonly known as breadfruit is a starchy multipurpose underutilized tropical fruit that belongs to the family Moraceae. The fruit grows abundantly in southern parts of India such as Karnataka and Kerala. Breadfruit is round, oval or oblong in shape and weighs about 6 kgs. The colour of the ripe fruit varies from yellow to yellowish brown. The flesh of the fruit is creamy, soft, sweet to taste and has a characteristic flavour (Zerega *et al*. 2005).The fruit is an excellent source of dietary fiber and micronutrients. Breadfruit contains several functional compounds such as dihydroxychalcones, prenylated flavonoids, flavones, morin, moracin, phenolic compounds, steroids, stilbenes and terpenes that provide a diverse range of health promoting properties (Wang *et al*. 2006, Sikarwar *et al*. 2014). Medicinal properties of breadfruit include antimicrobial, anticancer, anti-inflammatory, antioxidant, anticholingeric and estrogen regulating

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properties (Baba *et al*. 2016, Tamegnon *et al*. 2017).

Waste management involves converting plant based by-products into commercially available novel products that are useful to the community with the additional benefit of decreasing environmental pollution (Chatterjee 2014). Recent area of research involves exploiting the possibility of using fruit peels as functional components in different industries as they represent a natural source of organic acids, secondary metabolites, sugars and volatile organic compounds. Despite being rich in nutritional and medicinal properties, breadfruit still remains as an underexploited fruit in most of the tropical areas. Furthermore, peel and seed extracts of this fruit has also received very little research attention. Hence, the objective of the study was to evaluate and compare the antioxidant and antibacterial activities of pulp and peel extracts of *A. altilis.*

METHODOLOGY

Preparation of extract: Breadfruit was purchased from a local market in Kerala. The peels were separated manually and shade dried for 4-5 days at room temperature. 50 g of dried peels and 50g of fresh pulp of breadfruit were soaked in 100 mL of ethanol for 72 hrs using maceration technique. The supernatant was filtered and concentrated using a rotary vacuum evaporator. The dry residue was preserved at 5°C until further use.

Chemicals: 2, 2- diphenyl-1-picrylhydrazyl, gallic acid, quercetin, folin ciocalteu reagent, trichloroacetic acid, ferric chloride and ascorbic acid were purchased from Sigma Aldrich and Hi Media Chemicals. All other organic solvents and reagents used were of analytical grade.

Qualitative analysis of phytochemicals: The extracts were screened for the presence of phytochemicals such as alkaloids, glycosides, saponins, phenols, flavonoids, terpenoids, steroids, quinones and tannins using standard methods (Raaman 2006).

Estimation of TPC: TPC was estimated using Folin-Ciocalteau reagent method (Singleton *et al*. 1999). Hundred µL of the extracts (1mg/mL) were mixed with 1 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water). After 5 min, 1 mL of 20% Na_2CO_3 was added. The mixture was incubated at room temperature for 30 min and the absorbance was measured at 760 nm. TPC is expressed in terms of µg/mg GAE.

Estimation of TFC: TFC was estimated using $AlCl_3$ reagent method (Chang *et al*. 2002). The extracts (500µg/mL) were mixed with 0.5 mL of 5% $NaNO_3$ and incubated for 5 min. Lastly, 0.3mL of 10% $AlCl_3$ was added followed by subsequent addition of 1 mL of 1M NaOH and incubated for 15 min. The absorbance was measured at 510 nm. TFC is expressed in terms of µg/mg QE.

Antioxidant activity

DPPH$^{\cdot}$ radical scavenging activity: Various concentrations of the extracts were mixed with 1 mL of 0.1 mM of DPPH$^{\cdot}$ dissolved in methanol. The entire setup was left in dark at room temperature and the absorption was measured after 30 min. Absorbance was read at 517nm. Results are expressed in terms of percentage inhibition of DPPH$^{\cdot}$ free radical (Blois 1958).

Ferric (Fe^{3+}) reducing power activity: Various concentrations of the extracts were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min. Later, 1mL of 10% trichloroacetic acid and 1mL of 0.1% of freshly prepared ferric chloride was added. The reducing power is expressed as absorbance value measured at 700 nm (Yen and Chen 1995).

Phosphomolybdenum reduction assay: Various concentrations of the extracts were mixed with 1 mL of the reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The test tubes were capped and incubated in a water bath at 95°C for 90 min. The tubes were cooled and the absorbance was measured spectrophotometrically at 695 nm. The reducing power is expressed as absorbance value measured at 695 nm (Prieto *et al*. 1999). For all antioxidant assays, the standard used was ascorbic acid.

Antibacterial activity: The antibacterial activity was determined using agar well diffusion method. Bacterial strains used in the study were *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 441)*, Staphylococcus aureus* (MTCC 96), *Shigella flexneri* (MTCC 1457), *Proteus vulgaris* (MTCC 426) and *Klebsiella pneumonia* (MTCC 109). Freshly prepared nutrient agar was poured into the petri plates and was allowed to solidify. Bacterial inoculum suspension was streaked over the surface of the media using a sterile cotton swab. Wells were made using a sterile steel borer. The standard used for antibacterial activity was tetracycline. The inoculated plates were incubated for 24 hours and observed for the presence of clear zone. The antibacterial activity is expressed in terms of zone of inhibition in millimetres (Langfield *et al*. 2004).

Gas chromatography – mass spectrometry (GC-MS): The extracts were injected into a HP-5 column (30 m x 0.25 mm with 0.25 µm film thickness) agilent technologies 6890 N JEOL GC Mate II GC-MS model. Helium was used as the carrier gas at a constant flow rate of 1 mL/min and the injector was operated at 200°C. The column oven temperature was maintained at 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV, ion source temperature of 250°C, interface temperature of 250°C and mass range of 50-600 mass units. National Institute Standard and Technology database having more than 62,000 patterns was used for interpretation of identified compounds.

RESULTS

Phytochemical analysis: Plants synthesize a wide range of chemical compounds known as phytochemicals or secondary plant metabolites. Preliminary qualitative analysis of phytochemicals showed the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, saponins, tannins and quinones in both pulp and peel extracts of breadfruit. Upon quantification, TPC of peel extract was found to be two times greater (158 ± 5.32 µg/mg GAE) than the amount present in pulp extract (88.20 ± 2.96 µg/mg GAE). TFC of pulp and peel extract was found to be 51.29 ± 1.82 µg/mg QE and 54.30 ± 8.54 µg/mg QE.

Antioxidant activity: $DPPH^{\cdot}$ radical scavenging activity of peel and pulp extracts of *A. altilis* was found to be concentration dependent (Table 1). The peel extract exhibited greater radical scavenging activity when compared to the pulp extract. IC_{50} value of peel and pulp extracts was found to be 98µg/mL and 246.54µg/mL respectively. Results of reducing power assays indicate that the reducing power of peel and pulp extracts of *A. altilis* increased linearly with increase in concentration (Table 2 and 3). However, the reducing power of peel extract was higher than the pulp extract.

Antibacterial activity: Table 4 illustrates the

Table 1: $DPPH^{\cdot}$ radical scavenging activity of pulp and peel extracts of *A. altilis*

Concentration µg/mL	Pulp extract	Peel extract	Ascorbic acid
50	8.20 ± 2.65	27.61 ± 2.66	43 ± 2.69
100	27.63 ± 1.24	51.34 ± 2.09	60 ± 0.98
150	41.3 ± 3.49	78.15 ± 1.70	82 ± 2.01
200	46.52 ± 2.44	82.7 ± 0.95	85 ± 1.20
250	50.77 ± 0.14	85.25 ± 0.38	90 ± 0.54
300	63.44 ± 1.46	89.27 ± 0.76	96 ± 3.25

Values are the mean of triplicates

Table 2: Fe^{3+} reducing activity of pulp and peel extracts of *A. altilis*

Concentration µg/mL	Pulp extract	Ascorbic acid	Concentration µg/mL	Peel extract	Ascorbic acid
	Absorbance at 700 nm	Absorbance at 700 nm		Absorbance at 700 nm	Absorbance at 700 nm
50	0.16 ± 0.01	0.45 ± 0.03	20	0.35 ± 0.01	0.47 ± 0.05
100	0.28 ± 0.03	0.54 ± 0.01	40	0.49 ± 0.02	0.52 ± 0.02
150	0.38 ± 0.04	0.69 ± 0.02	60	0.61 ± 0.01	0.59 ± 0.01
200	0.41 ± 0.01	0.75 ± 0.04	80	0.66 ± 0.01	0.65 ± 0.04
250	0.50 ± 0.01	0.81 ± 0.05	100	0.71 ± 0.01	0.78 ± 0.01
300	0.55 ± 0.01	0.90 ± 0.02	120	0.74 ± 0.01	0.86 ± 0.03

Values are the mean of triplicates

Table 3: Phosphomolybdenum reducing activity of pulp and peel extracts of *A. altilis*

Concentration µg/mL	Pulp extract	Peel extract	Ascorbic acid
	Absorbance at 695 nm	Absorbance at 695 nm	Absorbance at 695 nm
50	0.29 ± 0.02	0.40 ± 0.01	0.50 ± 0.02
100	0.41 ± 0.02	0.48 ± 0.04	0. 62± 0.04
150	0.58 ± 0.05	0.64 ± 0.01	0.72 ± 0.03
200	0.67 ± 0.04	0.70 ± 0.01	0.77 ± 0.01
250	0.73 ± 0.05	0.77 ± 0.03	0.82 ± 0.02
300	0.74 ± 0.04	0.79 ± 0.01	0.89 ± 0.01

Values are the mean of triplicates

Table 4: Antibacterial activity of pulp and peel extracts of *A. altilis*

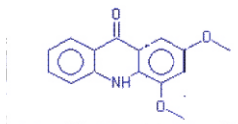
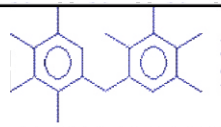
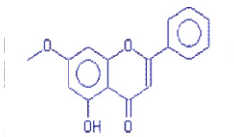
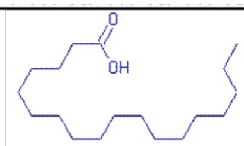
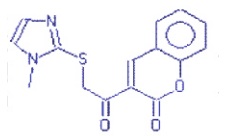
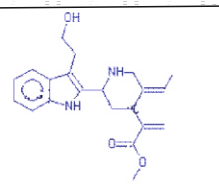
Extracts	Pathogens	Zone of inhibition (nm)				
		Standard	250µg	375µg	500µg	625µg
Pulp extract	*Escherichia coli*	27	10	12	14	15
	Bacillus subtilis	30	14	15	16	18
	Shigella flexneri	31	13	14	16	17
	Klebsiella pneumonia	27	10	11	12	15
	Staphylococcus aureus	29	12	14	15	18
	Proteus vulgaris	29	12	13	14	18
Peel extract	*Escherichia coli*	22	15	16	18	20
	Bacillus subtilis	20	11	12	13	18
	Shigella flexneri	20	13	14	15	16
	Klebsiella pneumonia	22	15	16	17	20
	Staphylococcus aureus	28	20	21	22	23
	Proteus vulgaris	20	16	17	18	20

antibacterial potential of pulp and peel extract of *A. altilis*. The antimicrobial activity of pulp and peel extracts of *A. altilis* increased gradually with increase in concentration. However, on comparison, the peel extract had greater potential to inhibit the growth of micro-organisms. Maximum inhibitory activity was observed for *Staphylococcus aureus* (23mm) followed by *Proteus vulgaris* (20 mm) and *Escherichia coli* (20mm) at a concentration of 625µg/mL. For pulp extract, maximum inhibitory activity was observed for *Staphylococcus aureus* followed by *Proteus vulgaris* and *Bacillus subtilis* (18 mm at a concentration of 625µg/mL).

GC-MS analysis of pulp extract of *A. altilis*: The retention time, name, structure, molecular weight and molecular formula of volatile compounds present in pulp extract of *A. altilis* is given in table 5.

GC-MS analysis of peel extract of *A. altilis*: Spectral analysis of ethanolic peel extract of *A. altilis* showed the presence of thirteen volatile compounds. The retention time, name,

Table 5: Compounds identified from pulp extract of *A. altilis*

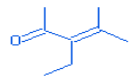
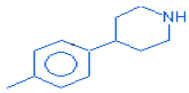
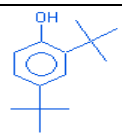
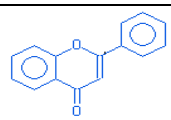
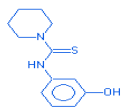
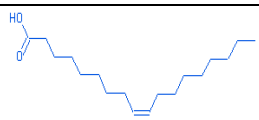
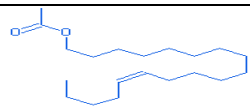
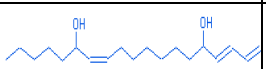
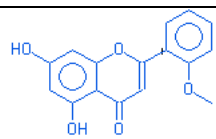
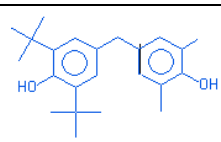
Retention Time	Name	Structure	Molecular weight	Molecular formula
10.18	2, 4-dimethoxy-10 H-acridin-9 –one		255	$C_{15}H_{13}NO_3$
13.12	Benzene, 2,3,4,5-tetramethyl -1[2,3,4,5-tetramethyl [benzyl]		280	C_6H_6
16.05	4H-1-benzopyran- 4-one-5-hydroxy-7-methoxy-2- phenyl		268	$C_{16}H_{14}O_4$
16.65	Octadecanoic acid		284	$C_{18}H_{36}O_2$
18.27	Oleic acid		282	$C_{18}H_{34}O_2$
18.47	Coumarine, 3- [2-(1-methyl-2-imidazolythia)-1-oxoethyl]		300	$C_{15}H_{12}N_2O_3S$
21.78	4-priperdineacetic acid, 5-ethyllidene- 2-[3-(2-hydroethyl)-1H- indol- 2yi]-methylene, methyl ester		354	C_7H_{13}

molecular weight, molecular formula and structure of the detected components is given in table 6.

DISCUSSION

Besides consumption of commonly available local fruits, consuming underutilized fruits are also important as they are rich in micronutrients and numerous unique non-nutritive components. India is one such country where different varieties of underutilized fruits are cultivated. However, these fruits have received little attention with reference to use as natural antioxidant and antimicrobial sources. The present study was carried out to compare the antioxidant and antibacterial properties of pulp and peel extracts of *A. altilis*. Fruits categorised as underutilized or neglected species are an excellent source of phytonutrients. Breadfruit is a rich source of phenolic constituents such as catechins, chlorogenic acid, cinnamic acid, castalagin, ellagic acid, epicatechins and gallic acid. Results of the study indicate that the pulp and peel extracts of *A. altilis* showed the presence of

Table 6 : Compounds identified from peel extract of *A. altilis*

Retention Time	Name	Structure	Molecular Weight	Molecular Formula
12.65	3-penten-2-one,3-ethyl-4 – methyl		126	$C_8H_{14}O$
14.17	3-cyclohexen -1-ol-4 methyl -1-[1-methylethyl]		154	C_6H_{12}
15.92	Piperidine,4-[4-methylphenyl]		175	$C_{18}H_{21}N_3$
17	Phenol,2,4-bis(1,1-dimethyethyl]		206	$C_{14}H_{22}O$
17.7	Flavone		222	$C_{15}H_{10}O_2$
18.68	Piperidine-1-thiocarboxamide,N-[3-hydroxyphenyl]		236	$C_{28}H_{39}N_3O_3$
19.52	Oleic acid		282	$C_{18}H_{34}O_2$
20.17	12-methyl-E,E-2,13-octadecadien-1-ol		280	$C_{19}H_{36}O$
21.4	13-octadecen-1-yl acetate		310	$C_{20}H_{38}O_2$
22.05	E,E Z-1,3,12-nonadecatriene-5,14-diol		294	$C_{19}H_{34}O_2$
22.85	4H-1-benzopyran-4-one,5,7-dihydroxy-2-[2methoxyphenyl]-		284	$C_{16}H_{12}O_5$
24.8	Phenol,2,6-bis(1,1-diethylethyl)-4[(4-hydroxy-3,5-diethyl phenyl)methyl]-		340	$C_{29}H_{44}O_2$

alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, saponins, tannins and quinones. Upon quantification, TPC and TFC of pulp extract were found to be 88.20 ± 2.96 µg/mg GAE and 51.29 ± 1.82 µg/mg QE. Shrikanta *et al*. (2015) estimated the polyphenol and resveratrol content of three neglected fruits namely *Syzygium cumini*, *Artocarpus heterophyllus* and *Morus rubra*. The results showed that *Morus rubra* had the highest resveratrol content (50.61 µg g (-1) dry weight).

Antioxidants function as hydrogen donors, metal chelators and as singlet oxygen quenchers. $DPPH^{\cdot}$ radical scavenging assay is widely used to investigate the antioxidant property of several natural plant based compounds. The ability of pulp and peel extracts of *A. altilis* to scavenge $DPPH^{\cdot}$ free radical increased linearly in a dose dependent manner. The peel extract had greater scavenging activity (89.27%) when compared to the pulp extract (63.44%) at a concentration of 300µg/mL. IC_{50} value of peel and pulp extracts was found to be 98µg/mL and 246.54µg/mL. Jalal *et al*. (2015) reported the IC_{50} value of methanolic pulp extract of *A. altilis* to be 55µg/mL. Differences in the IC_{50} value could be due to different solvents and extraction methods used for evaluating the antioxidant property. Results of Fe^{3+} reduction activity indicate that the reduction capability of pulp extract (maximum absorbance = 0.55 ± 0.01 at 300µg/mL) was less when compared to peel extract (maximum absorbance = 0.74 ± 0.01 at 120µg/mL). The end point of phosphomolybdenum reducing assay is reduction of Mo (VI) to Mo (V) along with the formation of bluish green complex. The results indicate that peel extract had greater reducing power. Overall, the peel extract showed higher antioxidant activity. This might be due to the presence of higher amount of phenolic compounds and flavonoids present in the peel extract. Furthermore, spectral analysis of peel extract of *A. altilis* showed the presence of alkaloid, flavonoid and phenolic compounds which could have contributed to greater antioxidant activity.

Different parts of a plant have numerous distinctive functional components that are widely used in the development of antimicrobial drugs. In the present study, the antibacterial activity of pulp and peel extracts of *A. altilis* increased with increase in concentration. For peel extract, maximum inhibitory activity was observed for *Staphylococcus aureus* (23mm at 625µg/mL). With regard to pulp extract, maximum inhibitory activity was observed for *Staphylococcus aureus* followed by *Proteus vulgaris* and *Bacillus subtilis* (18 mm at 625µg/mL). Results of the present study are in par with the findings of (Jiyauddin *et al*. 2014) who reported that methanolic fruit extract of *A. altilis* showed antibacterial activity against *Staphylococcus aureus* (zone of inhibition of 15mm). Risari *et al*. (2017) reported that leaf extracts of *A. altilis* significantly inhibited the growth of *Staphylococcus epidermidis, Escherichia coli and Propionibacterium acnes*. Underutilized fruits are known for their antimicrobial activity. A Similar trend of antimicrobial activity has been documented in earlier studies on different underutilized fruits. Patel and Rao (2012) in their study concluded that fruit extracts of underexploited fruits such as *Manilkara hexandra* and *Mimusops elengi* exhibited good antibacterial activity. Similarly, fruit extract of *Syzygium calophyllifolium* (walp fruit), an important wild edible fruit used by the tribes of Western Ghats exhibited good antibacterial activity against *Escherichia coli* (32 mm), *Salmonella typhi* (27mm) and *Staphylococcus aureus* (27.3 mm) at 100 mg/mL concentration (Sathyanarayanan *et al*. 2018).

In the field of food preservation, natural compounds possessing antioxidant and antimicrobial activity are considered as re-emerging alternatives. Agricultural waste substrates such as peels and seeds are rich in phenols and flavonoids (Malviya *et al*. 2014, Sathya 2014, John *et al*. 2017, John *et al*. 2018). Due to their ability to neutralize the

harmful effect of free radicals, inhibit lipid peroxidation and impede the growth of pathogenic micro-organisms, these waste by-products have a potential role in food industry. Singh and Immanuel (2014) prepared paneer samples by incorporating natural antioxidant components extracted from peel extracts of three fruits namely lemon, pomegranate and orange. The results showed that the extracts at 2% level was acceptable and had greater ability to prevent peroxide formation. The ability to prevent peroxide formation decreased from pomegranate peel> lemon peel> orange peel. In another study, Conte *et al*. (2007) used lemon peel for preserving mozzarella cheese. Results showed an increase in the shelf life of all packaged mozzarella cheeses, indicating that lemon peel significantly inhibited the growth of different kinds of pathogenic micro-organisms. Pavithra *et al*. (2017) in their study observed that dried papaya peel powder incorporated into chapatti increased the antioxidant content of the chapatti. Currently the pulp of breadfruit is spray dried and the resulting flour obtained is used for making bars, cookies and pastas. However, the peels and seeds of breadfruit are discarded. The results of the present study clearly point out the antioxidant and antibacterial activities of breadfruit peel. Peel extracts of *A. altilis* can be efficiently utilized as a functional component in formulating food products or as a preservative agent in food industry due to its antioxidant and antimicrobial activities.

CONCLUSION

Exploitation of underutilized fruits is the best solution to overcome social problems such as poverty, nutritional insecurity and unemployment. Presence of various unique bioactive compounds present in breadfruit has remarkably contributed to antioxidant and antibacterial property. Furthermore, effectual utilization of waste by-products in the development of value added products is considered to be a natural sustainable approach in reducing environmental pollution along with managing biological waste substrates. The present study evidently indicates the antioxidant and antibacterial properties of breadfruit peel extract thereby highlighting the role of agriculture waste substratum in the production of value added commodities. Nevertheless, scientific studies focusing on the bioavailability of functional compounds isolated from peel extracts of underutilized fruits are essential to authenticate their health promoting properties

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