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Studies on the Antioxidant Content and Antimicrobial Activity of Cucumber (*Cucumis sativus*) Peels Collected from Bagmari, Kolkata, West Bengal, India

Debasree Ghosh[1,*], Hamidur Rahaman Mondal[2]

Abstract

Cucumber (Cucumis sativus) is widely consumed across the globe, yet its peels are commonly discarded as waste, despite possessing a wealth of bioactive compounds. Recent studies have highlighted that these peels contain substantial amounts of antioxidants, antibacterial, anti-inflammatory, and anti-carcinogenic properties, making them a promising candidate for value-added applications. In this study, methanolic extracts (80% v/v) of cucumber peels were systematically analyzed to assess their total phenolic content (TPC), total flavonoid content (TFC), and ascorbic acid concentration. The analyses were carried out using well-established protocols, including the Folin–Ciocalteu method for TPC, the Aluminum Chloride colorimetric method for TFC, and spectrophotometric analysis for ascorbic acid quantification. The results demonstrated that the peel extracts are rich in phenolic compounds and flavonoids, along with a notable amount of ascorbic acid, indicating strong antioxidant potential. Furthermore, the extracts exhibited significant antibacterial activity against selected microbial strains, underscoring their potential as natural antibacterial agents. These findings suggest that cucumber peels, often overlooked and discarded, could serve as a sustainable source of natural preservatives in the food industry. Moreover, the use of such agro-waste not only promotes environmental sustainability by reducing organic waste but also adds economic value through the development of functional food ingredients or nutraceuticals. Further research is warranted to optimize extraction techniques, explore in-depth biological activities, and evaluate the practical applications of cucumber peel extracts in real food systems and pharmaceutical formulations.

Keywords: Cucumber peel, phytochemicals, vitamin C, antimicrobial activity, flavonoids

INTRODUCTION

The fruit and vegetable industry generates significant peel waste, leading to nutritional, economic, and environmental challenges (Kumar et al., 2020) [1]. India, a major producer of fruits and vegetables, contributes substantially to lignocellulosic biomass through cultivation, harvesting, processing, and consumption activities. This biomass can be repurposed as a low-cost biosorbent, feedstock for biofuels and biochemicals, and a substrate for enzyme and metabolite production (Pathak et al., 2017) [2]. Notably, the processing of fruits and vegetables results in 25–30% waste, with peels containing valuable bioactive compounds like enzymes, polyphenols, and vitamins. Efficient utilization of fruit peels can mitigate environmental issues and enhance health through enriched food products (Hussain et al., 2022) [3].

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Phytochemicals, naturally occurring in fruits and vegetables, offer protection against diseases (Ayodele et al., 2015) [4]. These secondary metabolites, categorized by chemical composition, structure, biosynthesis, or solubility, are vital for discovering therapeutic and industrial compounds, such as alkaloids, flavonoids, phenolics, saponins, steroids, and tannins (Chanda and Ramachandra, 2019; Pradeep et al., 2014) [5, 6]. Antioxidants, which prevent cellular damage from free radicals, are divided into enzymatic and non-enzymatic types, with natural antioxidants, like flavonoids and phenolics, that are playing key roles in antiviral effects and treatment of conditions, such as COVID-19 (Sehwag and Das, 2013; Flieger et al., 2021) [7, 8].

Phenolic acids, present in various plant-based foods, exhibit significant in vitro antioxidant activity and offer antidiabetic, cardioprotective, and antimicrobial benefits (Robbins, 2003; Kumar and Goel, 2019; Oluwole et al., 2022) [9–11]. Flavonoids, abundant in plants, contribute to combating diseases, such as cancer and Alzheimer's (Kumar and Pandey, 2013; Panche et al., 2016) [12, 13]. Ascorbic acid (vitamin C), essential for human health and found in many fruits and vegetables, functions as a redox cofactor and catalyst in numerous biochemical reactions (Pauling, 1970; Steinberg and Rucker, 2013; Berretta et al., 2020) [14–16].

Microbial infections remain a significant health challenge, with antimicrobial resistance spreading globally (Chaturvedi, 2024) [17]. Antimicrobials and antibiotics have historically reduced infection-related mortality, and recent studies highlight the rich antimicrobial properties of fruit and vegetable waste, particularly peels and seeds (Sakr et al., 2014; Rayamajhi et al., 2010; Narender et al., 2018) [18, 19, 20]. This research explores the potential of fruit peel waste in enhancing health and addressing environmental issues.

AIMS AND OBJECTIVES

The aims and objectives of the present study are:

- To assess the phytochemical activity of cucumber collected from Bagmari (Maniktala), Kolkata, West Bengal.
- To assess the susceptibility of cucumber collected from Bagmari (Maniktala), Kolkata, West Bengal against antibiotics: Streptomycin, Kanamycin and Penicillin.
- To assess the ascorbic acid content of cucumber collected from Bagmari (Maniktala), Kolkata, West Bengal.

METHODOLOGY

Materials

- All materials used were of AR/GR graded.
- All media used for anti-microbial activities for samples were Hi-media, Hi-media Laboratories Pvt. Ltd. Works, Dindori, Nashik, MH, Mumbai- 400086, India.

Sampling

Cucumber peel was taken as a sample for this study. The description of the sample is as follows:

- English name –Cucumber.
- Species name – *Cucumis sativus.*
- Family name – Cucurbits.
- Local name – Sosha.
- Parts used – Peel.
- Status – Cultivated.

Fresh cucumbers were bought from a fruit market, near Bagmari Bazar, Kolkata, West Bengal, India. Cumbers were brought to the laboratory by putting them in a sterile zip-locked bag. Before the experiment began, the superficial parts of cucumbers were cleaned thoroughly with the help of distilled water and tissue paper.

PREPARATION OF SAMPLE EXTRACT

For Determination of Antioxidant Content

For Total Phenolic Contents (Singlaton and Rossi, 1965; Bao et al., 2005) [21, 22]

The peels were separated from the pulp of cucumber and the weight was measured using petit balance with the help of butter paper.

- Appropriate amount of sample (Fresh weight) + 10 ml 80% methanol(V/V).
- Kept overnight in the refrigerator at 4℃.
- Cold centrifuged at 10000 rpm for 10 min.
- The supernatant was filtered and collected in the test tubes.

For Total Flavonoid Contents

The peels separated from the pulp of cucumber and the weight was measured using petit balance with the help of butter paper.

- Appropriate amount of sample (Fresh weight) + 10 ml 80% methanol (V/V).
- Kept overnight in a refrigerator at 4℃.
- Cold centrifuged at 10000 rpm at 4℃ for 10 min.
- The supernatant was filtered and collected in the test tube.

For Determination of Ascorbic Acid Content

The peels of cucumber were separated from cucumber pulp and weight was determined using petit balance and butter paper. With the aid of a mortar and pestle, the needed quantity of peel (5 g) was crushed [23].

For Determination of Antimicrobial Activity

Cucumber peels were removed from cucumber pulp, and weight was determined using a tiny balance and butter paper. Using a mortar and pestle, 5g of cucumber peel was crushed. It was subsequently diluted along with 10 ml of sterile distilled water and utilized as a sample [24].

ANALYSIS

For Total Antioxidant Content

For Total Phenolic Contents

For determination of total phenolic contents, the Folin–Ciocalteu method was used. The number of total polyphenols in the peel extract was calculated employing gallic acid as the reference. 80% methanol (V/V) was used to make the extract solution. Sample concentration was 50 mg/ml. 1ml of the aforementioned extract solution was combined with 10%, 1ml of the Folin–Ciocalteu (FC) reagent, and 0.8 ml of a 7.3% sodium carbonate (Na_2CO_3) aqueous solution. Then, 10ml of volume was created using distilled water. The reaction compound's absorbance was estimated at 765 nm in a spectrophotometer (Cystonic UV vis 118) following 30 minutes incubation at a room temperature. The total phenol content was demonstrated as mg of gallic acid equivalents (GAE)/g of the dry weight of the sample [21].

For Total Flavonoid Contents

Quercetin was used as a reference to determine the total flavonoid concentration in the extracted peel using a modified colorimetric method. 80% methanol was used to make the extract solution. 100 mg/ml was the sample's concentration. This extract was combined along with 0.15 ml of a 5% sodium nitrate ($NaNO_2$) solution in 1 ml of the mixture. $AlCl_3$ solution (10% aluminum chloride) in 0.15 ml was added after 6 minutes. The volume was brought to 10ml by adding 2 ml of 4% sodium hydroxide (NaOH) once more after 6 minutes. After thoroughly combining the solutions, they were left to stand at room temperature for 15 minutes while the reaction compound's absorbance at 510 nm was evaluated in a spectrophotometer. The quantity of quercetin equivalents (QE) per gram of dry weight of the sample was used to measure the total flavonoid content (Bao et al., 2005) [22].

For Determination of Ascorbic Acid Content

6% metaphosphoric acid is used for estimating Vit-C content of the sample. Preparation of Sample extract clean cucumber was taken and dried with paper towel [23]. Appropriate amount was taken mortar and pestle and crushed. It was then extracted with

- 6% metaphosphoric acid was used.
- The supernatants were de canned into polypropylene tubes and filtered to Whatman no. 1 filter paper.
- 2 ml prepared sample extract + 2 ml acetate buffer(pH 4.0) and dye(sodium salt of 2,6 dichlorophenol indophenol) was taken in a separating funnel (mixed well).
- 10 ml xylene was added and mixed well. Stand for 6 seconds to separate the water layer.
- Then the water layer was removed from the separating funnel.
- The colour in xylene was measured in a spectrophotometer at 500 nm.

For Determination of Antimicrobial Activity

- *Chemicals and Reagents:* Streptomycin (25 mcg/disc), Kanamycin (30mcg/disc) and Penicillin (10 units/disc) were obtained from Thermo Fisher Chemicals, West Bengal, India. Media, such as nutrient agar (Hi-media) and bacterial strains were obtained from cucumbers bought from Bagmari Bazar, Kolkata, India [24].
- *Instrumentation:* Laminar air flow chamber of model MFIH3X2 MICRO-FILT, India (Pune) was used for the subculturing of microbes, Incubator model O-CIS 6 and Autoclave model 220/230 VOLT/1/CY-AC, India.
- *Sterilization of Glass Wares:* All the glasswares (test tubes, pipettes, petri dishes, beakers and conical flasks etc.), nutrient agar and glass spreader were sterilized in the Autoclave at 120℃ for 10 minutes at 15 psi pressure.

Preparation of Media

In a sterilized conical flask, 100 ml of sterilized distilled water were used to dissolve 5 g powdered nutrient agar (Hi-media). With the aid of an autoclave, the media was sterilized at 120℃ under 15 psi for 10 min. Under strictly adherent aseptic circumstances, melted and sterile nutritional agar was put into several sterilized petri plates and allowed to harden for 30–45 min. The molten agar was poured into the plates until they were about 2/3 full, or about 20 ml per plate. To check for contamination, sterile nutrient-agar in petri plates was allowed to incubate in the refrigerator for 24 h. Brown paper was used to seal the plates [25].

Disc Diffusion Method

The disc diffusion method, which is preferred over the Kirby–Bauer method, was used to assess the antimicrobial activity of produced peel extracts (Willey et al., 2008) [26]. Himedia agar plate's whole surface was inoculated with recently produced bacteria. The relevant antimicrobial assay disc (Streptomycin 25 mcg/disc, Kanamycin 30 mcg/disc and Penicillin 10 units/disc) were applied to the dried agar surface using sterilized forceps after it had dried for around 5 min. The diameter of the zone of inhibition measured to the closet millimeter using a translucent scale to estimate the microbial growth following 24 h of incubation at 37℃ (Bhatt et al., 2014) [24].

Statistical Analysis

Each experiment was repeated three times for each sample and results were presented with their means and standard deviations (SD). The statistical analysis was done using One Sample t-test and one way ANOVA test with the help of the Statistical Package for the Social Sciences (SPSS), Software Version 16.0.

RESULT AND DISCUSSION

Antioxidant Content of the Sample

Phenolic Content

The Total Phenolic Content (TPC) in cucumber peel was found to be 279.66 mg GAE/g, significantly higher ($P < 0.001$) compared to other parts of the cucumber. Various studies support this finding (Tables 1 and 2):

- Sari et al. (2021) and Nasrin et al. (2015) [27, 28] reported phenol contents of 262.31 mg/g and 23.08 mg GAE/g, respectively, in cucumber.
- Insanu et al. (2022) [29] found 2.34 g GAE/100 g using the DPPH method.
- Yunusa et al. (2018) [30] observed higher phenolic content (23.08 mg GAE/g) in ethanolic peel extract, which also had a significantly higher FRAP value.
- Sir Elkhatim et al. (2018) [31] demonstrated a strong correlation between TPC and antioxidant activity in cucumber peels.

Differences in results may stem from variations in cucumber varieties, fruit age, environmental factors, and analytical techniques. Factors, such as plant maturity, particle size, solvent polarity, and extraction methods also influence phenolic extraction. Environmental conditions, like soil type, rainfall, temperature, and humidity, impact phenolic content which decreases with ripening while anthocyanin concentrations increase (Manach et al., 2004) [32]. These findings suggest that cucumber peels are valuable sources of bioactive compounds for food and pharmaceutical applications.

Table 1. Mean ± SD of Total Polyphenol Content (mg GAE/100 g FW) in cucumber peel collected from Bagmari, Kolkata, West Bengal, India.

Parameter	Polyphenol Content (mg GAE/g) Mean ± SD (n = 3)	Significance
Cucumber Peel	2.796 ± 4.50	S*

*Note: *P < 0.001.*

Table 2. Analysis of variance of phenolic content (one sample t-test).

Parameter	t	df	Sig (2-Tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	*Upper*
Cucumber Peel	107.423	2	0.000	279.66	268.46	290.86

Flavonoid Content

The study on cucumber peel revealed a flavonoid content of 155.33 mg QE/g, significantly present ($P < 0.001$). Cucumber exhibits various pharmacological activities, such as anticancer, antiulcer, analgesic, and antioxidant effects (Dhande et al., 2013) [33]. It contains flavonoids and other phytochemicals. Another study using the ferric chloride colorimetric method found a flavonoid content of 2.14 ± 0.56 mg/g (Uzuazokaro et al., 2018) [34]. Phytochemical screening with the Shinoda test showed PBS peel extract MCF-7 value of 290.0 mg/ml and H1299 value of 52.0 mg/ml (Foong et al., 2015) [35]. Ethanolic cucumber peel extract had the highest flavonoid content (14.02 mg QE/g) and was significant ($p < 0.05$) (Yunusa et al., 2018) [30] (Tables 3 and 4). Factors, like variety, geographic location, harvest time, and processing techniques, influence cucumber composition (Uthpala et al., 2020) [36]. Flavonoid types include flavones, isoflavones, flavanones, flavonols, flavan-3-ols (flavanols), and anthocyanins, and their total content changes with these components. Physical factors and oxidation also impact flavonoid content and food quality (Manach et al., 2004) [32].

Table 3. Mean ± SD of Total Flavonoid Content (mg QE/100 g FW) in cucumber peel collected from Bagmari, Kolkata, West Bengal, India.

Parameter	Flavonoid Content (mg QE/g) Mean ± SD (n = 3)	Significance
Cucumber Peel	1.553 ± 3.05	S*

Note: P < 0.001.

Table 4. Analysis of variance of total flavonoid content (one sample t-test).

Parameter	t	df	Sig(2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	*Upper*
Cucumber Peel	88.066	2	0.000	155.33	147.74	162.92

Ascorbic Acid Content

Cucumber peels from Kolkata, West Bengal, India, were found to contain 4.630 mg/100 g of ascorbic acid. Statistical analysis confirmed a significant presence of ascorbic acid ($P < 0.001$). Various studies support the claim that cucumbers contain ascorbic acid, with different varieties showing different levels (Tables 5 and 6). For instance, the Heera hybrid variety has the highest ascorbic acid content at 5.35 mg/100 g, while the K-75 variety has the lowest at 3.45 mg/100 g (Reddy et al., 2022) [37].

Sharaa and Mussa (2019) [38] measured ascorbic acid using a 5% metaphosphoric acid-10% acetic acid solution, finding 27.90 ± 0.41 mg/100 g in fresh cucumber. Biswas et al. (2021) [39] used a redox titration method with iodine and iodate solutions, reporting 0.94 ± 0.21 mg AAE/g FW. Another study by Omoniyi and Alli (2021) [40] found 143.360 ± 101.450 mg/100 g.

Ascorbic acid concentration is influenced by photosynthesis and carbohydrate production, with higher light exposure increasing vitamin C levels. Conversely, high nitrogen fertilizer rates tend to lower vitamin C concentration (Mozafar, 1993) [41].

Variations in ascorbic acid content can be attributed to several factors:

- *Measurement methods:* Different techniques and equipment can yield varying results.
- *Variety of cucumber:* Different breeds have varying nutrient levels.
- *Growing conditions:* Environmental factors, like water, soil quality, and sunlight, affect nutrient content.
- *Ripeness:* Nutritional content changes as fruits ripen, affecting vitamin C levels.
- *Pesticides and fertilizers:* The use of these can impact nutrient content significantly.

Table 5. Mean ± SD of ascorbic acid content (mg/100 g FW) in cucumber peel collected from Bagmari, Kolkata, West Bengal, India.

Parameter	Ascorbic Acid Content (mg/100g) Mean ± SD (n = 3)	Significance
Cucumber Peel	4.630 ± 0.51	S*

*Note: *P < 0.001.*

Table 6. Analysis of variance of total ascorbic acid content (one sample t-test).

Parameter	t	df	Sig (2-Tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	*Upper*
Cucumber Peel	15.430	2	0.004	4.630	3.339	5.921

Antimicrobial Sensitivity (Tables 7 and 8, Figure 1)

The study investigated the antimicrobial activity of cucumber peel, comparing it with different antibiotics. Results showed that cucumber peels had significantly higher antimicrobial activity with streptomycin compared to kanamycin, and no activity with penicillin. Streptomycin and kanamycin, being broad-spectrum antibiotics, were more effective, while penicillin, a narrow-spectrum antibiotic, was ineffective due to its action against specific Gram-positive or Gram-negative organisms. The variability in antimicrobial effectiveness could be influenced by external factors, like pesticides and fertilizers, which might enhance bacterial resistance and affect the results.

Supporting literature indicates that cucumber peel extracts exhibit varying antimicrobial effectiveness, with zones of inhibition ranging from 11–21 mm. This variation may be linked to the phenolic and flavonoid compounds in cucumber peel, which have antioxidant properties and can interact with bacterial cell walls, disrupting microbial membranes and influencing bactericidal effectiveness. Studies by John et al., 2018, El-Desoukey et al., 2018 [42, 43] and others corroborate these findings.

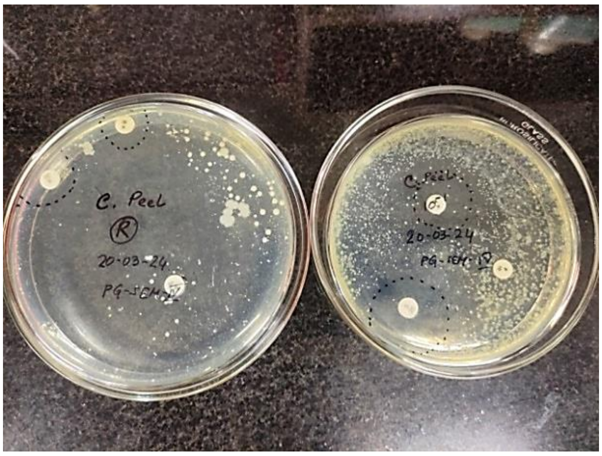

Figure 1. Formation of zone of inhibition in raw sample and 10^{-1} dilution.

Table 7. Mean ± SD diameter of zone of inhibition, caused by extracts of cucumber peel against gram negative bacteria at different test concentration collected from Kolkata, West Bengal, India.

Sample	Concentration	Antibiotics	Zone of Inhibition (mm) Mean ± SD
Cucumber Peel	Raw (1 ml)	Penicillin	0
		Kanamycin	15.33 ± 1.15
		Streptomycin	20.33 ± 1.52
	10^{-1}(0.1 ml)	Penicillin	0
		Kanamycin	21.33 ± 1.15
		Streptomycin	26.66 ± 1.52

Table 8. Analysis of variance of total antimicrobial sensitivity (one way ANOVA test).

	Condition	Sum of Square	df	Mean Square	F	Significance
Antibiotics	Between Group	10.833	8	1.354	10.446	0.001
	Within Group	1.167	9	0.130		
	Total	12.000	17			
Sample conc.	Between Group	1.833	8	0.229	0.296	0.636
	Within Group	2.667	9	0.296		
	Total	4.500	17			

FUTURE ASPECT OF THE STUDY

Cucumber peels hold promising future applications across various fields. Rich in fiber, vitamins, and minerals, they are expected to become valuable nutritional supplements. Their bioactive compounds, including flavonoids and antioxidants, may lead to new pharmaceutical and functional food products with potential health benefits. The peels' unique properties could also drive innovation in biodegradable packaging, reducing plastic use. Additionally, cucumber peel extracts are likely to see increased use in cosmetics for their soothing and hydrating effects. As antimicrobial agents, they may extend food storage life and contribute to sustainable waste management through composting or bioremediation. Furthermore, bio-based fibers from cucumber peels could find applications in eco-friendly textiles, enhancing sustainability in various industries.

CONCLUSIONS

From this research study, we can conclude that the cucumber peel is rich in flavonoids, phenolic acids, and vitamin C, which contribute to its antioxidant properties. These compounds help reduce oxidative stress and can be used as natural antioxidants in food, dietary supplements, and functional foods. Humans lack the enzyme to produce ascorbic acid, so it must be supplemented through fruits and vegetables, including cucumber peel. Based on the results of the quantitative estimation the phenolic acid content is found to be present in greater amounts than flavonoids. Vitamin C from cucumber peel has various health benefits, including anti-atherogenic, anti-carcinogenic, anti-diuretic, and immunomodulatory properties. Cucumber peel extracts also have antimicrobial properties, making them useful in preserving food without synthetic chemicals. Based on the results of the quantitative estimation the phenolic acid content is found to be present in greater amounts than flavonoids. These findings suggest that fruit peels can be effectively utilized to manage environmental issues and create functional foods, nutraceuticals, and pharmaceuticals for chronic disease prevention.

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