# Antioxidant, Anti-inflammatory and Free radicals scavenging activity of methanolic extract of *Musa paradisiaca* L peels

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***Abstract***

*Musa paradisiaca commonly known as banana is grown and eaten as ripe fruit around the world. Peels are a waste product that makes up roughly 18–33% of the entire fruit. Banana peels have shown various biological properties. Therefore, the phytoconstituents, antioxidant potentials, anti-inflammatory activities and free radical scavenging properties of banana peels were identified in order to make use of them as a source of useful components. The presence of alkaloids, flavanoids, proteins, carbohydrates, tannins, terpenoid, saponins and glycosides is revealed by phytochemical analysis of methanolic extracts of banana peel. While terpenoids and saponins have antibacterial properties, alkaloids, flavanoids and tannins are phytochemicals with excellent antioxidant qualities. There were 137.6 ± 39.59 mg GAE/g of total phenolic content and 256.6 ± 29.30 mg QE/g of flavonoid content. At 1000 µg/ml conc., the methanolic extract of Musa paradisiaca peels exhibited the maximum antioxidant activity which was dose dependent.*

*Furthermore, ascorbic acid (the reference control) and the antioxidant activity by peel extracts were comparable. To ascertain the scavenging capabilities of free radicals, we assessed the scavenging activities of nitric oxide, superoxide and hydroxyl radicals. The nitric oxide, superoxide and hydroxyl radical scavenging capabilities were found to be dose dependant. According to the study's findings, banana peel has anti-inflammatory properties at all doses. Thus, our results showed that banana peels have the strongest in vitro antioxidant, anti-inflammatory and free radical scavenging properties. However, extensive research is required to fully understand its potential biomedical applications.*

**Keywords:** *Musa paradisiaca*, antioxidant potentials, free radicals, anti-inflammatory activity, nitric oxide.

## Introduction

Food waste and by-products are considered to be a serious problem with negative social, economic and environmental impacts. Reducing the amount of food waste is a national priority. Food insecurity is still a major problem despite international efforts. Seventeen percent of food is thought to be lost or wasted during the consumer and retail phases[1]. Improving the effectiveness, safety, quality and sustainability of our food systems requires reducing food loss and waste. In order to create breakthroughs for the food sector, researchers must actively work to recover and revalue crop waste by-products. This strategy can improve the resilience of food systems and decrease food waste.

Numerous by-products are produced during the processing of fruits and vegetables, including a sizable amount of waste in the juice business. These waste materials, which include leaves, peels, undesired pulp, seeds, cull fruits and stones are rich in bioactive compounds such as proteins, polysaccharides (cellulose, pectin and starch), vitamin C and phytochemical compounds such flavonoids, anthocyanins, carotenoids and phenolic acids[2-8].

Fruit and vegetable wastes have been found to contain significant levels of secondary metabolites and these waste materials have been extracted to look for phenolic compounds, dietary fibers and other physiologically active metabolites[9,10]. According to scientific studies, the peels, seeds, fruits and vegetables contain a significant amount of phytochemicals and vital nutrients[9]. For instance, the phenolic content of mango and jackfruit seeds, as well as the skins of grapes, avocados and lemons, can be up to 15% higher than that of fruit pulp[11,12].

Thus, the waste from fruits and vegetables might be used to produce biologically active metabolites that could be used in the textile, culinary, pharmaceutical and cosmetics industries. Using fruit peels properly would not only alleviate many environmental issues, but it will also enhance health by producing enhanced food products.

Our bodies constantly produce free radicals, either naturally or as a result of exposure to environmental stressors and other variables. These free radicals have been linked to a number of illnesses including cancer, atherosclerosis, arthritis, Parkinson's disease, Alzheimer's disease, aging and other age-related issues[13]. Complex defense systems for radical detoxification are present in mammalian cells. Antioxidants are substances that scavenge free radicals and stop the harm they can do. Despite these natural defenses, it seems more beneficial to use the additional antioxidants found in food, particularly from fruits, vegetables and whole grains[14]. The use of natural items for therapeutic and preventative medicine is becoming more popular because of their low adverse effects[15].

Many fruits' peels have drawn interest as a natural source of antioxidants and phytochemicals, which are abundant in substances that have the ability to scavenge free radicals. In tropical and subtropical areas, bananas (*Musa* spp., Musaceae family) are one of the primary fruit crops grown for their edible fruits[16]. Banana fruits are available all year round and 116 million tonnes of bananas were produced worldwide in 2019. 125-gram average fruit weight contain about 75% water, while the remaining 25% is dry substance[16]. When ripe, banana fruits range in size and color from yellow to purple to red. However, although wild bananas have numerous huge, hard seeds in their fruits, practically all cooking bananas have fruit without seeds[16,17]. Fruits can be consumed fresh, boiled, or crushed and used in baking[16,17].

In addition, green or unripe bananas are used to make starch and cook a variety of foods[16,17]. A percentage of ripe bananas are lost or damaged during transportation to markets and banana peels and plant components are used in animal feed. It was reported that banana peel may have antibacterial and antioxidant properties[18]. Because different fruit portions contain varied antioxidant and antibacterial components, the chemical makeup of banana peels determines their possible uses. Copper, zinc, sodium, potassium, calcium, phosphorus and iron are all present in unripe banana peels[19].

In addition, banana peels are a good source of potassium, essential amino acids, proteins, polyunsaturated fatty acids and dietary fiber[20]. Thus, in view of above beneficial effects, the aim of the present study was to investigate the phytoconstituents, total phenolic content (TPC), total flavonoid contents (TFC) and free radical scavenging activity of methanolic banana fruit peel extracts. Further, *in vitro* antioxidant potentials and anti-inflammatory activities were examined.

## Material and Methods

**Plant source:** The *Musa paradisiaca* (banana) was purchased from the local market of Jhansi District, Uttar Pradesh, India. The fruit was washed thoroughly with tap water and then with distilled water. The peels of the fruits were air dried in the dark at room temperature. The dried peel was ground into uniform powder using mixer and grinder. The powder was used for extraction preparation.

**Methanolic Extraction Procedure:** The powdered peels were percolated using 80% of methanol in the Soxhlet apparatus at 60-65°C. This extract was evaporated to dryness in a water bath at 40°C temperature and stored in air tight bottles.

**Phytochemical Analysis:** The presence or absence of secondary metabolites was carried out as described previously[21-23]. The alkaloids, carbohydrates, reducing sugars, flavonoids, glycosides, tannin, saponin, protein, triterpenoids and steroids were determined in the methanolic extracts of banana peel.

**Total Phenolic Content (TPC):** The Folin-Ciocalteu method[24] was used for the determination of TPC. 100 µl of different concentrations extracts was mixed with 500 µl of water and 100 µl of Folin-Ciocalteu reagent. The mixture was incubated for six minutes. Further, 500µl of distilled water and 1 ml of 7% sodium carbonate were added to the reaction mixture. The absorbance was taken after 90 minutes at 760 nm spectrophotometrically. The gallic acid equivalents (mg GAE/g) were used to calculate the TPC. Each test was carried out in triplicate.

**Total Flavonoid Content (TFC):** The aluminum chloride complex formation assay, which was performed by Piyanete et al[44], was used to determine the TFC of the methanolic extract of banana peels. The quercetin was utilized as a reference, the flavonoid content was calculated as mg QE/g. After mixing 100 µl of the quercetin dilution with 500 µl of distilled water and 100 µl of 5% sodium nitrate, the mixture was allowed to stand for 6 minutes. Subsequently, 200 µl of 1M NaOH solution was added after 150 µl of a 10% $AlCl_3$ solution had been added and then incubated for 5 minutes. This reaction mixture's absorbance was measured at 510 nm. All the procedures were performed in triplicate.

**Total antioxidant capacity:** The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was performed to evaluate the free radical scavenging activity of the banana peel extracts[23]. The peel extracts were exposed to 3 mL of 0.1 mM DPPH dissolved in methanol. After vigorously agitating the tubes, place them at ambient temperature for duration of 30 minutes in a lightless environment. A UV-visible spectrophotometer was used to measure the absorbance at a wavelength of 517 nm. In the experiment, distilled water served as the control while ascorbic acid was used as the reference. The percent free radical scavenging activity of extracts and the positive control ascorbic acid was determined with the following formula:

Free radical scavenging activity (%) = [(Ac−As)/ Ac×100]

where Ac is Absorbance of control at 517 nm and As is Absorbance of the sample.

**Nitric oxide radical scavenging assay:** Free radicals generated from sodium nitroprusside (SNP) were measured according to the earlier described method[27] with some modifications. Different concentrations of reaction mixture containing SNP (15 mM) in PBS (pH 7.3) with and without sample, were incubated at 25°C for 210 mins. After adding the Griess reagent, the reaction mixture was incubated for 10 minutes at room temperature. The standard used was ascorbic acid. The absorbance was measured at 560 nm using a UV-Vis microplate reader.

**Superoxide anion scavenging assay:** Based on a previously described method with minor modifications[25], the reduction of NBT determined the total antioxidant capacity of methanolic extract. Phosphate buffer (20 mM, pH 7.4), PMS

(60µM), NBT (156µM) and different conc. of extract were all included in the 1 mL reaction mixture. After incubation for 5 min at 25°C temperature, the absorbance was taken at 560 nm against an appropriate blank solution. BHT was used as reference control.

**Hydroxyl radical scavenging activity:** Hydroxyl radicals scavenging activity was assessed according to Keshari et al[32]. 450 µl of sodium phosphate buffer (200 mM, pH 7.0), 150 µl $H_2O_2$ (10 mM), 150 µl $FeSO_4$-EDTA (10 mM), 525 µl of distilled water and 75 µl of plant sample solution (1000–31.25 µg/ml in methanol) were added in the test tubes. The tube was then kept for four hours at 37°C. To stop the reaction, 750 µl of TBA (1% in 50 mM NaOH solution) and 750 µl of trichloroacetic acid (2.8%), were added. After ten minutes in the boiling water bath, the mixture was cooled with tap water. At 520 nm, the absorbance was then measured. Methanol served as the blank and ascorbic acid as the standard. The formula for total antioxidant activity was used to calculate the hydroxyl radical scavenging activity.

**Anti-inflammatory activity:** The anti-inflammatory potential of peel extract was studied as described by Padmanabhan et al[43] using the protein denaturation method. The potent nonsteroidal anti-inflammatory drug (diclofenac sodium) was used as the standard. The reaction mixture, consisting of 2 ml of sample, 2.8 ml of phosphate-buffer saline (PBS, pH 6.4) and 2 ml of egg albumin (fresh chicken egg (1 mM)), was incubated at 27°C for 15 minutes. The mixture was heated in a water bath for 10 minutes at 70°C for denaturation. After cooling the sample, absorbance was measured at 660 nm. Each test was performed three times. Percent inhibition of protein denaturation was determined using the following formula:

$$\% \text{ inhibition} = ((Ac-As)/Ac) \times 100$$

where As is Absorbance of sample and Ac is Absorbance of control.

## Results

**Screening of Phytoconstituents:** Phytochemical screening of the methanolic extracts of banana peels shows the presence of different secondary metabolites as determined by biochemical tests (Table 1). The presence or absence of these secondary metabolites depends on the qualitative detection methods used. Secondary metabolites are essential for humans and animals alike.

**Determination of total phenols and flavonoids content:** The total phenol content was 137.6± 39.59 mgGAE/g of extract and the total flavonoid content was 256.6 ± 29.30 mgQE/g of extract. These results provide a comprehensive profile of the antioxidant activity of methanol extract of *Musa paradisiaca* peels with respect to their phenols and flavonoids content (Table 2).

**Antioxidant activity:** The results indicated that the peel extracts have antioxidant potential as compared with ascorbic acid. The antioxidant property of peel extract was increased when concentration of extract was increased and it was dose dependent (Figure 1). The antioxidant property observed may be due to the high phenolic and flavonoid content.

**Nitric oxide, superoxide and hydroxyl radical scavenging activity:** The nitric oxide, superoxide and hydroxyl radical scavenging activity of the methanolic extract of *M. paradisiaca* peels were dose dependent (Figure 3). The free radical property of peel extract was increased when concentration of extract was increased.

***In vitro* Anti-inflammatory Activity:** The anti-inflammatory potential of banana peel extract was evaluated using a protein denaturation assay and compared with the reference drug, Diclofenac sodium. The protein denaturation was observed with banana peel extracts (Figure 2). Conventional drugs showed the higher anti-inflammatory activity (93.1±6.2%), as compared to peel extracts (64.2± 6.77%) at 100 µg/ml conc.

## Discussion

The peel of bananas is a useful resource because it is high in dietary fiber and bioactive substances[30-32]. Since several of these natural ingredients have strong antibacterial, antimicrobial and antioxidant qualities, banana peels are a good option for pharmaceutical and nutraceutical uses[33-35]. A variety of ailments, including as burns, anemia, gastrointestinal issues, ulcers, inflammation, diabetes, respiratory conditions and irregular uterine bleeding, have historically been treated with the peel[36]. Remarkably, the peel has a significant concentration of phenolic compounds and dietary fiber, which increases its potential health advantages, such as its antibacterial, antioxidant, prebiotic and antibiotic qualities[37-39]. The amount of phenolic content in banana peel is remarkably 1.5–3 times higher than that in the pulp[40]. As a result, it has enormous potential for use in pharmaceuticals, dietary supplements and food and feed additives in the future.

Biorefinery techniques can increase resource efficiency, reduce waste and support sustainable practices by extracting and using several useful components from biomass[41-43]. The most researched types of phytochemicals found in fruits and vegetables are secondary metabolites. They belong to a family of organic compounds that are extensively found in the kingdom of plants. They fall into diverse groups and are linked to varied characteristics making impacts on human health[44].

Several studies have concentrated on the molecules found in banana pulp. Gallic acid, ferulic, sinapic, salicylic, carotenoids, flavonoids, biogenic amines like dopamine, phytosterols and a considerable amount of ascorbic acid are among the phenolic components shown to be present[45-49].

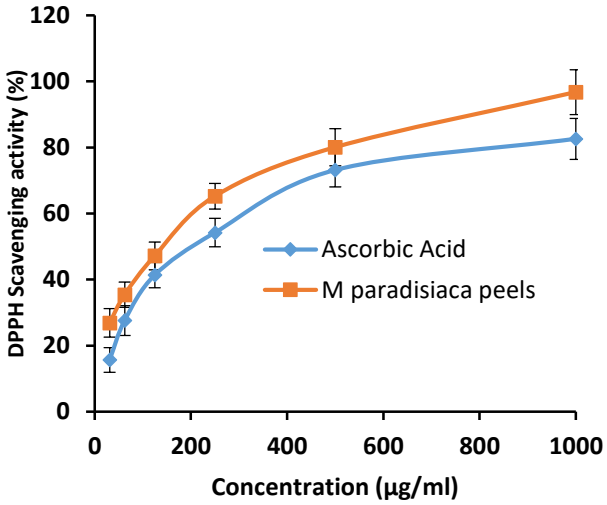


**Figure 1: The Antioxidant property of methanolic extract of *M. paradisiaca* peels**

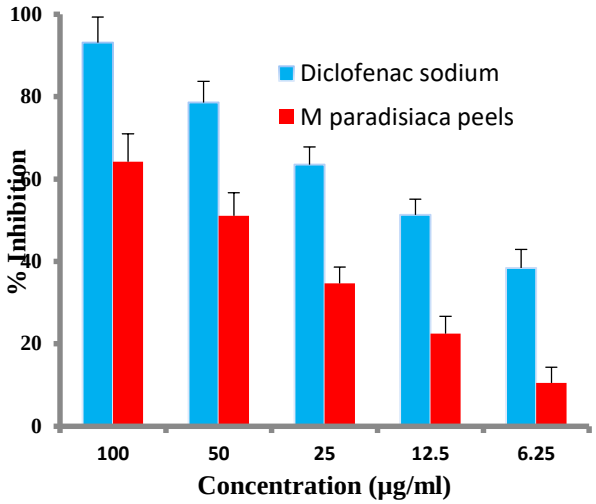


**Figure 2: Anti-inflammatory activity of methanolic extract of *M. paradisiaca* peels vs Diclofenac sodium**

**Table 1**
**Qualitative phytochemical analysis of the methanolic extracts of *Musa paradisiaca* peels**

| Phytochemical Test | Methanolic Extract | Phytochemical Test | Methanolic Extract |
|---|---|---|---|
| **Alkaloids** | | **Glycosides** | |
| Mayer's | + ve | Borntrager's | - ve |
| Wagner's | + ve | Legal's | + ve |
| Hager's | + ve | Keller-killiani | - ve |
| **Carbohydrates** | | **Protein and A.A.** | |
| Molisch | + ve | Ninhydrin | + ve |
| Barfoed's | - ve | Biuret | - ve |
| **Reducing Sugars** | | **Tannin and phenolic** | |
| Fehling's | + ve | Ferric Chloride | + ve |
| Benedict's | + ve | Lead Acetate | + ve |
| | | Dilute iodine solution | + ve |
| **Flavonoids** | | **Triterpenoids and Steroids** | |
| Alkaline Reagent | + ve | Salkowski's | + ve |
| Lead Acetate | + ve | | |
| **Saponin** | | **Hydrolysable tannin** | |
| Froth | + ve | | + ve |

(+) indicates presence while, (–) indicates the absence of the components

**Table 2**
**Total Flavonoid and Phenolic Content of methanolic extract of *Musa paradisiaca* peels**

| Total Flavonoid Content (mgQE/g) | | Total Phenolic Content (mgGAE/g) | |
|---|---|---|---|
| **Conc. (µg/ml)** | ***Musa paradisiaca* peels** | **Conc. (µg/ml)** | ***Musa paradisiaca* peels** |
| 1000 | 241 | 150 | 132 |
| 500 | 281 | 120 | 127 |
| 250 | 221 | 90 | 206 |
| 125 | 292 | 60 | 118 |
| 62.5 | 248 | 30 | 105 |
| **Mean ± SD** | **256.6 ± 29.30** | **Mean ± SD** | **137.6± 39.59** |

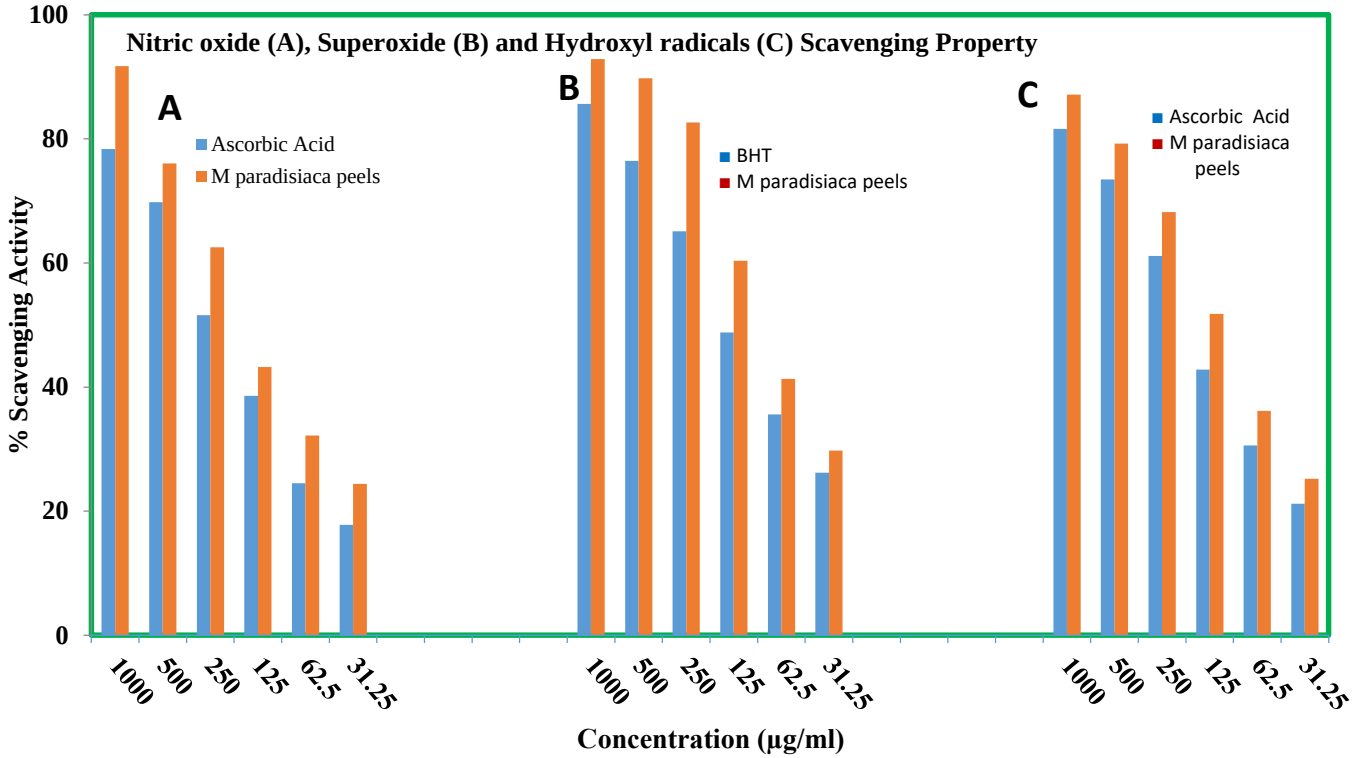


**Figure 3: (A) The Nitric oxide, (B) superoxide and (C) Hydroxyl radicals scavenging activity of methanolic extract of *M. paradisiaca* peels**

Since these components are more common in bananas, they have a higher potential for antioxidants than other berries, herbs and vegetables[50-52]. This fruit peel has long been used in traditional medicine to treat inflammation, burns and anemia[53].

Plants contain natural substances called phytochemicals that have a variety of biological effects on human health. Research has demonstrated that the phenolic chemicals, carotenoids, flavonoids, tannins, terpenes, alkaloids, glycosides and phytosterols found in banana peels provide substantial nutritional advantages that improve human health[54]. The methanolic extract of banana peels contained a number of secondary metabolites according to our findings (Table 1).

Within living cells, free radicals are constantly being produced for regular metabolic functions. This free radical oxidizes essential biological components, primarily proteins, lipids and DNA, which can lead to a variety of diseases. *In vitro* and *in vivo* antioxidants neutralize the free radicals. Superoxide dismutase, glutathione peroxidase and catalase are examples of antioxidant enzymes that are constantly at work within cells to prevent the production of free radicals[55]. A useful indicator of bioactivity is the presence of phytochemicals in a plant sample, particularly phenolics, flavonoids, alkaloids and glycosides[56].

The most significant bioactive natural byproducts of secondary metabolites are flavonoids and phenolic acids which have anti-aging and antioxidant properties. They can scavenge free radicals and lower the risk of cancer[57]. Phenylalanine ammonia lyase (PAL) is primarily responsible for the synthesis of phenols in plants from phenylalanine. In essence, phenolics are a variety of natural antioxidants that are utilized as nutraceuticals. They are present in apples, green tea and red wine and are known to have a significant anti-cancer effect. They are also believed to protect heart disease to a considerable extent and occasionally act as anti-inflammatory agents. The other examples of phenolic

compounds are flavones, rutin, naringin, hesperidin and chlorogenic acid[58].

Flavonoids have been shown to be more potent antioxidants *in vitro* than tocopherols and ascorbates and they have the perfect structure for scavenging free radicals. They are effective reducing agents with the ability to delocalize unpaired electrons and stabilize radicals produced from polyphenols. Flavonoids are potent metal chelators and free radical scavengers. They also have anti-inflammatory, anti-ulcer, antitumor and anticancer properties. They have an effect on the signaling pathways in cells that regulate the cell cycle, differentiation and apoptosis[59].

The body naturally produces endogenous antioxidants, which aid in scavenging dangerous free radicals and shielding cells from oxidative stress. By decreasing free radicals, encouraging the development of healthy cells, shielding cells from premature and aberrant aging, combating age-related molecular degradation and strengthening the immune system, which prevents or controls chronic diseases, exogenous antioxidants aid in reversing this imbalance[60]. The body's defenses against a variety of diseases are improved by bioactive molecules which through a variety of methods and characteristics, operate as antioxidants, anti-inflammatory, anticancer and antidiabetic agents[54].

The antioxidant activity measured in this study is similar to the reference control and dose dependent (Figure 1). The overall amount of flavonoids and phenols is associated to the antioxidant activity. Thus, in the methanolic extract, we assessed the TPC and TFC. While the flavonoid content was 256.6 ± 29.30 mg QE/g, we found that the total phenolic content was 137.6 ± 39.59 mg GAE/g (Table 2). Non-steroidal anti-inflammatory drugs, or NSAIDs, are commonly used to reduce inflammation; nevertheless, prolonged usage can have serious side effects such as nephritis, hepatitis and gastrointestinal issues[61]. Although opioids are a potent alternative to NSAIDs, they exacerbate mental health conditions such as addiction, depression and withdrawal symptoms[62]. Thus, we need a potent medication that effectively reduces pain and inflammation without having any adverse side effects.

It has been demonstrated that *M. poradisiaca's* apigenin, vitamins A, C, E and B-complex vitamins, as well as trace minerals like magnesium, calcium, zinc and selenium, decreases inflammatory responses[63]. According to Bindu et al[10], medications used to treat inflammation have been associated with serious side effects like stomach ulcers, rashes, diarrhea and liver and kidney problems. For this reason, it is critical to look into the anti-inflammatory properties of natural products like medicinal plants. They found that both high and low dosages of *M. poradisiaca* leaf extract significantly reduced the rats' paw edema caused by carrageenan and that the extract's flavonoids, phytosterols and tannins may be responsible for anti-inflammatory properties.

Several methods were used to evaluate *Musa* species' anti-inflammatory properties such as inhibition of heat-induced hemolysis, hypotonicity-induced hemolysis, membrane stabilization, albumin denaturation and protease inhibition[65]. The above mentioned approaches result in inhibition of inflammation. For example, it is known that denaturation of proteins results in inflammation, whereby proteins lose their secondary and tertiary structures when exposed to heat, acid, base, or organic salt. Therefore, the abundance of bioactive substances in *M. poradisiaca* peels, along with their potent antioxidant and anti-inflammatory properties, makes them excellent choices for nutraceutical uses. These adaptable peels may find use in a range of functional meals, nutritional supplements, or natural health products that promote well-being and prevent inflammatory and oxidative stress-related illnesses.

## Conclusion

According to the current study, banana peels naturally contain a variety of phytochemicals that are good to health including flavonoids and total phenolics. Antioxidant potentials, anti-inflammatory properties and free radical scavenging activity were also noted and compared to reference controls. Therefore, it is strongly advised that fruit peels be used in food products in the right amounts. Their phytochemicals lower the risk of degenerative diseases including cancer and cardiovascular disease. The nutritional value and biological activity of the peel will be increased by more genetic engineering-based research.

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