Evaluation of Antioxidant Activity of *Musa Balbisiana's* Peel Extract

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Abstract

Antioxidants found in food, such as adaptogens, polyphenols, vitamins A, C, D, E, and anthocyanins, and slow down the aging process by reducing the reactive oxygen species (ROS) production in cells. Research is being done on the antioxidative qualities of many plants and plant extracts. Naturally occurring antioxidants, polyphenols are essential in preventing oxidative stress brought on by the body's free radicals. This study examined the peel extract of Musa balbisiana for its total phenolic, total flavonoid, and antioxidant properties. Peels from bananas constitute a substantial agro-industrial waste. Polyphenols from this waste could be extracted and used to produce future anti-aging formulations. Bananas include a variety of bioactive compounds, including antioxidants, which scavenge free radicals and bolster the body's defenses. Phenols, carotenoids, and vitamin C are the most prevalent antioxidants found in bananas. Banana contains pulp of high levels antioxidants, such as carotenes, vitamin C, dopamine, and norepinephrine. In this research, a study was conducted using water, ethanol, and a 50% hydroalcoholic solvent to extract polyphenols from Musa balbisiana peels. Total flavonoid content (TFC), total polyphenol content (TPC) and 1, 1-diphenyl-2-trinitrophenylhydrazine (DPPH) free radical scavenging activity. The results of this study show that 50% hydroalcoholic solvent extraction produced more polyphenols than ethanol or aqueous solvent extraction. Total flavonoid and total phenolic content of each extract were determined using aluminum chloride and the Folin-Ciocalteu method. Antioxidant activity of the extracts was measured by DPPH assay. *Musa balbisiana* peel extract has a high level of biocompatible phenolics, which makes it a potential antiaging ingredient.

Keyword: Musa Balbisiana, Antioxidant Activity, Total Polyphenolic Content (TPC) Total Flavonoid Content (TFC), DPPH Radical Scavenging Assays.

1. INTRODUCTION

Many diseases, such as atherosclerosis, diabetes, arthritis, age-related macular degeneration, some cancers, inflammation, genotoxicity, and Alzheimer's disease, are now associated with reactive oxygen species (ROS) and reactive nitrogen species (RON). These include hydroxyl radicals, superoxide ions, nitric oxide radicals, singlet oxygen, and hydrogen peroxide. Although the mechanism is unclear, these diseases may be caused by the interaction of RNS species and ROS with biomolecules such as lipids, proteins, and DNA (Shukla et al., 2009; Septembre-Malaterre et al., 2016). In current years, there has been a rise in interest in natural antioxidants, particularly those found in fruits and vegetables. According to epidemiological research, a diet richer in natural antioxidants (Vitamin E, ascorbic acids, phenolics and carotenoids) can help prevent heart disease, cataracts, cancer, and illnesses associated with aging (Steffen et al., 2003). According to WHO, a sizable portion of the populace in poor nations gets their primary medical care from natural resources. Numerous studies conducted recently have demonstrated an inverse link between the manifestation of disease state and the consumption of polyphenols or foods rich in polyphenols (Sellick et al., 2011; Kumari et al., 2016a; and 2016b). Furthermore, it has been demonstrated that phenolic chemicals found in food, such as quercetin, apigenin, and chlorogenic acid, lower the risk of acute and chronic illnesses (Bhandarkar et al., 2019; Agunloye et al., 2019). The anti-inflammatory and antioxidant qualities of polyphenols have been linked to these therapeutic responses (Agunloye et al., 2019).

Indigenous to India and other areas of asia, banana or Musa balbisiana Colla. (Family: Musaceae), has been long used in traditional medicine by the tribal people of northeast India (Kalita et al., 2016). Several banana species are traditionally used to treat heart conditions, inflammation, diabetes, hypertension, dysentery, and diarrhea, according to literature evaluations. Bananas are rich in bioactive substances such as flavonoids, phenols, carotenoids, amines, vitamin C and vitamin E. All of these substances have antioxidant effects and are beneficial to human health. (Pereira and Maraschin, 2015; Singh et al., 2016). The main bioactive substances with antioxidant qualities and health benefits found in banana fruit are phenols. Catechin, gallic acid, tannins, anthocyanins and epicatechin, are among the many phenolics found in bananas. Due to its high phenolic content,

banana rhizome is utilized both medicinally and as food (Kandasamy and Aradhya, 2014). Numerous phenolics, including sinapic, gallic, ferulic, salicylic, vanillic, p-hydroxybenzoic, gentisic, p-coumaric acids, and syringic, have been found to be important components of bananas (Russell et al., 2009). Cyanidin Quercetin, myricetin, and kaempferol, are among the flavonoids found in bananas. These compounds are beneficial to health primarily because they scavenge free radicals, reactive oxygen species, and nervous system toxins (Kevers et al., 2007). (Vu et al., 2018) have conducted a review on the phenolic chemicals compound found in peels of banana and their possible health benefits. They have recommended that the food and pharmaceutical industries exploit this banana fruit processing business has valuable by-product. In enterprises that produce goods based on bananas, around 40% of the trash weight of fresh bananas is produced (Nagarajaiah and Prakash, 2011). It is the root source of significant environmental issues. It is necessary to use and take advantage as a cheap of banana peels, sustainable, and natural source of essential ingredients.

2. MATERIAL AND METHODS

Reagents and Chemical

All reagent and solvent were of analytical grade. Sigma Aldrich supplied all of the chemicals and reagents, including ascorbic acid, sodium carbonate, aluminum chloride, gallic acid, and DPPH (2,2-diphenyl-1-picrylhydrazyl).

Collection and Authentication of Plant Material

Peels from Musa balbisiana were collected in May month from the Bhopal market. The peels were authenticated by Head, botanist and Professor, Janta PG College, APS University, Rewa M.P. and provided a herbarium specimen no. for future reference.

Extraction

After rinsing the *Musa balbisiana's* peels in tap and distilled water to get rid of any remaining dirt, the peels were chopped into little pieces. Then dried for 48 hours at 50 ^{o}C in a hot air oven. The peels were processed and dried into a fine powder with a moisture level of less than 10%. Next, the powder peel was placed into plastic zip bags, making ensuring the bags were sealed tightly (Chaudhry et al., 2022).

With a few modifications, the procedure described in the literature (Adeel et al., 2022), was used to make the *Musa balbisiana's* peel extracts. 250g of peel powder was soaked in 1L of three different solvent (Ethanol, 50% Hydroalcoholic solvent (Ethanol: water in the ratio 50:50), and water) separately, and the samples were then left to overnight at room temperature. The extract was filtered through Whatman No. 1 filter paper and centrifuged for 10 min at 4 °C at 6000 rpm after being agitated on a hot plate for three hours at 200–250 rpm using a magnetic stirrer at 35 °C. A rotary evaporator was used to filter, separate, and then concentrate the supernatant. The extracts were refrigerated after being dried to 40 °C.

Determination of Total Phenolic Contents (TPC)

The usage of the Folin–Ciocalteu approach, the overall phenolic content material of Musa balbisiana's peel extract with all three solvents had been ascertained for my part to put it in brief. Folin-Ciocalteu reagent is 5 mL of 10% (w/v) become combined with 1 mL of solution one at a time (one hundred–500 µg/mL). After 5 min, the mixture was mixed with 2.0 mL of 75% Na2CO3 and incubated for 10 min at 50 °C with occasional stirring. The sample was then cooled and the absorbance was measured at 765 nm using a UV spectrophotometer. (Shimazu, UV-1800) to quantify the white sample without extractables. (Lee et al., 2015). The results have been given in milligrams in keeping with gram (mg GAE/g) of gallic acid equivalents for the dry extract.

Determination of Flavonoid Contents

The flavonoid content of each of the three solvent-extracted Musa balbisiana peel extracts was measured separately. Mix 5.6 mL of distilled water, 0.2 mL of 10% (w/v) AlCl3 in methanol, and 0.2 mL of potassium acetate (1 M) with 1 mL of the extraction fluid (25-200 µg/mL). Record the absorbance at 415 nm after half an hour of incubation at room temperature and compare with the blank control. The findings were displayed as milligrams/gram of dried extract's quercetin equivalents (mg QE/g) (Aryal et al., 2019).

Antioxidant activity

Preparation of stock solution:

Dissolve 100 mg to prepare a stock solution of 1.0 mg/ml Musa balbisiana's peel extract of each solvent individually in 100 ml of ethanol. Then, using a dilution procedure, five different concentrations of stock solutions were created, with each concentration being 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml.

Preparation of standard solution

To make a 1.0 mg/ml standard solution, 100 ml of ethanol and 100 mg of ascorbic acid were mixed. Next, five concentrations of the standard solution were created by diluting it: 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml.

DPPH Free Radical Scavenging Activity Assay

In a cuvette, starting from an initial concentration of 20 µg/ml, mix 1 ml of 0.1 mg/ml DPPH solution and 2 ml of 20 µg/ml stock solution. After that, the test and drug were placed in the oven for 30 min at 24 ° C in a place protected from light. After 30 min of incubation, absorbance at 517 nm was measured using a UV

spectrophotometer. (Shimazu, UV-1800). For every stock solution of three extract and standard solution concentration, the aforementioned procedures were carried out three times. By comparing the sample with ascorbic acid, the standard antioxidant, the inhibition of percentage of the sample was ascertained. The IC_{50} value indicated concentration of banana peel extract required to achieve 50% optical density. Moreover, the UV-Vis spectrophotometer was calibrated using ethanol as a blank (Nadirah et al., 2018). Following this formula, the DPPH radical scavenging activity was determined.

Radical scavenging activity= (Abs Control−Abs Sample)/Abs Control×100%

Where

Abs Control = Absorbance of Control

Abs Sample = Absorbance of Sample

3. RESULT AND DISCUSSION

Total Phenolic and Flavonoid Content

As a strong antioxidant, polyphenols are thought to have positive impacts on human health by warding off degenerative illnesses. Table 1 and Figures Nos. 1 and 2 show the total phenolic and flavonoid content of Musa balbisiana's peel extracts as a function of solvent. It is evident that the overall flavonoid and phenolic content was impacted by the solvent system, which included ethanol, water, and 50% ethanolic extract. The type of solvent used has an impact on TPC and TFC in various extracts. The overall phenolic and flavonoid concentration varied when the solvent polarity was altered, indicating the varying solubility of phenols and flavonoids in the various solvent environments.

Table 1: Total Phenolics and Flavonoids content in Banana Peel Extract

Musa balbisiana's Peel	Aqueous Extract	Ethanol extract	50% ethanolic extract
Total Phenol Content (TPC) (mg GAE/g dry extract wt)	7.11 ± 0.24	12.14 ± 0.38	17.21 ± 0.16
Total Flavonoid Content (TFC) (mg QE/g dry extract wt)	3.52 ± 0.43	6.5± 0.64	8.28 ± 0.12

The values are means ± S D of three replicates.

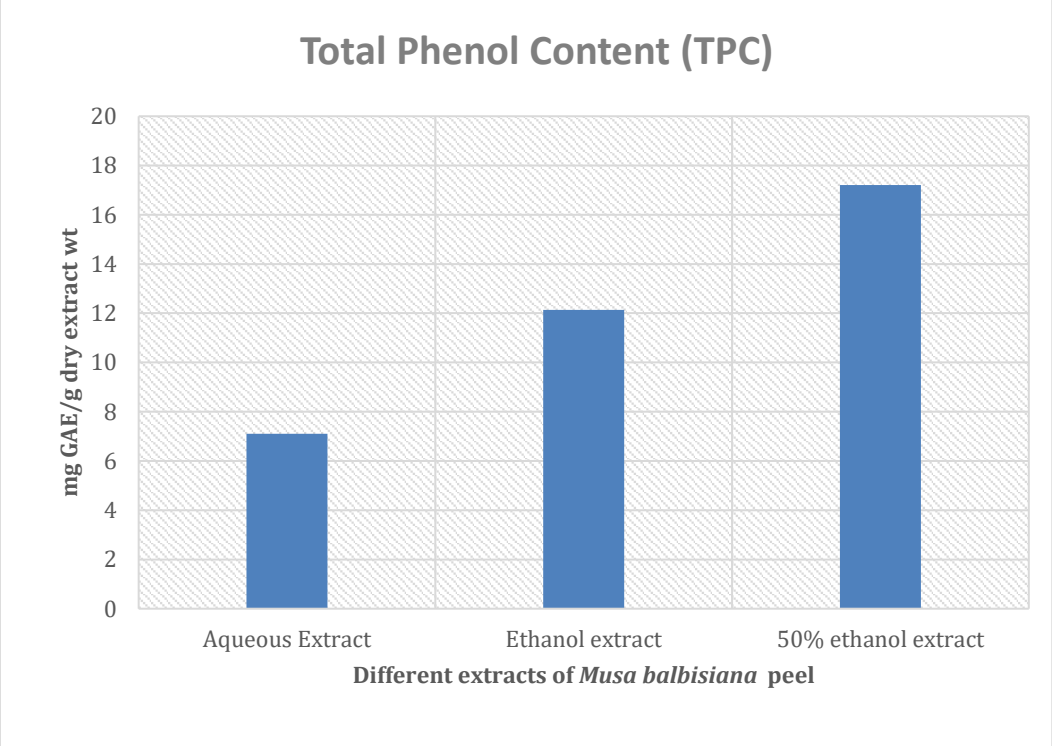

Figure 1. Different extracts of Total Phenolic content of Musa balbisiana Peel

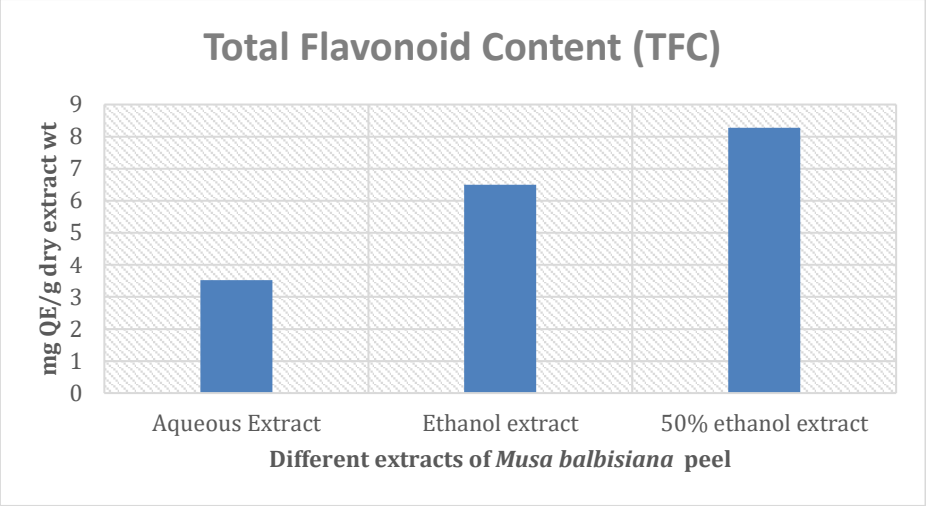

Figure 2. Different Extracts of Total Flavonoid Content of Musa Balbisiana Peel

According to reports, the 50% hydroethanolic extract of guava leaves had increase in concentration of phenolic compounds than the water extract (Qian and Nihorimbere, 2004). According to (Stanojević et al. 2009), there was a decrease in phenolic component concentration of Hieracium pilosella in the following order: 50% hydroethanolic extract > 80% hydromethanolic extract > water extract. An additional investigation found that 40% hydroethanolic extract had increase the concentration of phenolic compounds (Ito et al. 2012). Our findings are consistent with a number of previous researches that looked at the connection between Polyphenolic content and 50% hydroalcoholic solvent.

Antioxidant Activity

Using the DPPH radical scavenging assay of antioxidant activity of, Musa balbisiana peel extract with various solvents were examined and shown in figure no.3. Based on the decrease in absorbance (A) at 517 nm, the activities were calculated to scavenge the stable DPPH free radical. This study used the DPPH radical scavenging assay, which assesses an antioxidant extract's capacity for scavenging by measuring its ability to transfer electrons and act as a hydrogen donor. Antioxidant chemicals and DPPH free radical react to cause DPPH to change color from deep purple (1,1-diphenyl-2-picrylhydrazyl) to pale yellow (1,1-diphenyl-picrylhydrazine), reducing absorbance (Abdullah et al., 2012).

By using the linear equation of y = 0.3539x - 2.024 for Aqueous extract, y = 0.5852x - 3.867for ethanol extract, y = 0.7108x + 9.508 for 50% hydroalcoholic extract and y = 0.5454x + 42.605 for ascorbic acid, IC_{50} value is calculated.

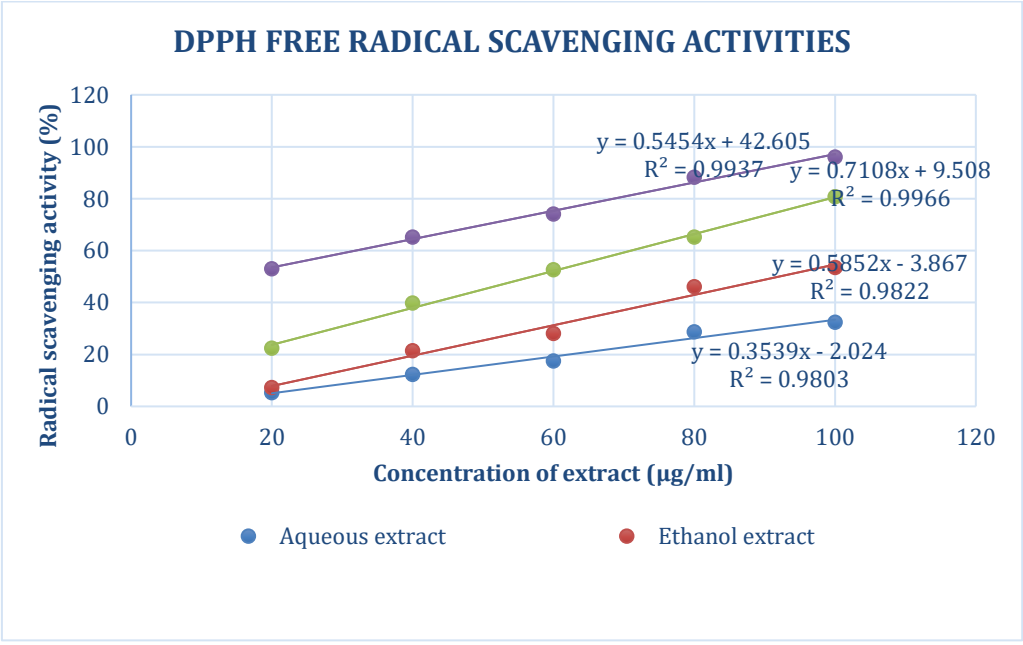

Figure 3. % of Inhibition of Ascorbic Acid, Water, Ethanol And 50% Ethanol Extract of Musa Balbisiana Peel

Table 2. DPPH scavenging activity and IC_{50} value of Ascorbic acid, water, ethanol and 50% ethanol extract of Musa balbisiana peel

Concentration of extract (µg/ml)	DPPH Scavenging Activity(%)			
	Aqueous extract	Ethanol extract	50% ethanol extract	Ascorbic acid
20	5.26 ± 0.31	7.21 ± 0.22	22.38 ± 0.17	53.02± 0.16
40	12.24 ± 0.27	21.43 ± 0.16	39.81 ± 0.31	65.23 ± 0.23
60	17.42 ± 0.22	28.11 ± 0.27	52.62 ± 0.77	74.11± 0.57
80	28.72 ± 0.11	46.04 ± 0.71	65.21 ± 0.62	88.24± 0.61
100	32.41 ± 0.21	53.42 ± 0.12	80.76 ± 0.53	96.06± 0.23
IC_{50} (µg/ml)	147.00	92.04	56.96	13.55

The IC_{50} value and percentage inhibition of DPPH radical for each extract of Musa balbisiana peel were displayed in table 2. The lower the IC50 value higher the antioxidant activity of the sample. If the IC50 value of the antioxidant sample is less than 50 µg/ml, the antioxidant is very strong; for example, a sample with an IC50 value of 101 to 150 µg/ml; (Fidrianny et al., 2014). Our findings are consistent with a number of researches that looked at the connection between antioxidant capability and phenolic chemicals. A prior investigation discovered that the phenolic component composition affected antioxidant ability (Kosińska et al. 2012). According to a different study, antioxidant capacity and phenolic component concentration are positively correlated. (Kim et al., 2008).

4. CONCLUSION

A rich source of phenolic compounds of Musa balbisiana peels are significant antioxidant activity. In comparison to the traditional maceration method, the current study finds that improved extraction of phenolic and flavonoid may be accomplished by magnetic stirrer at 200–250 rpm at 3 hours for 35 °C on a hot plate using a very short amount of time. Additionally, the current study finds that solvents at lower concentrations exhibit greater extraction activity and that using an absolute solvent might not guarantee a fair extraction. An extract produced with 50% concentration of ethanol is superior to one produced with ethanol and other concentrations facilitates better extraction than water. Because there are more polyphenolic components in the 50% ethanolic extract of banana peel than in the aqueous and ethanol extract, the 50% ethanolic extract has greater antioxidant activity. Future research should be designed, according to the authors, to estimate the effect of other cutting-edge technique on the extraction of bioactive components from powdered Musa balbisiana.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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