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Phytonutrient screening and evaluation of *in-vitro* antibacterial activity of onion and garlic peels: A comparative study with the prospects of waste to wealth

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Abstract
The current world survey states that it has become a major issue of huge wastage of fruits and vegetables in our ecology and economy. The by-products of these vegetables can be a major source of nutrients with having potential phytochemical, antioxidant, and antimicrobial potential. Hence, the significance of our study is to do the comparative analysis of phytonutrients and *in-vitro* medicinal activity by using the discarded onion and garlic peels as sustainable resources. The results of the current study depict onion peel extracts contain highest concentration of polyphenols (174.95 ±20.42 mg GAE/g DW), flavonoids (49.97±1.77 mg QE/g DW), tannins (31.72±0.02 mg TAE/g DW), carbohydrates (30.39±1.30 mg GE/g DW), proteins (38.07±0.86 mg BSAE/g FW), whereas, garlic peel extracts contain highest concentration of antioxidants (37.54±1.05 mg AAE/g DW). Both the onion and garlic peel extract show the maximum zone of inhibition against *E. coli*. The outcome of the study comes up as a very innovative and beneficial way to cope up with the current life-style disorder and to reduce the risk of diseases.

Keywords: *Allium cepa*, *Allium sativum*, vegetable wastes, phytonutrients, antioxidants, anti-bacterial

1. Introduction
Vegetables have always been a great source of vitamins, dietary fibres, minerals, phytochemicals and other nutritive compounds which aids in fulfilling our daily dietary demand and inhibiting several diseases [1]. The majority of the time, however, we are unaware of the fact that the vegetable wastes that are produced have a lot of nutritional value in addition to pharmacological properties like antibacterial, antifungal, anticancer, and anti-mutagenic properties [2]. The pharmaceutical and food industry can be a good platform for using these biodegradable agro-waste materials in the purpose of making nutraceuticals, medicines and other value-added products following certain procedures so that all those important compounds present in waste materials can be utilized in human health by consuming these medicines [3]. Many scientific investigations have reported that the unused part of vegetables are major sources of important phytochemicals such as; polyphenols, flavonoids, tannin, antioxidants, amino acids, alkaloids.

Onion is considered one of the oldest cultivated vegetables originated in central Asia and now is the most common household vegetable all around the world. Onion (*Allium cepa*) is also known as bulb onion or common onion which belongs to genus: *Allium* and family: Amaryllidaceae. Onion generates a huge amount of waste every year that it has become quite challenging for researchers trying to establish efficient technique for reusing biomass. Onion skin itself contains high quantity of polyphenols, flavonoids, and antioxidants properties. It is also major source of dietary fibres, vitamin A, C, E. The abundance of such compounds in onion peels help in preventing cancer, obesity, diabetes, cardiovascular disorder [4]. Garlic is a widely distributed plant all around the world and at the ancient age garlic considered to be one of the best remedies in several epidemics such as; dysentery, cholera, influenza. Garlic (*Allium sativum*) belongs to genus: *Allium* and family: Amaryllidaceae which was originated in central and southwest Asia. The huge garlic peels wastes that are generated can be a major source of polyphenolic compounds, antioxidant properties, alkaloids. Garlic contains an organic sulphur compound called 'Allicin' which is responsible for its pungent smell. The antioxidant properties present in garlic are able to fight off harmful free radicals which cause oxidative stress in human body as well as other lethal diseases.

It also has antifungal, antiviral, antiparasitic, anti-inflammatory and cardiovascular properties [5]. At the ancient time when there was no existence of antibiotics, physicians used to rely upon garlic because of its diverse importance and efficacy in the field of medicine.

Therefore, our present study is mainly focused on 'converting vegetables waste into resource' by determining the phyto-nutritional compounds and evaluating *in-vitro* antioxidant and antibacterial activity of onion and garlic peel extracts on a comparative way. [Figure 1]

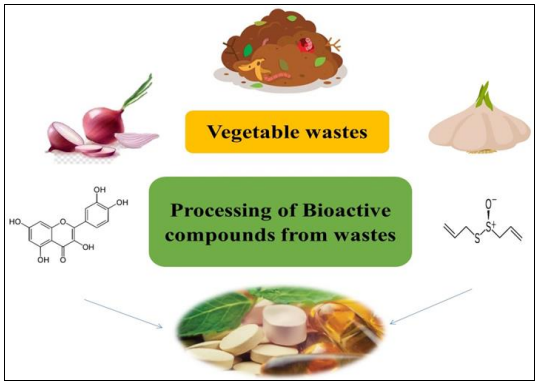

Fig 1: The Schematic Abstract

2. Materials

2.1 Chemicals

For the experiment, the chemicals used had an analytical grade from Merck Life Science Mumbai. Folin-ciocalteu, aluminium chloride and ascorbic acid were obtained. SD Fine- Chem Limited in Mumbai supplied Gallic acid and sodium hydroxide. From SRL Pvt. Ltd Quercetin, sodium nitrite, sodium carbonate, and tannic acid were purchased. DPPH was collected from Sisco Research Laboratories Pvt. Ltd., Maharashtra. Besides these, Luria Bertani agar, Luria broth, Anthrone reagent, and Bovine Serum Albumin (BSA) were bought from Himedia, Mumbai.

2.2 Collection of the samples

The current experiments were executed in the Biotechnology laboratory in Techno India University, Kolkata from May to October, 2022. Red Onion (*Allium cepa*) & Garlic (*Allium sativum*) samples [Figure 2] were purchased from the local vegetable market of H.B. Town, Sodepur, North 24 Parganas [22.6938° N, 88.3931° E]. The outer red scales of red onion and white scales of garlic were taken out and thereafter sundried for few days.

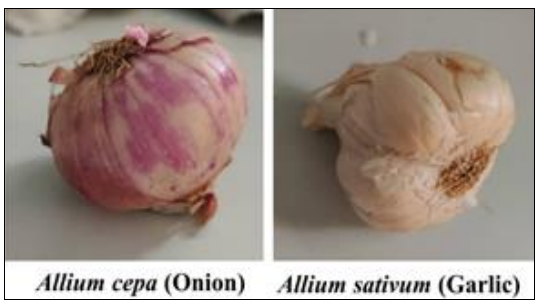

Fig 2: Collected Vegetable Samples

3. Methods

3.1 Preparation of the peel sample

After complete drying of water content from the seeds, they were subjected to mechanical grinding until a coarse powder was processed. Solvent extraction using the powdery form was done with double distilled water (aqueous) and 100% ethanol respectively [6].

3.2 Technique for solvent extraction

For the preparation of seed extracts, to 50 ml of solvent (ethanol and distilled water), 1 gm of each seed powder (raw and ripe seed) was added. Following this, overnight extraction was carried out at room temperature by keeping the conical flasks on a shaker. Filtration of the extracts was carried out using Whatman No.1 filter paper and kept at 4 ˚C for future biochemical analyses.

3.3 Quantitative analysis

3.3.1 Quantitative phytochemical analysis

3.3.1.1 Quantification of total polyphenol concentration

A small modification to the Folin-Ciocalteau method's basic protocol [7] was used for the quantitative determination of the total polyphenolic compounds, which was carried out in triplicate. At 765nm, the absorbance was measured. Gallic acid standard was used to produce the calibration curve. The total amount of polyphenols was stated as mg gallicacid equivalents/g of dry material.

3.3.1.2 Quantification of total flavonoid concentration

By slightly modifying the conventional methodology of the aluminum chloride colorimetric method [8], the total flavonoid content was measured in triplicate. At 510 nm, absorbance was measured. Quercetin was used as the reference substance to create the calibration curve. The amount of flavonoids was represented as mg of quercetin equivalents/g of dry material.

3.3.1.3 Quantification of total tannin concentration

With little alterations to the standard Folin-Ciocalteu method [9], quantitative estimation of total tannin content was done. At 700 nm, absorbance was measured. Tannic acid was used as the reference substance to create the calibration curve. The amount of tannin was represented as mg of tannic acid equivalent/g of dry material.

3.3.2 Quantitative nutritional analysis

3.3.2.1 Quantification of total carbohydrate concentration

Quantification was done via the Anthrone method. Glucose was used to prepare the standard curve of the experiment. The absorbance was measured at 610 nm. The expression of the result was mg Glucose equivalents/g of dry material [10].

3.3.2.2 Quantification of total protein concentration

Quantification was done via the Bradford assay. Bovine serum albumin (BSA) was used to prepare the standard curve of the experiment. The absorbance was taken at 595 nm. The expression of the result was mg BSA equivalents/g of dry material [11].

3.3.3 *In-vitro* antioxidant assay (DPPH radical scavenging assay)

The stability of the 2, 2-diphenyl-2-picrylhydrazyl free radical scavenging activity of the extracts was measured using the standard approach to assess the presence of antioxidant capabilities in natural products [12]. At 517 nm, absorbance was detected. By employing ascorbic acid as the standard, the calibration curve was modified. Triplicates of this experiment are run. The following formula was used to determine the inhibition percentages:

% inhibition = (Control OD- Sample OD)/ Control OD *100

3.3.4 *In-vitro* Antimicrobial Property

For the purpose of determining in-vitro antibacterial activity, disc diffusion techniques were used. Gram-positive bacteria *S. aureus* and *E. coli*, which were acquired from the laboratory of the Microbiology Department at Calcutta University, were utilized in this investigation. For this test, sterile Analytical grade water was used to create the sample extracts, which were then filtered through 0.2-m what man Filter paper. Every microorganism was sub-cultured in a volume of 100 µl in 5 ml of sterile Luria broth, which was then incubated for 24 hours at 37 °C. 20 µl of test bacteria were smeared and seeded onto sterile LB Agar plates that had been preheated during the log phase of newly sub cultured tubes. With the help of sterile forceps, sterile paper discs were applied to the top of inoculated agar plates. The sample extracts were then pipetted out in 20 µl aliquots onto the paper discs affixed to the agar surface. The plates were given a few minutes to dry before being incubated for 24 hours at 37 °C. The area of the inhibition zone (in mm.) created by the extracts surrounding the disc was used to measure the antibacterial activity. Every test was run in triplicate [13-15].

3.3.5 Statistical Analysis of Data

The results of all quantitative experiments were performed in sets of triplicate and were represented as average ± standard deviation. Statistical analyses like mean, standard curve, standard deviations were done using the software Microsoft Excel. At P value less than 0.05, statistical significance was acknowledged.

4. Results and Discussion

4.1 Quantitative assay

The present course of study aims to determine the antioxidant, phytonutritional and antimicrobial potential of two vegetative species peel extracts. Since it is well established that the antioxidant property of plants/vegetables largely depends on the total polyphenols (TPC) and total flavonoids (TFC) content, that we quantify their concentration using standard protocols and correlate them with their free radical scavenging activity and nutritional property depends on Carbohydrates, proteins and amino acid. Standard curve equation and R^2 value for total polyphenol, total flavonoid, total tannin, total carbohydrate, total protein and amino acid content as well as DPPH free radical scavenging assay are given in tabulated form [Table 1], which highlighted the strength and accuracy of further quantitative estimation assays.

Table 1: Standard curve equation along with R^2 value for each quantitative assay

Serial No.	Name of quantitative assay	Standard curve equation	R^2 value
1	Polyphenols	7.2117x-0.0158	0.9917
2	Flavonoids	0.4543x+0.0208	0.9769
3	DPPH	68.901x-0.119	0.9579
4	Tannin	1.0697x+0.0095	0.9980
5	Carbohydrate	5.430x+0.234	0.9947
6	Protein	7.0523x+0.6714	0.9947

4.1.1 Quantitative phytochemical analysis

4.1.1.1 Total Polyphenol Concentration

Quantification was done using the Gallic acid standard curve of the experiment (R^2=0.9917). From Figure 3(A), it was observed that the amount of concentration of polyphenols in onion (174.95±20.42 mg GAE/g DW) was more than garlic (22.02±0.32 mg GAE/g DW) in aqueous solution. So, we can state that the error bar in onion is high which indicates that standard error of the mean is highest and the error bar in garlic is too low or negligible which indicates that standard error of the mean is almost cannot be seen.

On the contrary, concentration of polyphenols in onion (211.37±5.25 mg GAE/g DW) was more than garlic (25.15±4.15 mg GAE/g DW) in ethanol solution. So, we can state that the error bar in onion is comparatively greater than the error bar of the garlic which means standard error of the mean in onion's value is higher than the standard error of the mean in garlic.

4.1.1.2 Total flavonoid concentration

Quantification was done using the Quercetin standard curve of the experiment (R^2=0.9769). From Figure 3(B), it was observed that the amount of concentration of flavonoids in onion (49.97±1.77 mg QE/g DW) was more than garlic (3.81±0.21 mg QE/g DW) in aqueous solution. So, we can state that the error bar in onion is high which indicates that standard error of the mean is highest and the error bar in garlic is very much low which indicates that standard error of the mean is too lowest.

On the other hand, the amount of concentration of flavonoids in onion (99.13±9.11 mg QE/g DW) was more than garlic (3.77±0.64 mg QE/g DW) in ethanol solution. So, we can state that the error bar in onion is high which indicates that standard error of the mean is highest and the error bar in garlic is very much low which indicates that standard error of the mean is lowest.

4.1.1.3 Total tannin concentration

Quantification was done using the Tannic acid standard curve of the experiment (R^2=0.9980). From Figure 3(C), it was observed that the amount of concentration of tannin in onion (31.72±0.02 mg TAE/g DW) was more than garlic (3.22±1.92 mg TAE/g DW) in aqueous solution. So, we can state that the error bar in onion is also high which indicates that standard error of the mean is highest and the error bar in garlic is low which indicates that standard error of the mean is lowest.

Similarly, it was observed that the amount of concentration of tannin in onion (25.33±0.18 mg TAE/g DW) was less than garlic (1.28±0.24 mg TAE/g DW) in ethanol solution. So, we can state that the error bar in onion is low which indicates that standard error of the mean is lowest and the error bar in garlic is high which indicates that standard error of the mean is highest.

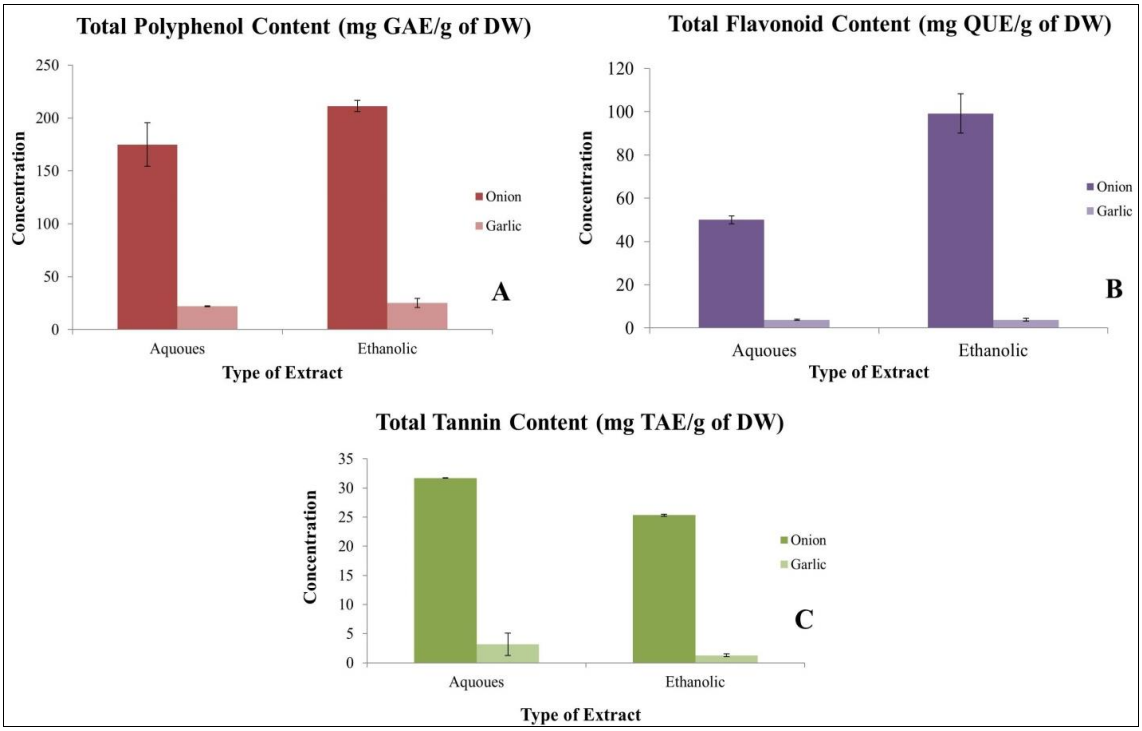

Fig 3: Comparative Result of Different Phytochemical Constituents [A: Total Polyphenol Content; B: Total Flavonoid Content; C: Total Tannin Content]

4.1.2 Quantitative nutritional analysis

4.1.2.1 Total carbohydrate concentration

Quantification was done using the Glucose standard curve of the experiment (R^2=0.9947). From Figure 4(A), it was observed that the amount of concentration of carbohydrate in onion (30.39±1.30 mg GE/g DW) was more than garlic (16.41±0.57 mg GE/g DW) in aqueous solution. So, we can state that the error bar in onion is high which indicates that standard error of the mean is highest and the error bar in garlic is low which indicates that standard error of the mean is lowest.

It was also found that the amount of concentration of carbohydrate in onion (233.28±9.51 mg GE/g DW) was less than garlic (265.78±33.78 mg GE/g DW) in ethanol solution. So, we can state that the error bar in onion is low which indicates that standard error of the mean is lowest and the error bar in garlic is high which indicates that standard error of the mean is highest.

4.1.2.2 Total protein concentration

Quantification was done using the BSA standard curve of the experiment (R^2=0.9947). From Figure 4(B), it was observed that the amount of concentration of protein in onion (38.07±0.86 mg BSAE/g FW) was more than garlic (33.17±0.33 mg BSAE/g FW) in ethanol solution. So, we can state that the error bar in onion is high which indicates that standard error of the mean is highest and the error bar in garlic is low which indicates that standard error of the mean is lowest.

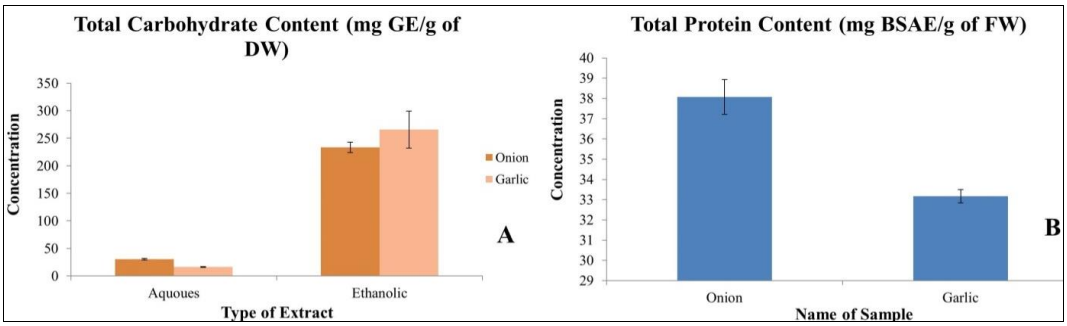

Fig 4: Comparative Result of Different Nutritional Components [A: Total Carbohydrate Content; B: Protein Content]

4.1.3 Results of *In-vitro* antioxidant assay

The inhibition percentage as calculated using the Ascorbic Acid standard curve (R^2=0.9579) From Figure 5, it was observed that the amount of concentration of antioxidants in onion (6.98±0.35 mg AAE/g DW) was less than garlic (37.54±1.05 mg AAE/g DW) in aqueous solution. So, we can state that the error bar in onion is low which indicates that standard error of the mean is lowest and the error bar in garlic is high which indicates that standard error of the mean is highest.

In case of Ethanolic extract, it was observed that the amount of concentration of antioxidants in onion (23.06±0.66 mg AAE/g DW) was less than garlic (38.18±1.47 mg AAE/g DW). So, we can state that the error bar in onion is low which indicates that standard error of the mean is lowest and the error bar in garlic is high which indicates that standard error of the mean is highest.

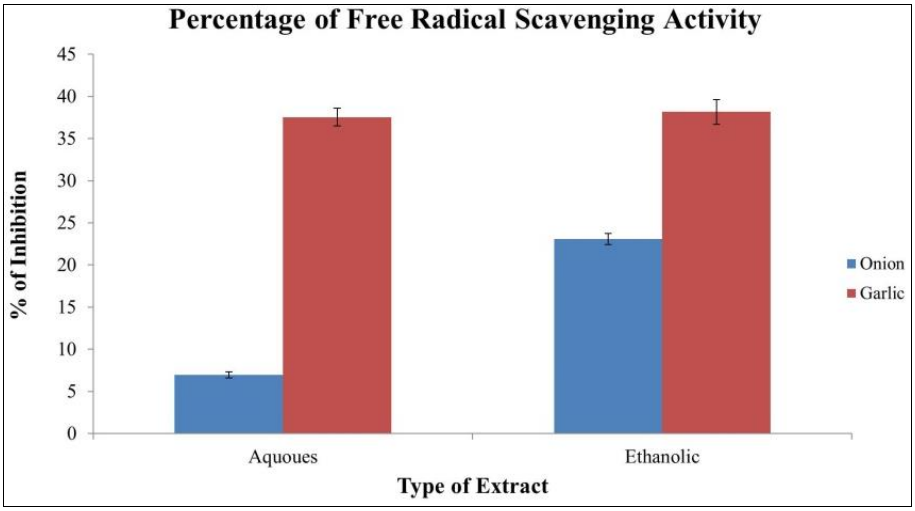

Fig 5: Comparative Result of Antioxidant Capacity (% of Inhibition)

4.1.4 Results of antimicrobial activity

We evaluated the antibacterial properties by taking both onion and garlic peels extracts. The two strains of bacteria *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) were taken to determine their susceptibility against garlic and onion peel extracts. The clear round zones that have been created around the paper discs indicate 'Zone of Inhibition'. It implies that at that particular zone the bacterial strains are susceptible to those antimicrobial compounds. The results obtained were represented in Table 2, 3 and Figure 6. In garlic peels the maximum zone of inhibition i.e., net zone 6.666667 mm was exhibited against *E. coli* and in onion peels the maximum zone of inhibition i.e., net zone 4 mm was exhibited against *E. coli* as well.

Table 2: Comparative Table Showing *In-Vitro* Antibacterial Activity of Onion Peels Against Two Strains of Bacteria

Organism	Zone of Inhibition (mm) mean ±sd	
	Control	Extract
Staphylococcus aureus	13.3333±0.57735	15±1.732051
Escherichia coli	13.3333±0.57735	17.3333±0.57735

Table 3: Comparative Table Showing *In-Vitro* Antibacterial Activity of Garlic Peels Against Two Strains of Bacteria

Organism	Zone of Inhibition (mm.)	
	Control	Extract
Staphylococcus aureus	12±0	13.66667±0.57735
Escherichia coli	7.33333±1.154701	14±1.732051

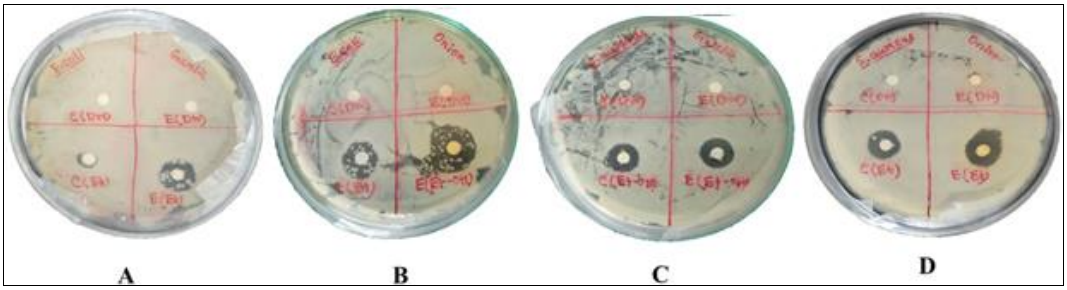

Figure 5: Agar Plates Showing Zone of Inhibition against Onion and Garlic Peel Aqueous and Ethanolic Extract With respect To Control (Double Distilled Water and 100% Ethanol) [A, C]: Garlic Extracts against *E. coli* and *S. Aureus*; [B, D]: Onion Extracts against *E. coli* and *S. Aureus*.

5. Conclusion

Agricultural wastes are a good supply of mediators to support microbial growth; therefore they can be employed in the manufacture of single-cell protein. Between the two samples, the onion peel showed a substantial amount of polysaccharide. The powdered onion peel contained a significant concentration of nutritive and phytochemical substances, according to the results. It was also discovered to have a significant mineral deposit, including calcium, potassium, magnesium, and sodium. For growth, protein is an essential dietary component that provides nitrogen, potassium, sulphur, and other elements. Onion peel can be regarded as a rich source of nitrogen because it included a significant amount of nutritional components. Antioxidants and these bioactive substances are employed to improve the nutritional and healing capacity of processed foods. The study's findings confirm that the main source of antioxidants is traditional vegetable consumption. Both peel extracts showed sizable antibacterial activity that was equivalent to one another. Based on the findings, it is evident that Allium species vegetables are a rich source of naturally occurring antioxidants and different phenolic and flavonoid components, which enhance their nutritional and therapeutic qualities. Plant antimicrobial compounds may be used as bio-insecticides and bio-preservatives in the food business, as well as potential application against a variety of foodborne infections. Therefore, if employed properly in the food and pharmaceutical industries, these chosen vegetable wastes may be transformed into profit.

Accordingly, the study reveals that the vegetable peels that we reject and label as "Waste" can actually be used to create herbal medicines with positive health effects, turning them from "Waste" into "Wealth."

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