

COMPARATIVE STUDY ON NUTRITIONAL CONTENT, PHYTOCHEMICALS PROPERTIES AND BIOACTIVITY OF GREEN BANANA PULP AND PEEL

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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Dedication

I dedicate this work to those persons who made my MS journey successful, specially to my supervisor.

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The Author

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LIST OF ABBREVIATIONS

AlCl ₃	Aluminium Chloride	
AOAC	Association of Official Analytical Chemists	
CF	Crude Fiber	
СР	Crude Protein	
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate	
DV	Daily Values	
FOS	Fructooligosaccharides	
GAE	Gallic Acid Equivalent	
GC	Gas Chromatography	
HPLC	High Performance Liquid Chromatography	
ITIS	Integrated Taxonomy Information System	
mg/g	Milligram per Gram	
mmol/L	Millimoles per Litre	
NaOH	Sodium Hydroxide	
QE	Quercetin Equivalent	
TFC	Total Flavonoids Content	
ТРС	Total Polyphenol Content	
v/v	Volume in Volume	
µg/gm	Microgram per Gram	

ABSTRACT

Bananas, well-regarded for their nutritional value and traditional medicinal uses, are one of the popular fruits among consumers. Paradoxically, the banana peel, a substantial byproduct in the banana processing industry, is typically overlooked and discarded as waste. However, banana peels are a hidden gem of nutrition and versatility. This study was undertaken with the objective of conducting a comprehensive comparison of the nutritional composition, phytochemical attributes, and bioactivity of green banana pulp and peel extracts. Banana peel powder contained higher levels of approximately $(7.18\pm0.02\%)$ crude protein, $(8.56\pm0.04\%)$ crude fat, $(26.77\pm0.096\%)$ crude fiber, and $(10.12 \pm 0.106 \%)$ ash content. Contrarily, Banana pulp powder had a larger percentage of moisture (6.09 \pm 0.06%) and carbohydrate content (81.76 \pm 0.065%). Significantly, the total flavonoid content in banana peel (226.22 mg QE/100g) surpassed that of banana pulp (58.21 mg QE/100g) and banana pulp contained the highest total polyphenol content (24.06 mg GAE/100g) than that of banana peel (8.69 mg GAE/100g). Both samples displayed comparable antioxidant capacities. Phytochemical screening indicated the presence of essential compounds, including carbohydrates, proteins, tannins, and flavonoids, in both extracts. Notably, glycosides were absent in both, while saponins were not detected in banana pulp. Furthermore, both banana pulp and peel extracts demonstrated antimicrobial activity against the tested microorganisms (S. aureus and E. coli). Specifically, the ethanol extract of banana pulp displayed the most substantial inhibition zones. This study highlights the superior nutritional profile of green banana peel compared to pulp, along with promising phytochemical and bioactive properties. Consequently, banana pulp and peel powder have the potential to become valuable resources with diverse applications, promoting healthier diets, sustainable agriculture, and eco-friendly innovations.

Keywords: Banana peel, nutritional content, antioxidant, phytochemical screening, bioactive and sustainable agriculture.

Chapter 1: Introduction

1.1 Background

Nature has bestowed upon us a comprehensive repository of healing solutions to alleviate human ailments. Roughly 80% of the global population relies entirely or in part on traditional medicine as their main source of primary healthcare (Kunwar and Adhikari, 2005). Per the World Health Organization (WHO), in Bangladesh, approximately 90% of patients are attended to by practitioners of the conventional healthcare system, while in Burma, it's around 85%, and in India, it's roughly 80% (Siddiqui, 1993). Medicinal plants contain abundant secondary metabolites (promising pharmaceutical compounds) and essential oils with therapeutic significance. The key benefits of using medicinal plants for treating different illnesses include their safety, cost-effectiveness, efficacy, and ready accessibility (Atal and Kapoor, 1989; Siddiqui, 1993). The medicinal properties of plants are attributed to their phytochemicals, which exert specific physiological effects on the human body. Phytochemicals are natural compounds found in plants used for both nutrition and medicine, contributing to illness prevention and overall health maintenance (Dillard and Germane, 2007). These phytochemicals possess qualities such as antioxidant or hormone-like actions, aiding in the combat against various diseases, including cancer, heart disease, diabetes, and high blood pressure, while also preventing the harmful effects of carcinogens on target tissues (Daniel et al., 2011). Medicinal plants have been instrumental in treating numerous diseases, sparking ongoing exploration for diverse plant extracts that could serve as potential sources of novel antimicrobial agents (Yuhui et al., 2003). The primary benefits of utilizing medicinal plants for treating a range of medical conditions include their safety, cost-effectiveness, efficacy, and widespread accessibility (Atal and kapoor, 1989; Siddiqui, 1993). Across diverse human societies worldwide, over 35,000 plant species are harnessed for medicinal purposes. As a result, scientists are progressively focusing on traditional medicine, seeking fresh avenues for developing improved medications to combat microbial infections.

The dependency on plants of human and animals for their sound livelihood is from very ancient ages. The reason is not only because of food and oxygen supply but also for their various use in daily life such as medicinal uses. Sometimes Plants are compared to the "treasure box" hidden with a lot of active components that can be utilized for generating new drugs. Another feature of medicinal plants are a cheap and accessible source to synthesize a variety of active chemicals that are useful in preventing and treating a wide range of illnesses. Humans have long depended on plants for medicinal purposes, as many plant species contain compounds with therapeutic properties. This dependence on plants for medicine is known as herbal medicine or phytotherapy. The use of plants for medicinal purposes is deeply rooted in human history and has been practiced by various cultures worldwide for thousands of years back to ancient civilizations, such as the Egyptians, Greeks, Chinese, and indigenous cultures. These societies relied on botanical knowledge to treat various ailments and conditions. Many traditional medicine systems, such as Ayurveda in India, Traditional Chinese Medicine (TCM) in China, and Indigenous healing practices globally, are based on the use of plant-based remedies (Balick and Cox, 2000). These systems have been passed down through generations and continue to be practiced today. Extraction from different parts of plant such as leaf, bark, root, flower, seed, and stem can be used for medical purposes.

Antibiotic resistance in microbes is an alarming and most debated issue of human health. In recent years, the bizarre ability of microbes to develop resistance to various antibiotics has become an increasing threat for successfully treating the infectious diseases due to pathogenic microbes. Consequently, researchers worldwide have been focusing on the herbal products, as a way to develop better drugs against multi drug resistant microbe strain (Singh et al., 2010). Medicinal plants are possible sources of new drugs and possess boundless values for developing pharmaceutical products, phytomedicines, and dietary supplements (Sam at al., 2008). Even though, the antimicrobial properties have been tested on many plant species, the majorities have not been sufficiently evaluated yet (Mahesh et al., 2008). Recent researches have uncovered that the fruit and vegetable peels are potential antimicrobial agents (Chanda et al., 2010; Mohamed et al., 1994). Interestingly, in some fruits, the seeds and peels are found to have even higher antimicrobial activity than the pulp (Jain et al., 2011).

Fruit processing generates substantial amounts of waste, including peels, seeds, skins, and cores. There is a problem as some legal constraint often make it difficult

to dispose the waste. This waste, if not managed effectively, can have negative environmental impacts. However, it also presents opportunities for sustainability through various strategies such as waste reduction, reuse, recycling, resource recovery and value-added product development. Numerous studies and examples demonstrate the potential value that can be derived from fruit processing waste, making it an important area of focus for the food industry's sustainability initiatives. High-value goods from the recovery of these waste are commercially viable and the utilization of these wastes as by products for further investigation on the manufacture of food additives or supplements with high nutritional content have drawn a significant concern. By-products are a significant source of sugars, minerals, organic acids, dietary fiber, and phenolic, which are known to offer a variety of health benefits, including anticancer, antiviral, antibacterial, cardio protective, and antimutagenic effects.

Banana, scientifically known as Musa spp., is one of the most widely consumed fruits across the globe, renowned not only for its sweet taste but also for its abundant nutritional content and potential health benefits. Bananas, an everyday tropical fruit, are not only delectable but also nutritionally significant. Beyond their sweet and creamy taste, bananas are nutritionally dense, making them a valuable source of essential vitamins, minerals, and carbohydrates (Shin et al., 2013). As a source of essential nutrients, bananas are accepted as a prominent part of human diets (Nair et al., 2015). However, while the banana fruit is widely recognized for its nutritional value, the banana peel has often been relegated to the status of agricultural waste. The conventional consumption of whole bananas often leads to substantial food waste in the form of discarded peels, which contain a wealth of untapped nutritional and bioactive potential. In recent years, there has been a growing interest in utilizing not only the banana pulp but also its often-overlooked counterpart, these banana peels as a valuable source of bioactive compounds with potential health-promoting properties to create value-added products, such as banana peel powder, which can not only reduce waste but also offer unique health benefits (Shah et al., 2018). The presence of bioactive compounds, such as antioxidants and anti-inflammatory agents, in banana and banana peel powders has significant implications for human health. These compounds have the potential to reduce oxidative stress, lower the risk of chronic diseases, and enhance overall well-being (Ogunsina et al., 2017). A

variety of industrial applications exist for bananas and banana peels, encompassing activities such as producing fibers, generating biofuels, applying them in pharmaceuticals and cosmetics, developing biodegradable packaging materials, and employing them in water purification, among others (Reddy and Yang, 2005; Nourmoradi et al., 2014). This comparative evaluation of nutritional components, bioactive properties, and antibacterial activities of banana powder and banana peel powder represents a significant contribution to the understanding of the multifaceted potential of this tropical fruit.

1.2 Significance of this study

In our country, there has been notably minimal research conducted on bananas and banana peels, particularly in terms of their potential applications in addressing human ailments. Bananas have a well-established tradition of use in traditional medicine. They are believed to offer numerous health benefits, including their potential antibacterial properties. These traditional assertions can find scientific backing through the examination of the nutritional composition, quantitative and qualitative analysis, and exploration of their antibacterial properties, all of which can help identify potential health benefits. Investigating the nutritional and bioactive properties of banana peel powder contributes to sustainable resource utilization. Understanding the nutritional components of both banana and banana peel powders can provide valuable insights into their potential as dietary supplements and can contribute to nutritional diversity. This information is crucial for addressing nutritional deficiencies, especially in regions where bananas are a staple food. Antibiotic resistance is a global health concern. Investigating the antibacterial activities of banana and banana peel powders may lead to the development of natural antimicrobial agents. These could be used in the food industry to enhance food safety or in the development of alternative treatments for bacterial infections. Ultimately, the study's findings can have positive implications for public health by promoting the consumption of nutrient-rich foods and the development of natural remedies for bacterial infections, contributing to overall well-being.

1.3 Aim and Objectives

- 1. To evaluate nutritional composition, bioactive properties (TFC, TPC) of banana pulp and peel.
- 2. To analyze the antioxidant properties of banana pulp and peel extract.
- 3. To investigate the antibacterial effect of ethanoic extracts of banana pulp and peel powder against *E. coli* and *Staphylococcus aureus*.

Chapter 2: Review of Literature

2.1 Overview of Banana

Bananas (Musa spp.) are typical climacteric fruit with rich nutrient contents which are considered as a healthy fruit. They belong to the family Musaceae and are characterized by their elongated, curved shape and bright yellow skin when ripe (Nair et al., 2015). Mature banana plants can grow to 3m (10ft) tall or more. They are one of the top 10 world food crops contributing to cash and food crop in the tropics and subtropics. Bananas are considered a perennial food crop, though the exact duration of a banana plant's productivity can vary depending on factors such as the banana variety, climate, and agricultural practices. The top banana-producing country in the world is India, followed by China, the Philippines, Ecuador, and Brazil. Bangladesh is also a significant producer of bananas. Bananas are important in nutrition, therapeutics, traditional medicine and the pharmaceutical and food industries. The different chemical constituents like apigenin glycosides, myricetin-3-O-rutinoside, kaempferol-3-O-rutinoside, dopamine and serotonin have been reported in different parts and varieties of banana (Sidhu et al., 2018). Pharmacological actions of bananas have been seen such as antiulcer, antimicrobial and antioxidant activities. Almost all the parts of this plant, i.e. fruit, leaves, flower bud, trunk, pseudo-stem can be utilized. In summary, bananas are not only a delicious and versatile fruit but also a valuable addition to the diet due to their rich nutritional content, including carbohydrates, vitamins, minerals, and bioactive compounds.

2.2 Distribution and habitat

Bananas (*Musa spp.*) are tropical and subtropical plants with a wide distribution across the globe. They are primarily native to Southeast Asia and are believed to have originated in regions that now encompass Malaysia, Indonesia, and the Philippines (Pillay et al., 2007). However, due to extensive cultivation and trade, bananas are now grown in numerous countries around the world. The natural habitat of wild banana species is characterized by warm and humid tropical climates. They thrive in regions with average temperatures between 80°F to 100°F (27°C to 38°C) and high annual rainfall, typically ranging from 78 inches to 98 inches (2,000 mm

to 2,500 mm) (Stover and Simmonds, 1987). These conditions are essential for the growth and fruiting of banana plants.

Bananas are commonly found in lowland areas, riverbanks, and rainforests, where they benefit from the protection of taller vegetation and a constant supply of moisture (Pillay et al., 2007). In their natural habitat, banana plants often grow in clumps, forming dense stands. This dense growth provides them with stability and protection against wind and other environmental factors. However, bananas have been extensively cultivated and adapted to a wide range of environmental conditions. They are now grown in diverse regions worldwide, including tropical and subtropical countries in Asia, Africa, the Americas, and even some parts of Europe (FAO, 2019). In cultivation, bananas are often planted in well-drained soils and require regular irrigation to ensure consistent growth and fruit production.

2.3 Taxonomy of Banana

According to the Integrated Taxonomy Information System (ITIS), the taxonomy of the common banana, *Musa spp.*, is as follows:

Domain	Flowering plant
Kingdom	Plantae (Plants)
Sub Kingdom	Tracheobionta (Vascular plants)
Class	Liliopsida (Monocotyledons)
Subclass	Zingiberidae
Super division	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Order	Zingiberales
Family	Musaceae (Banana family)
Genus	Musa L. (banana)
Speices	Various species within the Musa genus

Table 2.1: Taxonomy of Banana

There are multiple species and varieties within the *Musa* genus, each with its own unique characteristics and uses. Commonly cultivated banana species include *Musa acuminata* (sweet or dessert bananas) and *Musa paradisiaca* (plantain bananas). The exact species and variety of banana may vary depending on the cultivated type.

2.4 Description of banana peel

Banana (*Musa spp.*) peels, often dismissed as mere waste, represent a remarkable yet underexplored resource with multifaceted applications. These discarded outer layers of banana fruits, typically discarded after consumption, have recently garnered attention for their diverse and untapped potential. Banana peels, constituting more than 30% of the fruit's weight, are frequently disposed of as waste, contributing to environmental pollution issues in landfills (Osma et al., 2007). Nonetheless, these peels harbor valuable compounds that hold potential for diverse applications within the food industry. Harnessing this by-product as a value-added resource is crucial for ensuring the enduring sustainability of the banana industry. Banana peels are not only a rich source of essential nutrients and bioactive compounds but also possess properties that extend their utility far beyond the fruit they protect (Pereira and Maraschin, 2015). These compounds have shown promise in various potential applications, including as a nutritional supplement, natural antioxidant, biodegradable packaging material, and even in phytomedicine.

2.4.1 Composition of Banana Peel

Banana peels consist of several components, including water, carbohydrates, dietary fiber, and a variety of bioactive compounds. Typically, banana peels represent approximately 30-40% of the total banana weight (Bajpai et al., 2015).

- Water Content: Banana peel has a high water content, which contributes to its moisture and succulence (Bajpai et al., 2015).
- **Carbohydrates:** Carbohydrates, predominantly in the form of starches and sugars, constitute a significant portion of the banana peels composition (Mondal et al., 2011).
- **Dietary Fiber:** Banana peels serve as a commendable source of dietary fiber, encompassing both soluble and insoluble varieties. This fiber content

plays a pivotal role in promoting digestive well-being (Mukherjee et al., 2011).

2.4.2 Bioactive compounds in Banana Peel

Banana peels contain various bioactive compounds that have garnered research interest due to their potential health benefits:

Polyphenols: Banana peels house polyphenolic compounds like catechins, epicatechins, and proanthocyanidins, known for their antioxidative capabilities (Kumar and Saurabh, 2018).

Flavonoids: Additionally, flavonoids such as luteolin, quercetin, and kaempferol grace banana peel with their presence, contributing to both its anti-inflammatory and antioxidative effects (Jebson and Cliver, 2018).

Carotenoids: Carotenoids like β -carotene not only lend the peel its characteristic yellow hue but also hold promise for potential health advantages (Gupta et al., 2018).

Tannins: Furthermore, banana peels contain tannins with astringent qualities, opening doors to potential therapeutic uses (Gupta et al., 2018).

2.5 Potential Applications of Banana Peel

The versatile composition and bioactive components found in banana peels have led to its exploration for various applications:

Dietary Supplement: Due to their rich dietary fiber content and micronutrient profile, banana peels have been considered as a viable option for dietary supplementation (Mukherjee et al., 2011).

Natural Antioxidant: The antioxidative properties inherent in banana peel polyphenols and flavonoids hold promise for both food preservation and potential health benefits (Kumar and Saurabh, 2018).

Biodegradable Packaging: Research endeavors have explored the feasibility of utilizing banana peel extracts in the creation of environmentally friendly biodegradable packaging materials (Sessou et al., 2018).

Phytomedicine: Several studies have investigated the therapeutic potential of banana peel extracts, exploring their applications in areas such as wound healing and diabetes management (Gupta et al., 2018).

2.6 Nutritional Components of Banana Fruit

Bananas are renowned for their carbohydrate content, providing a quick and convenient source of energy (Nair et al., 2015). Moreover, they are rich in essential vitamins such as vitamin C, B-vitamins (particularly vitamin B6), and minerals like potassium and magnesium (Shin et al., 2013). The potassium content of bananas is especially noteworthy, as it plays a pivotal role in maintaining healthy blood pressure levels (Hossein-nezhad and Holick, 2013). Bananas provide the following key nutrients as shown below:

Table 2.2: Nutrition facts of Banana (USDA, 2021)

Serving size 100g		% Daily Values (DV)
Calories	89 kcal	4%
Total Carbohydrate	22.8 g	
Dietary Fiber	2.6 g	
Sugars	12.2 g	
Total Fat	0.3 g	
Protein	1.1 g	2%
Vitamin C	8.7 mg	15%
Vitamin B6	0.4 mg	18%
Vitamin B9 (Folate)	20	5%
Panthothenic Acid	0.3 mg	3%
Potassium	358 mg	10%
Magnesium	45mg	8%
Manganese	0.3 mg	13%

2.7 Bioactive Properties of Banana

Bioactive properties refer to the beneficial physiological effects that certain compounds or substances found in food, plants, and natural sources exert on the human body. These bioactive compounds are often associated with promoting health and preventing or mitigating various diseases. They play a pivotal role in the field of nutrition, functional foods, and pharmacology due to their potential to enhance well-being and overall health. Bioactive compounds encompass a wide range of chemical substances, including phytochemicals (found in plants), antioxidants, polyphenols, essential fatty acids, vitamins, minerals, and peptides. These compounds are known to exert various biological activities, and their consumption is linked to numerous health benefits. Antioxidants, such as vitamins C and E, and polyphenols like flavonoids, protect cells from oxidative stress and damage caused by free radicals (Halliwell and Gutteridge, 2007). Bioactive compounds, including omega-3 fatty acids and curcumin, exhibit anti-inflammatory properties, reducing chronic inflammation implicated in various diseases (Serhan et al., 2008). Essential oils, found in many herbs and spices, have antimicrobial effects, which can help combat bacterial, fungal, and viral infections (Bakkali et al., 2008). Banana peels contain various phytochemicals, including polyphenols, carotenoids, and flavonoids, which exhibit antioxidant, anti-inflammatory, and potentially antimicrobial properties (Ogunsina et al., 2017; Sarker et al., 2020).

2.8 Antioxidant properties

Antioxidants are a diverse group of compounds that play a crucial role in protecting the body against oxidative stress and the damage caused by free radicals. These highly reactive molecules, known as free radicals, can harm cells, proteins, and DNA, contributing to various diseases and aging processes. Antioxidants work by neutralizing these harmful free radicals, helping to maintain cellular health and reduce the risk of chronic diseases (Lobo et al., 2010). Antioxidants encompass a wide range of compounds, including vitamins (e.g., vitamin C and vitamin E), minerals (e.g., selenium and zinc), phytochemicals (e.g., polyphenols and flavonoids), and enzymes (e.g., superoxide dismutase). These antioxidants can be obtained through a balanced diet rich in fruits, vegetables, nuts, and whole grains. Their role in maintaining health and preventing chronic diseases underscores the importance of a diet rich in these protective compounds. Bananas, both in their fruit and peel, contain antioxidant compounds that contribute to their health benefits. Studies have indicated that the antioxidant compounds found in banana peels were twice as abundant compared to those present in the edible portion (Someya et al., 2002). They contain bioactive compounds, including polyphenols and flavonoids, which exhibit antioxidant properties. These compounds help neutralize harmful free radicals, reducing oxidative stress and potentially lowering the risk of chronic diseases (Kumar and Saurabh, 2018). Vitamin C, Vitamin B6 in banana fruit and polyphenolic compounds, such as catechins and proanthocyanidins, Flavonoids like luteolin and quercetin, Carotenoids like β -carotene in banana peel have potential health benefits due to their antioxidant properties (Abdul Kadir et al., 2015; Kumar and Saurabh, 2018). Bananas have the potential to serve as a valuable natural antioxidant source for combatting cancer and heart disease (Someya et al., 2002).

2.8.1 Functions of antioxidants

For plant-based diets to exert their protective effects, the presence of antioxidants is paramount. Research studies have consistently demonstrated that regular consumption of fruits and vegetables significantly reduces the risk of developing chronic diseases (Dembinska-Kiec et al., 2008). Moreover, an antioxidant-rich diet has been firmly linked to the provision of long-term health benefits (Sin et al., 2013). Antioxidants play a pivotal role in combating free radicals, thereby contributing to cancer prevention, safeguarding cellular integrity, and potentially extending overall lifespan (Kalcher et al., 2009). The intricate mechanism through which antioxidants operate involves a sophisticated antioxidant system designed to shield against the detrimental impacts of free radicals and their metabolic byproducts. This defense system encompasses processes such as lipidation of antioxidants, the donation of hydrogen and electrons, and the subsequent formation of antioxidant-lipid complexes.

2.8.2 Mechanism

When a substance intervenes to prevent the generation of free alkyl radicals during the initial phase or interrupts the propagation of free radicals, it can result in a delayed onset of lipid oxidation or a slower chemical progression. The formation of free radicals can be postponed through the application of peroxide stabilizers, singlet oxygen inhibitors, and metal chelating agents. Additionally, antioxidants and metal chelating agents serve to halt the propagation of the free radical chain reaction by providing hydrogen (Brewer, 2011).

The free radical chain reaction is as follows-

(1) R:H + O::O + Initiator
$$\rightarrow$$
 R• + HOO•
(2) R• + O::O \rightarrow ROO•
(3) ROO• + R:H \rightarrow ROOH + R•
(4) RO:OH \rightarrow RO• + HO•
(5) R::R + •OH \rightarrow R:R-O•
(6) R• + R• \rightarrow R:R
(7) R• + ROO• \rightarrow ROOR
(8) ROO• + ROO• \rightarrow ROOR + O2
(9) ROO• + AH \rightarrow ROOH + A•
(10) ROO• + A• \rightarrow ROOA.

2.9 Anti-Inflammatory Effects

Inflammation is a natural immune response that helps the body combat infections and repair tissue damage. However, when inflammation becomes chronic or excessive, it can contribute to a wide range of diseases, including arthritis, cardiovascular diseases, and neurodegenerative disorders. Anti-inflammatory activity refers to the ability of certain compounds or substances to reduce or suppress inflammation in the body. These substances, known as antiinflammatories or anti-inflammatory agents, play a crucial role in maintaining health and preventing or managing various inflammatory conditions (Serhan and Savill, 2005). Bioactive compounds found in bananas, including quercetin and dopamine, have exhibited anti-inflammatory properties, potentially aiding in the mitigation of inflammation in body (Ganeshpurkar et al., 2011). The bioactive constituents found in banana peels, such as polyphenols and flavonoids, possess anti-inflammatory characteristics, offering potential relief for conditions associated with inflammation. That's why Banana peel extracts have been explored for their potential in topical applications to reduce skin inflammation and irritation (Mahomoodally et al., 2019).

2.10 Antibacterial effect

Antibacterial effects, the ability to inhibit or kill bacteria, have become a focal point in both medical and scientific research. It encompass a broad spectrum of strategies and mechanisms employed to combat bacteria. These effects can be naturally occurring, as seen in various plants and microorganisms, or synthetically designed in the form of antibiotics. Throughout history, herbs and plants have been revered for their multifaceted roles in traditional medicine and culinary practices. Beyond their aromatic and culinary appeal, many of these botanical treasures have also demonstrated potent antibacterial properties. Studies have indicated that banana extracts, derived from the fruit pulp, possess antibacterial activity against a range of pathogenic bacteria. Compounds found in banana fruit, such as lectins and phenolic compounds, are believed to contribute to these antibacterial effects (Abdel-Salam et al., 2019). While the antibacterial potential of banana flesh is noteworthy, it is the frequently neglected banana peel that conceals fascinating antibacterial attributes. Recent research has unveiled that banana peel extracts encompass bioactive compounds, notably polyphenols and flavonoids, demonstrating antibacterial efficacy against a diverse range of pathogens. These discoveries unveil promising prospects for potential applications in healthcare and food preservation (Mahomoodally et al., 2019; Vigneshwari et al., 2015).

2.11 Health Benefit from banana and their peel

The potential advantages of including banana and banana-derived products in one's diet is explored below-

Rich Source of Dietary Fiber: Banana and banana peel are both rich sources of dietary fiber (Sarker et al., 2020). Dietary fiber plays a crucial role in maintaining digestive health by facilitating regular bowel movements, reducing the likelihood of constipation, and potentially lowering the risk of gastrointestinal disorders. Moreover, dietary fiber induces a sense of satiety, which can be beneficial for managing weight and controlling appetite (Burton-Freeman, 2000).

Improved Gut Health: The intake of banana-derived items, including peel, has been linked to enhanced gastrointestinal well-being. Banana peels contain prebiotic

substances such as fructooligosaccharides (FOS), which act as nourishment for beneficial intestinal microorganisms (Ogunsina et al., 2017). A balanced gut microbiome is associated with a range of health advantages, including improved immune function and decreased inflammation (Round and Mazmanian, 2009).

Antioxidant Properties: Both banana and banana peel exhibit antioxidant properties due to the presence of bioactive compounds such as polyphenols and carotenoids (Sarker et al., 2020). Antioxidants help protect cells from oxidative damage caused by free radicals, which is associated with aging and the development of chronic diseases (Pizzino et al., 2017). Consuming antioxidant-rich foods like banana products may contribute to overall health and well-being.

Cardiovascular Health: The presence of potassium in bananas, as well as banana peel, is recognized for its role in promoting cardiovascular well-being through the regulation of blood pressure (Hossein-nezhad and Holick, 2013). Ensuring an adequate intake of potassium can be beneficial in reducing the likelihood of hypertension and associated cardiovascular ailments (Geleijnse et al., 2012). Moreover, the fiber content found in banana-derived products might enhance heart health by aiding in the control of cholesterol levels (Brown et al., 1999).

Reduced Risk of Chronic Diseases: Regular consumption of banana products has been associated with a reduced risk of chronic diseases. The antioxidants and bioactive compounds in bananas may have protective effects against conditions such as cancer (Sarker et al., 2020). The presence of vitamin C in banana powder and the potential anti-inflammatory effects of banana peel compounds contribute to their disease-fighting properties (Ogunsina et al., 2017).

Weight Management: Utilizing bananas as a dietary supplement or incorporating them as an ingredient in various food items has the potential to aid in weight management by virtue of their substantial fiber content and their capacity to enhance the sensation of fullness. This may lead to prolonged satiety, potentially resulting in a reduction in total calorie consumption (Sarker et al., 2020). Integrating bananas into one's dietary regimen could extend the period of feeling satisfied, which may, in turn, lead to a decrease in overall calorie intake (Burton-Freeman, 2000).

Potential Antimicrobial Properties: Emerging research suggests that banana and banana peel extracts may possess antimicrobial properties, which could contribute to the prevention of bacterial infections (Rahimi-Madiseh et al., 2019). These properties may have implications for both food preservation and human health.

Chapter 3: Materials and Method

3.1 Duration and Location of study

The study was conducted from October 2022 to April 2023. The entire investigation was conducted in the labs of the Department of Applied Chemistry and Quality Assurance, Department of Food Processing and Engineering, Department of Physiology, Biochemistry and Pharmacology, Department of Animal Science and Nutrition as well as Poultry Research and Training Center (PRTC) at Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram. *E. coli* and *Staphylococcus aureus* bacteria that were used in this experiment were collected from the Research Lab of PRTC.

3.2 Samples Accumulation

Samples of Green Banana, locally known as Atia Kola were harvested at an optimal stage of maturity from local garden of Nalua, Satkania, Chattogram. Atia Kola contains soft seeds and provides relief against constipation and intestinal disorders (Islam and Hoque, 2004). In order to receive the banana in the best condition possible, special care was taken during collection and bananas were kept in the refrigerator of the lab till the next step of study. Further necessary components for the experiment were acquired from the laboratory's inventory.

3.3 Study Design

The research was conducted to assess and compare the nutritional composition, bioactive properties, and antimicrobial activity against specific bacteria that cause diarrhea between banana pulp and peel. According to this purpose, this study was involved in separation of banana pulp and peel, powder preparation from both pulp and peel sample after drying, proximate analysis (moisture, ash, crude fat, protein, crude fiber, and carbohydrate), solvent extraction using ethanol and determination of bioactive properties, antioxidant properties and antimicrobial activity for crude extract of both sample.

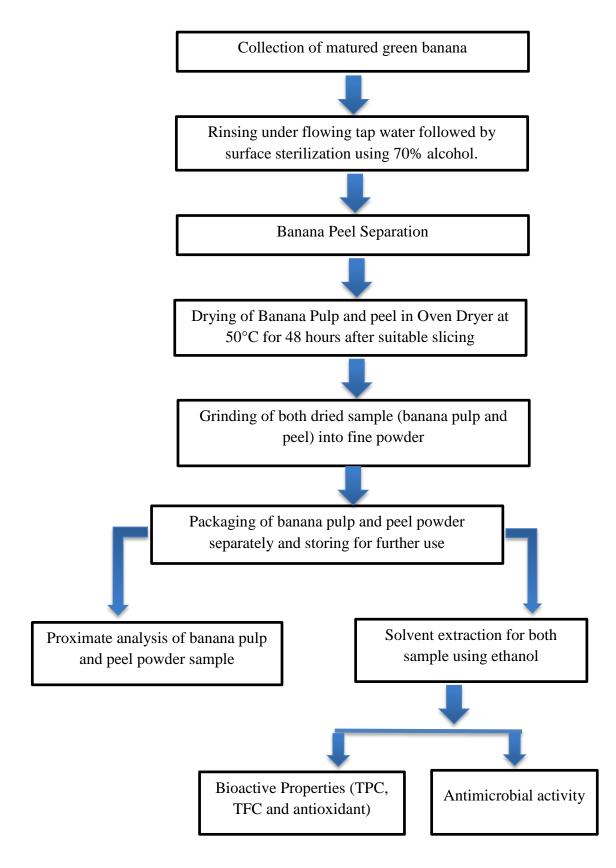


Figure 3.1: Study Design

3.4 Preparation of Banana pulp and peel powder

In the laboratory, bananas underwent a cleaning process involving rinsing with tap water, followed by surface sterilization using 70% alcohol, and a final rinse with sterile distilled water. Then peels were taken and pulps and peels were collected separately. Both the pulp and peel were sliced into appropriately sized or thinner pieces to expedite moisture removal. Subsequently, the separated pulp and peel samples underwent drying in a hot air oven set at 50 °C for a duration of 48 hours. Following the drying process, the samples were e grinded thoroughly using a blender and the powder samples were run through a fine (2 mesh) sieve to eliminate any leftover debris. The obtained powdered samples were stored in clean, airtight plastic bags at room temperature for future utilization.

3.5 Extract Preparation

The banana pulp and peel powder were extracted using Ethanol solvent at a ratio of 1:10 w/v. The solvent mixture was kept at incubator at 37 ° C for 48 hours. Then the extracts were collected following filtration using Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator. Finally, the crude extracts were stored in clean brown bottles at -20°C until further use.

3.6 Proximate Analysis

The proximate components of both sample (banana pulp and peel powder) were evaluated in accordance with AOAC standard technique (DM Basis). The moisture, ash, crude protein, crude fiber, and crude fat contents were determined using the dry ash method, oven drying method, Kjeldahl's method, gravimetric method, and soxhlet method, respectively.

3.6.1 Moisture content

The Association of Official Analytical Chemists' (AOAC, 2005) standard technique was used to calculate the moisture content.

Principle: Food staffs usually contain moisture. Simple heating at 104-105°C for 3–4 hours in the oven and cooling in a desiccator to absorb moisture is used to estimate moisture. The procedure is performed numerous times until the sample exhibits a stable weight.

Apparatus: Desiccator, hot air oven, the crucible, and weighing scale

Calculation: This is how the percentage of moisture was determined:

Moisture %=
$$\frac{Initial weight - final weight}{Sample weight} \times 100$$

3.6.2 Ash content

The total ash content was ascertained using the AOAC method 14.006 (2005). The mineral components are all mixed together in the ash fraction. Using this technique, all organic material is burned to oxidize it, and the amount of ash that remains is calculated.

Apparatus: Porcelain, a gas burner, and a muffle furnace

Calculation: The following phrase was used to determine the ash content:

Ash % of Sample=
$$\frac{The amount of ash in the supplied sample}{Sample weight} \times 100$$

3.6.3 Crude Proteins

The protein content of beetroot was calculated using AOAC method (2005).

Principle: The Kjeldhal method is employed to calculate nitrogen. By measuring the material's nitrogen content and multiplying the nitrogen factor by 6.25, the 22 protein content of food items can really be determined. Plant protein is thought to contain 16% nitrogen on average. As a result, the plant protein factor is 100/16-6.25. A known amount of the sample is almost always digested with H₂SO₄ in the presence of the digestion mixture (CuSO₄ and K₂SO₄ in the ratio of 1:20). Following diluting the digested material and trapping the released ammonia in a 2% boric acid solution; surplus acid is neutralized with alkali (40% NaOH, w/v). A standard (0.1N) HCl solution is used to titrate the recovered distillate. By multiplying by 6.25, one can calculate crude protein and calculate the percent nitrogen.

Apparatus: Kjeldahl digesting unit, condenser, and flask are examples of equipment.

Reagent:

- Sulfuric acid in concentrated form
- Digestion blend
- Solution of boric acid

- Solution of alkali.
- Mixture of indicators
- > 0.1 N standard HCI

Calculation: Calculated nitrogen and protein percentages are as follows:

Protein %= $\frac{Titration \ value \times Normality \ of \ HCI \ (0.1) \times 0.014}{Sample \ weight} \times 6.25 \times 100$

3.6.4 Crude Fat

To ascertain the samples' crude fat content, a soxhlet device was utilized in accordance with the AOAC (2005) technique.

Principle: Food samples are dissolved in organic solvents such as chloroform, methanol and the filtrate is then separated to determine the amount of fat present. Putting the filtrate into two funnels, separating the mixture, drying it to measure the extract, and then estimating the fat content.

Apparatus: Thimble, the solvent extractor 23

Calculation: The crude fat percentage was given as follows:

Fat % of sample = $\frac{Weight of the extract}{Weight of the sample} \times 100$

3.6.5 Crude Fiber

The AOAC method (2005) was used to calculate crude fiber.

Basic principle: Crude fiber, the water-insoluble fraction of carbohydrates, mainly consists of cellulose, hemicellulose, and lignin. The estimation of crude fiber in a fat-free food sample involves a sequential process. First, a known quantity of the sample is boiled in a weak acid solution $(1.25\% H_2SO_4)$ for 30 minutes. Then, it undergoes boiling in a weak alkali solution (1.25% NaOH) for another 30 minutes while maintaining a constant volume. Finally, the ash content is subtracted from the obtained residue to calculate the crude fiber content through this digestion process.

Apparatus: Leibig condenser, Reflux condenser, and Gooch crucible are the instruments.

Reagent necessary:

➤ 0.255N Sulfuric acid solution,

> 10% Potassium sulfate solution, grade Asbestos-Gooch.

Calculation: The weight loss reflects crude fiber.

Crude fiber
$$\% = \frac{Weight of residue with crucible - weight of ash with crucible}{Weight of sample (moisture and fat free)} \times 100$$

3.6.6 Total Carbohydrate

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100/100gm (AOAC, 2016). Hence, it was calculated using the formula below:

% Carbohydrate = 100 - (Moisture %+Ash%+Protein%+Fat %+Fiber %)

3.7 Phytochemical Screening

Various techniques can be used to discover active compounds within a crude extract, including methods like Thin-Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and more. However, the simplest and most straightforward approach is phytochemical screening. Phytochemical screening of a food sample is a comprehensive analytical approach aimed at identifying and quantifying various bioactive compounds present in plants or plant-derived food products. These bioactive compounds are often natural substances with potential health benefits and can include a wide range of phytochemicals such as polyphenols, flavonoids, alkaloids, terpenoids, saponins, and more. The purpose of phytochemical screening is to assess the nutritional quality, potential medicinal properties, and overall chemical composition of a food sample derived from plant sources. Ultimately, the screening of phytochemicals in food samples plays a crucial role in promoting a better understanding of the nutritional and health-related aspects of plant-based foods, contributing to the enhancement of public health and the development of innovative food products. Phytochemical screening of the crude extract of banana pulp and peel powder was carried out using standard phytochemical procedure (Ayoola et al., 2008; Mikail HG., 2010).

Test for Carbohydrate

A total of 0.5 ml of the crude extract was combined with 2 ml of Molisch's reagent, and the mixture was thoroughly shaken. Subsequently, 2 ml of concentrated H2SO4 was cautiously added to the test tube, allowing it to flow down the side. The

appearance of a violet ring at the interface between the two layers indicates the presence of carbohydrates.

Test for Protein

According to the Millon's Test, the crude extract was combined with 2 ml of Millon's reagent. The appearance of a precipitate that turned red upon mild heating served as confirmation of the presence of proteins.

Test for Phenols

The addition of 2 ml of alcohol and 2-3 drops of ferric chloride solution to 1 ml of the crude extract resulted in the development of a blue-green or black coloration, which indicated the presence of phenols.

Test for Tanin

To detect the presence of tannins, 0.5 ml of the crude extract was mixed with 1 ml of distilled water and 2-3 drops of ferric chloride solution. The formation of a black coloration indicates the presence of tannins.

Test for Saponins

In a test tube, 1 ml of the crude extract was vigorously shaken with 5 ml of distilled water, and the formation of a persistent foam was regarded as an indicator of the presence of saponins.

Test for Flavonoids

A mixture was prepared by combining 0.5 ml of the crude extract with 2 ml of a 2% NaOH solution. This resulted in the formation of a vivid yellow color, which subsequently became colorless upon the addition of a few drops of diluted acid. It ensures the presence of flavonoids.

Test for Glycosides

A mixture of 2 ml of chloroform and the crude extract was prepared. Then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. The emergence of a reddish-brown color served as an indicator for the presence of glycoside

3.8 Determination of Bioactive Compounds

3.8.1 Measurement of Flavonoids (TFC)

The assessment of total flavonoid content in the samples followed the aluminum chloride colorimetric method detailed by Chang et al. (2002). For the preparation of the extract stock solution at a concentration of 1 mg/ml, a test tube was employed, initially filled with 1.5 ml of 95 percent C_2H_5OH , and subsequently diluted in 0.5 ml increments. Subsequently, 0.1 ml of 10% AlCl₃, 0.1 ml of 1 mol/L potassium acetate, and 2.8 ml of distilled water were introduced into the test tube. This mixture was left at ambient temperature for a duration of 30 minutes.

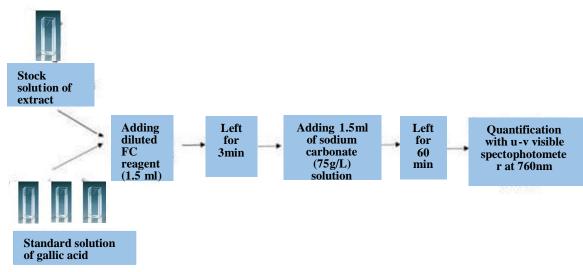


Figure 3.3: Determination of Total Flavonoids content (TFC)

3.8.2 Measurement of Polyphenols (TPC)

The determination of total phenolic content (TPC) in the extracts was conducted using a modified version of the Folin-Ciocalteu (FC) reagent method, as outlined by Al-Owaisi et al. (2014), with slight adaptations. In this procedure, 1 milliliter of the ethanoic extract was combined with 1.5 milliliters of FC reagent within a falcon tube and allowed to stand at room temperature for three minutes. Subsequently, 1.5 ml of 7.5% Na₂CO₃ was introduced, and the mixture was left to settle for 60 minutes. The absorbance was measured at a wavelength of 765 nm using a UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, Japan), with C₂H₅OH employed as the reference blank. TPC in the extracts was determined through calculations establishing the equivalence of TPC to milligrams of gallic acid equivalents (GAE) per gram of extracts. To ensure precision and reliability, the measurements were repeated three times, and the means and standard deviation were computed.

3.9 Antioxidant Capacity Measurement

The antioxidant activity of the extracts was evaluated using the DPPH test, with slight modifications from the procedure described by Azlim et al., 2010. Approximately 6 mg of DPPH was dissolved in 100 ml of 100% methanol to prepare a methanoic DPPH solution. Subsequently, 1 ml of the methanoic extract was mixed with 2 ml of the DPPH solution. The mixture was gently shaken and allowed to stand at room temperature in the dark for 30 minutes. Using a UV-VIS spectrophotometer, the absorbance was measured at a wavelength of 517 nm. For the control, 1 ml of methanol was mixed with 2 ml of the DPPH solution, and methanol served as the blank. To assess the scavenging activity of the samples, the decrease in absorbance relative to the DPPH standard solution was used as an indicator. The antioxidant capacity of the extracts was evaluated based on their ability to scavenge DPPH free radicals. A standard calibration curve was created using TEAC composite (Trolox equivalent antioxidant capacity), which was also used as the standard. The results were expressed as milligrams of Trolox equivalents per gram of powder on a dry weight (DW) basis. For enhanced accuracy, the measurements were replicated three times to calculate means and standard deviation.

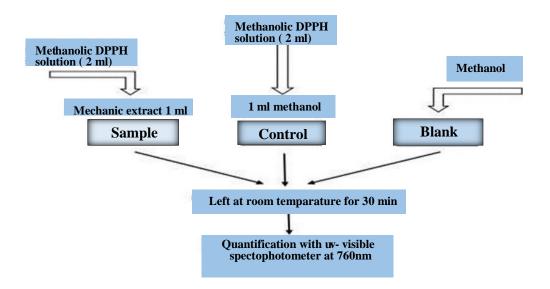


Figure 3.2: Determination of antioxidant capacity

3.10 Antibacterial activity

Preparation of Extract

The banana pulp and peel powder was mixed separately with Ethanol solvent at proper ratio and kept at incubator at 37 ° C for 48 hours. Then the extracts were collected following filtration using Whatman No. 1 filter paper and were concentrated using a rotary evaporator. Finally, the crude extracts were stored for further use (Zaiden et al., 2005).

Test Microorganisms

The antibacterial efficacy was evaluated against *Staphylococcus aureus* and *Escherichia coli*. Pure, isolated cultures of Escherichia coli and Staphylococcus aureus were sourced from the Poultry Research and Training Centre (PRTC) at Chattogram Veterinary and Animal Sciences University in Chattogram. The broad-spectrum antibiotics, Ciprofloxacin (CIP) and Gentamycin (CN), served as the standard reference for comparison.

Reagent and Apparatus

Reagents

- ▶ 1% Barium Chloride solution
- ➢ 1% Sulfuric acid
- ➢ Normal saline
- Distilled water

Media

- Blood Agar
- Mueller Hinton Agar

Apparatus

- Petri dishes
- Inoculating loop
- Screw capped test tubes
- ➢ Whatman no 1 filter paper
- Volumetric flasks
- > Pipette

- Beaker
- Spirit lamp
- > Tripod stands
- Electric weight machine
- ➢ Foil paper
- Spoon
- ➢ Marker pen
- ➢ Autoclave
- Incubator

McFarland standard preparation

A solution of 1% sulfuric acid and 1% barium chloride was made. A sterile test tube with a screw-on top was used to combine 99.5 ml sulfuric acid and 0.05 ml barium chloride solution for the 0.5% McFarland standard.

Preparation of culture suspension

Each isolate's inoculum was made from a subculture. In a sterile screw cap tube containing 2 ml of sterilized saline water, 4-5 colonies of each isolate were collected. Following that, the bacterial culture was emulsified in sterile normal saline, and the turbidity was set at 1.5*108 (CFU/ml equal to 0.5% McFarland standard).

Media preparation

In accordance with the label instructions, 38 grams of Mueller Hinton agar powder were precisely weighed and then mixed with 1 liter of distilled water. The resulting media were heated to ensure complete dissolution and thorough mixing. Once mixed, the media underwent autoclave sterilization and were subsequently transferred to a water bath to cool down. After achieving the desired temperature, the media were aseptically dispensed onto Petri plates and allowed to solidify. These Petri dishes were then incubated for a 24-hour period at 37°C to detect any signs of contamination.

Antimicrobial effect of samples against *Escherichia coli* and *Staphylococcus* aureus

The disc diffusion method was employed to examine the effectiveness of the extracts, and their impact was measured by taking note of the zone of inhibition surrounding the disc. Whatman No. 1 Filter paper was used to create discs with a diameter of 6 mm. 0.5 ml of each sample was used to impregnate the discs. The Mueller Hinton agar plates were uniformly inoculated by dipping a sterile cotton swab into the standardized bacterial suspension. They were left to dry for three to five minutes. Following that, each disc was put on the plates and lightly pressed to ensure full contact with the agar. To display overlapping of inhibitory zones, a space of at least 15 mm was kept between the plates' edges. The plates were incubated for 24 hours at 37 °C fifteen minutes after the discs were placed. Following incubation, the plates were inspected, and the diameter of the inhibitory zone for each isolate was measured.

3.11 Statistical Analysis

The data collected were organized, assigned codes, and recorded within a Microsoft Excel 2019 spreadsheet. Descriptive statistics, including the calculation of mean values and standard deviations, were performed for various parameters such as proximate analysis, bioactive compounds (TPC, TFC), and antioxidant capacity. Subsequently, statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences) software, version 19.0. This analysis employed One-way ANOVA (Analysis of Variance) procedures to determine the significance of variations at a 95% confidence interval. The level of significance chosen for the statistical analysis was set at 5% (≤ 0.05). Furthermore, the statistical analysis (Tukey's pairwise comparison) was conducted to identify specific differences among groups or treatments under consideration.

Chapter 4: Results

4.1 Proximate Composition

Table 4.1 displays the mean percentage with standard deviation (ME \pm SD) of the proximate composition value which includes moisture, protein, fat, crude fiber, ash and carbohydrate content of both banana pulp powder and banana peel powder.

Component	Banana Pulp	Banana Peel	P-value
	Powder	Powder	
Moisture (%)	6.09 ± 0.06^{b}	5.15 ± 0.04^{a}	< 0.05
Crude Protein (%)	4.36 ± 0.025^a	7.18 ± 0.02^{b}	< 0.05
Crude Fat (%)	0.80 ± 0.045^a	8.56 ± 0.04^{b}	< 0.05
Crude Fiber (%)	10.07 ± 0.042^{a}	26.77 ± 0.096^{b}	< 0.05
Ash (%)	2.98 ± 0.035^a	10.12 ± 0.106^{b}	< 0.05
Carbohydrate (%)	76.42 ± 0.065^{b}	$47.25\pm0.03^{\text{a}}$	< 0.05

Table 4.1: Proximate composition of Banana pulp and peel powder

Results are presented as mean \pm SD. *Different superscripted letters (a, b) in each row shows statistically significant differences (p value < 0.05) for all the samples.

According to this analysis, Banana pulp powder had a larger percentage of moisture $(6.09 \pm 0.06\%)$ and carbohydrate content $(81.76 \pm 0.065\%)$ where banana peel powder contained $5.15 \pm 0.04\%$ moisture content and $47.25\pm0.03\%$ carbohydrate content respectively. Contrarily, crude protein $(7.18\pm0.02\%)$, crude fat $(8.56 \pm 0.04\%)$, crude fiber $(26.77 \pm 0.096\%)$ and ash content $(10.12 \pm 0.106\%)$ of the banana peel powder was higher. The crude protein, crude fat, crude fiber and ash content of banana pulp powder was $4.36 \pm 0.025\%$, $0.80 \pm 0.045\%$, $10.07 \pm 0.042\%$ and $2.98 \pm 0.035\%$ respectively.

4.2 Bioactive properties

Bioactive components and antioxidant capacity were analyzed by using a UVvisible spectrophotometer. Results were subjected to descriptive statistical analysis followed by Tukey's comparison analysis. Results are shown in the below table:

Sample	Total flavonoids content (TFC) (mg QE/100g)	Total polyphenol content (TPC) (mg GAE/100g)	Antioxidant Capacity (% Inhibition)
Banana Pulp Powder	58.21 ± 0.113^{a}	24.06 ± 0.034^{b}	3.19 ± 0.003^{ab}
Banana Peel Powder	226.22 ± 0.071^{b}	8.69 ± 0.053^{a}	3.03 ± 0.001^{ab}

Table 4.2: Bioactive properties analysis test result

Results are presented as mean \pm SD. *Different superscripted letters (a, b) in each column shows statistically significant differences (p value < 0.05) for all the samples.

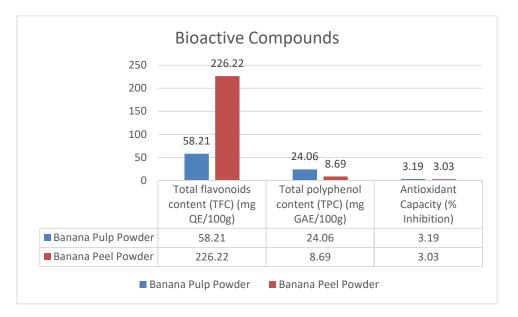


Figure 4.1: Bioactive properties of banana pulp and peel powder

According to the results, there was significant differences between banana pulp and peel powder in total flavonoid and polyphenol content. Banana peel contained the highest amount of total flavonoid concentration (226.22 ± 0.071 mg QE/100g) where banana pulp had (58.21 ± 0.113 mg QE/100g). Banana pulp had the highest total polyphenol content measurement (24.06 ± 0.034 mg GAE/100g). Banana peel had the lowest result (8.69 ± 0.053 mg GAE/100g). There was no significant differences in terms of antioxidant capacity between banana pulp and peel powder. The antioxidant capacity of banana pulp and peel from 3.03 to 3.19 mg TE/100g. Banana pulp has the highest antioxidant capacity (3.19 ± 0.003 mg TE/100g).

4.3 Phytochemical Screening

The phytochemical analysis of extracts from banana pulp and peel powder has unveiled intriguing insights into their chemical composition. The method used for phytochemical screening in this study was of a qualitative nature.

Chemical compounds	Banana Pulp Powder extract	Banana Peel Powder Extract
Carbohydrate	+	+
Protein	+	+
Tannins	+	+
Saponin	_	+
Glycosides	-	-
Flavonoids	+	+
Phenol	+	+

Both samples, banana pulp and peel powder extracts, share the presence of several essential phytochemicals, including carbohydrates, proteins, tannins, and flavonoids. However, glycosides were not detected in either sample, and banana pulp powder extract did not contain saponins.

4.4 Antimicrobial activity

The result of antimicrobial activity of both sample extracts against *E. coli* and *Staphylococcus aureus* are given in Table 4.4.

Sample	Escherichia Coli	Staphylococcus Aureus
Banana Pulp extract	15mm	13mm
Banana Peel extract	11mm	10mm

Table 4.4 illustrates the antibacterial effects of banana pulp and peel extracts against Staphylococcus aureus and E. coli. The ethanol extract of banana pulp exhibited the most significant inhibition zones, measuring 13 mm for *S. aureus* and 15 mm for *E. coli*. Conversely, the ethanolic extract of banana peel displayed inhibition zones of 10 mm against S. aureus and 11 mm against E. coli.

As part of the control group, Ciprofloxacin (CIP) was employed for *Escherichia coli* (*E. coli*) as positive control, while Gentamycin was utilized for *Staphylococcus aureus* (*S. aureus*) as negative control.

Chapter 5: Discussion

5.1 Proximate analysis

The results of the chemical analysis comparing banana pulp powder and banana peel powder provide valuable insights into the composition of these two distinct parts of the banana fruit. This analysis reveals significant differences in moisture content, carbohydrate content, and various other nutritional components between the two samples. These findings not only highlight the nutritional disparities between banana pulp and peel but also shed light on their potential applications in various industries, including food and agriculture.

One of the key findings of this analysis is the significant difference in moisture and carbohydrate content between banana pulp and peel powders. First and foremost, Banana pulp powder exhibited a higher moisture content of approximately 6.09%, indicating its relatively greater water content compared to banana peel powder, which had a lower moisture content of about 5.15%. This is expected, as the pulp is the fleshier part of the fruit, while the peel is drier. Similar to present study, the result of moisture content is agreeable with another study, which showed 6% moisture content for unripe banana flour (Rodríguez-Ambriz et al., 2008). The moisture content in green banana flour ranges within 4% to 8%, varying on bananas species and method of drying (Kumar et al., 2019) .This disparity in moisture content suggests that banana pulp retains more water, contributing to its succulence and texture and is crucial for product stability, storage, and shelf life. It suggests that banana pulp powder may require different handling and processing considerations than banana peel powder.

In terms of carbohydrates, banana pulp powder demonstrated a notably higher carbohydrate content of approximately 76.42%, while banana peel powder contained a lower carbohydrate content of approximately 47.25%. This substantial contrast in carbohydrate content reflects the difference in the primary constituents of these two banana components. A study showed carbohydrate content of similar ranges, particularly 80% carbohydrate in hot air dried banana flour (Asif-Ul-Alam et al., 2014). Banana pulp is rich in sugars, including glucose, fructose, and sucrose, contributing to its sweet taste, while banana peel contains more complex

carbohydrates, such as cellulose, hemicellulose, and pectin, which contribute to its fibrous and less sweet nature.

Furthermore, the analysis revealed that banana peel powder had higher levels of crude protein, crude fat, crude fiber, and ash content compared to banana pulp powder. The value of these content was similar with the result established on a study of composition of banana flour of different varieties (da Mota et al., 2000). Banana peel powder contained approximately 7.18% crude protein, 8.56% crude fat, 26.77% crude fiber, and 10.12% ash content. In contrast, banana pulp powder had lower values, with approximately 4.36% crude protein, 0.80% crude fat, 10.07% crude fiber, and 2.98% ash content. These differences highlight the potential nutritional advantages of banana peel powder. Higher protein content is valuable for individuals looking to supplement their diet with protein, while increased fiber content suggests a richer mineral profile in banana peel powder, which may have specific dietary benefits.

5.2 Bioactive properties

The findings from this study reveal noteworthy differences in the phytochemical composition between banana pulps and peel powder, shedding light on their potential health benefits and applications. In particular, the analysis focused on total flavonoids, total polyphenols, and antioxidant capacity.

Total flavonoid content (TFC) emerged as one of the key differentiating factors between banana pulp and peel powder. Banana peel exhibited a significantly higher concentration of total flavonoids (226.22 mg QE/100g) compared to banana pulp (58.21 mg QE/100g). Flavonoids are well-known for their antioxidant and anti-inflammatory properties, and the abundance of these compounds in banana peel suggests its potential as a source of natural antioxidants. This aligns with previous research findings that have highlighted the flavonoid-rich nature of banana peels (Behiry et al., 2019). In another research, banana pulp extracts (Fatemeh et al., 2012).

Conversely, banana pulp powder exhibited the highest total polyphenol content (24.06 mg GAE/100g) compared to banana peel powder (8.69 mg GAE/100g). This

finding suggests that banana pulp is a more concentrated source of polyphenols. Polyphenols are also potent antioxidants and have been linked to protective effects against oxidative stress-related conditions. This finding underscores the nutritional diversity between these two banana components. In a study, the researchers also observed significant differences in the flavonoid and polyphenol content between banana peel and pulp (Pereira and Maraschin, 2015). Their findings supported the notion that banana peel is a rich source of flavonoids, while banana pulp exhibited higher polyphenol content.

Interestingly, despite the differences in flavonoid and polyphenol content, there were no significant disparities in antioxidant capacity between banana pulp and peel powder. Both exhibited similar antioxidant capacities, ranging from 3.03 to 3.19 mg TE/100g, with banana pulp displaying the highest antioxidant capacity (3.19 mg TE/100g). Findings of a study emphasized the antioxidant potential of banana peel due to its flavonoid and polyphenol content, supporting our observations (Rebello et al., 2014).

5.3 Phytochemical Screening

The chemical composition of banana pulp and peel extracts reveals a complex profile of compounds that contribute to their nutritional and bioactive properties. Both banana pulp and peel contain essential components such as carbohydrates and proteins, which serve as sources of energy and vital nutrients. Tannins, present in both extracts, contribute to the astringency and potential health benefits of these banana components. Tannins are recognized for their broad-spectrum antimicrobial properties. They can inhibit bacterial growth at low concentrations and function as antifungal agents at higher concentrations. Tannins are referred to as polymeric phenolic compounds due to their ability to precipitate gelatin from a solution, a characteristic known as astringency (Peteros NP and Uy MM., 2010).

Saponins, detected in banana peel but not in pulp. Saponins have potential healthpromoting properties and may find applications in various fields. Saponins found in the peel extract are frequently employed in medical treatments for conditions such as epilepsy, excessive chlorosis, and migraines (Mikail HG., 2010). Glycosides, which were absent in both extracts, encompass a wide range of natural compounds with various functions. However, the absence of glycosides and the presence of saponins in banana peel powder extract suggest that the peel may hold specific bioactive compounds not found in the pulp. Flavonoids and phenols, present in both pulp and peel, are known for their antioxidant and anti-inflammatory properties, contributing to the potential health benefits associated with banana consumption. The presence or absence of these compounds highlights the unique chemical profiles of banana pulp and peel, influencing their suitability for dietary, medicinal, or industrial applications. It's important to consider these differences when exploring the diverse uses of banana components.

5.4 Antimicrobial activity

The evaluation of the in vitro antibacterial susceptibility of banana pulp and peel extracts against two common pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli (E. coli)*, has yielded insightful findings that hold potential implications for both food safety and healthcare applications. It is well known that the tropical fruits like banana are full in phytochemicals, which may be crucial for regulating and controlling the number of pathogens in both people and animals' gastrointestinal tracts.

One of the key findings is the contrasting antibacterial activity exhibited by banana pulp and peel extracts. The ethanol extract of banana pulp demonstrated remarkable inhibition zones, measuring 13 mm against Staphylococcus aureus and an even more substantial 15 mm against Escherichia coli. These results suggest that banana pulp extract possesses significant antibacterial properties, which could be attributed to the presence of bioactive compounds in the pulp. Conversely, the ethanolic extract of banana peel, while still displaying antibacterial activity, exhibited slightly smaller inhibition zones—10 mm against Staphylococcus aureus and 11 mm against Escherichia coli. The observed antimicrobial activity, as detected through the disc diffusion and broth dilution methods, may be attributed to the active compounds present in both the banana pulp and peel extract (Ishwar and Singh, 2000). The presence of phytochemicals in banana peels may explain their utilization by traditional medicine practitioners in various healthcare systems. These compounds have been employed in the treatment of bacterial infections such as cough, fever, cold, and venereal diseases. The outcomes of this research underscore the significance of organic solvent (ethanol) extracts, which displayed higher antimicrobial activity. This is likely because the antimicrobial compounds in the peels were either polar or non-polar and were more effectively extracted through the organic solvent medium. This observation aligns with the findings of previous studies on medicinal plants, which also indicated that organic solvents are more suitable for extracting phytochemicals (Natarajan et al., 2005).

As part of the control group, Ciprofloxacin (CIP) and Gentamycin were employed to test the susceptibility of Escherichia coli and Staphylococcus aureus, respectively. These antibiotics are commonly used in clinical settings to combat bacterial infections. The fact that the banana pulp and peel extracts exhibited inhibition zones comparable to these antibiotics underscores the potential of natural extracts as alternative antibacterial agents.

Green bananas offer a range of health benefits, including being a good source of dietary fiber, which supports digestive health and helps regulate blood sugar levels. They are also rich in essential nutrients like potassium, vitamin C, and vitamin B6, which contribute to heart health and immune function.

Chapter 6: Conclusion

Green bananas offer a range of health benefits, including being a good source of dietary fiber, which supports digestive health and helps regulate blood sugar levels. They are also rich in essential nutrients, which contribute to heart health and immune function. On the other hand, green banana peels, often underestimated and discarded, are a nutritional powerhouse. By recognizing their value, we can elevate our culinary adventures and promote a more sustainable and nutritious lifestyle. That's why the current study focused to explore the nutritional component, bioactive properties and in vitro antibacterial activity of banana pulp and peel extract. The findings from this study demonstrated the impressive phytochemical attributes of both portions of the banana, the pulp, and the peel. Notably, it highlighted the superior nutritional composition of banana peel when compared to the pulp. Banana peel powder exhibited elevated levels of crude protein, crude fat, crude fiber, and ash content, along with lower moisture and carbohydrate content. This discovery has encouraged us to reconsider the potential of banana peel as a valuable component in the creation of medications, cosmetics, enhanced products, and dietary supplements, rather than simply discarding it after consuming the banana pulp. Based on the results obtained from this experiment, it can be concluded that both green banana pulp and peel exhibit significant antibacterial capabilities. These findings suggest us to utilize green banana pulp and peel as natural antimicrobial agents in various applications, including food preservation, pharmaceuticals, and healthcare products and leaves a strong footprint for further exploration in this study.

Chapter 7: Recommendation and future perspectives

From this study, the following suggestions can be made for further experiments:

- \blacktriangleright The current studies could be repeated to confirm the experimental findings.
- Banana pulp and peel powder can be incorporated into functional foods and nutraceuticals to enhance their nutritional value. These products can address specific health concerns and provide consumers with convenient ways to access the health benefits of bananas.
- As banana peel showed higher fat content, so it can be regarded to a possible source of edible oil. Further research on oil extraction from banana pulp and peel could be done.
- Further research can explore how the phytochemical profiles of the sample extracts translate into functional properties and potential health benefits.
- Investigations into the safety and efficacy of these extracts for human consumption or medical applications are essential before any practical use can be considered.

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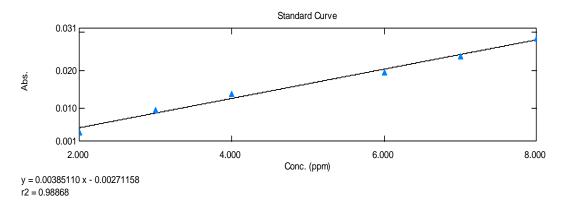
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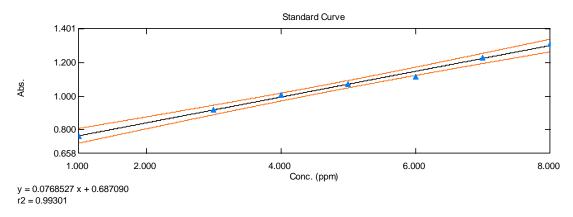
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Appendix I: Standard Curve for Flavonoids, Polyphenol and Antioxidant

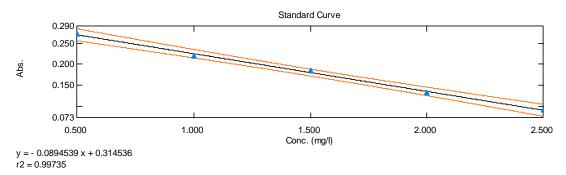


Standard Curve for Flavonoids (TFC Determination)

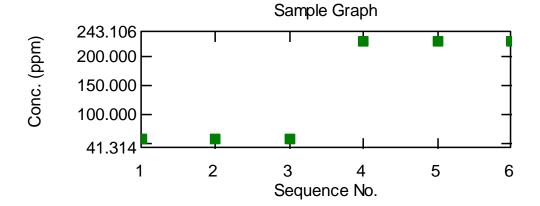
Standard Curve for Polyphenol (TPC Determination)



Standard Curve for Antioxidant Capacity Determination

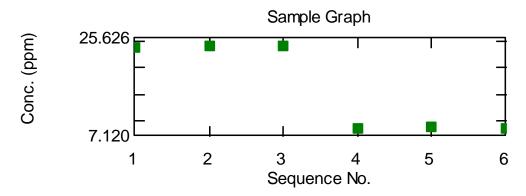


Appendix II: Sample Curve for Flavonoids, Polyphenol and Antioxidant

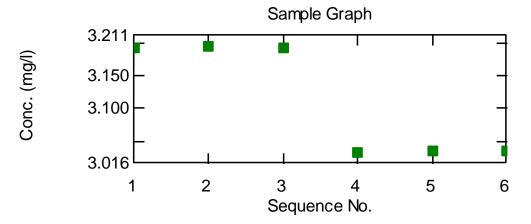


Sample Graph for Flavonoids (TFC Determination)

Sample Graph for Polyphenol (TPC Determination)



Sample Graph for Antioxidant Capacity Determination



Appendix III: Photo Gallery



a) Green banana pulp and peel before drying



b) Banana pulp and peel after drying



c) Banana pulp and peel powder



d) Banana Pulp and Peel powder Extract preparation



e) Phytochemical testing of extract



f) Antimicrobial Test activities

Brief Biography

Bappa Aich has passed the Secondary School Certificate (SSC) Examinations in 2012 with Grade Point Average (GPA) 5.00 followed by Higher Secondary Certificate (HSC) Examination in 2014 with GPA 4.70. He received the B.Sc. (Hon's) in Food Science and Technology in 2019 (held in 2020) from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of M.Sc. in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Quality Technology, Faculty of Food Science and Technology, CVASU. His career objective is to obtain and secure a challenging position as a Food scholar. He has profound interest to work in a challenging environment where his skill can be put to good use for problem solving and come up with innovative solutions.