INTRODUCTION

In our body various metabolic processes takes place like breakdown of fats and other food stuffs to release energy. Most of these metabolic processes utilize oxygen as a fuel and generates free radicals as by-products. These free radicals can be both useful and harmful to the body. They can destroy the various normal cells of our body by a phenomenon known as oxidation. The anti-oxidants function as reducing agents and are thus helpful in preventing this damage\(^1\). Free radicals are formed in the body by various mechanisms which may be endogenous or environmental. Various electromagnetic radiations can also generate free radicals. For example gamma rays can split water in the body to generate hydroxyl radical i.e. OH\(^-\). Though there are various ways by which antioxidants can exert their action.

But two mechanism of action are very important. First is the chain- breaking mechanism, in which the primary antioxidant donates an electron to the free radical and neutralizes it. In the second mechanism there is removal of ROS/reactive nitrogen species initiators by quenching chain-initiating catalyst. In both these mechanisms antioxidants neutralizes the free radicals and themselves become oxidized\(^4\).\(^5\).

Anti-oxidants usually function in various ways: both endogenous and exogenous anti-oxidants prevent the damage to cells by the free radicals; protect from cancer and heart diseases; protect from exercise induced damage and helps in the recovery process; various neurological diseases like Alzheimer’s disease, Parkinson’s disease etc. can be prevented; and prevent various other oxidative stress
induced diseases like Rheumatoid arthritis, Nephropathy and Pulmonary diseases\textsuperscript{5}. Various dietary components and nutrients act as natural antioxidants and are very useful to the body. They supplement the action of various endogenous antioxidants to combat the deleterious effects of free radicals. All the dietary nutrients are quite specific in its structure and function. Lack of these various antioxidants in the diet on long term basis can lead to various progressive and degenerative diseases in the body. It includes three components mainly i.e. vitamin C, vitamin E and carotenoids\textsuperscript{7,8}. Knowledge of phytochemicals of botanicals are important not for the discovery of therapeutic agents but also for disclosing new sources of economic materials. As commercialization of the natural medicine has occurred, it becomes an important issue to assurance the safety, quality and efficacy of medicinal plants\textsuperscript{9}.

**Taxonomic Classification of *Musa paradisiaca***

**Kingdom:** Plantae  
**Sub Kingdom:** Tracheobionta  
**Super Division:** Spermatophyta  
**Division:** Magnoliophyta  
**Class:** Liliopsida  
**Sub Class:** Zingiberales  
**Order:** Zingiberales  
**Family:** Musaceae  
**Genus:** Musa  
**Species:** paradisiaca

**Botanical Name:** *Musa paradisiaca* Linn.

*Musa paradisiaca* is one such plant family which has wide importance in terms of its fruits, flowers, leaves, stem etc.\textsuperscript{10}. Banana, an antique fruit crop known as “Apple of Paradise” plays an important and interesting roles in the history of human civilizations (Figure 1). All the parts of the plants are useful, even the hazardous waste pseudostem can also be used to make yarn, fabric, apparel as well as fertilizers, biochemicals, fish feed, paper, handicrafts, candy, pickles, etc.\textsuperscript{11,12}

![Image](image.png)

**Figure 1:** Bracts of *Musa paradisiaca*

**MATERIALS AND METHODS**

**Extraction method of bracts of *Musa paradisiaca***

The bracts of *Musa paradisiaca* were freshly collected from Indian vegetable market, Chennai, Tamil Nadu, India. The fresh bracts were well cleaned in sterile water and made into fine medium pieces, shade dried for few days and the bracts were soaked in ethanol for 72 hours. The maroon coloured supernatant was filtered and stored in a clean glass container, and later the supernatant was condensed in a hot plate at 40°C-45°C, which yields maroonish yellow coloured extract\textsuperscript{13,14}.

**Quantitative estimation of phenols and flavonoids**

**Determination of total phenols**

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds\textsuperscript{15} with slight modifications. One hundred µL of ethanol bract extract of *Musa paradisiaca* (1 mg/mL) was mixed with 900 µL of methanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of sodium carbonate (20% w/v) was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured using UV-Vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (GAE/mg of extract), which is a common reference compound.

**Determination of total flavonoids**

The total flavonoid content of ethanol bract extract of *Musa paradisiaca* was determined using aluminium chloride colorimetric method with slight modification as described\textsuperscript{16}. 500 µL of ethanol bract extract of *Musa paradisiaca* (1 mg/mL) was mixed with 500 µL of methanol, 0.5 mL of 5% (w/v) sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.5 mL of 10% (w/v) aluminium chloride solution was added and incubated for further 5 min at room temperature followed by addition of 100 µL of 1 M sodium hydroxide solution. The absorbance was measured at 510 nm using UV-Vis spectrophotometer. The total flavonoid content was expressed in terms of quercetin equivalent (QE/mg of extract), which is a common reference compound.

**In vitro antioxidant activities of ethanol bract extract of *Musa paradisiaca***

**DPPH’ radical scavenging activity**

The radical scavenging activity of ethanol bract extract of *Musa paradisiaca* was carried out by the reduction DPPH’ free radical method\textsuperscript{17}. One mL of ethanol bract extract of *Musa paradisiaca* with various concentrations (20-120 µg/mL) was mixed with 1 mL of 0.1 mM DPPH solution in
methanol. The mixture was then allowed to stand for 30 min incubation in dark. One mL of methanol mixed with 1 mL of DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

\[
\% \text{ of DPPH}^{-} \text{ radical inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

Superoxide (O$_2^{-}$) radical scavenging activity

Superoxide (O$_2^{-}$) radical scavenging activity was carried out by the method\textsuperscript{13} and the reaction mixture contains different concentrations (20-120 µg/mL) of ethanol Bract extract of *Musa paradisiaca* with 50 mM of phosphate buffer (pH-7.4), 200 µL of 1.5 mM of riboflavin, 200 µL 12 mM of EDTA and 100 µL 50 mM of NBT, added in that sequence. The reaction was started by illuminating the reaction mixture for 15 min in UV lamp. After illumination, the absorbance was measured at 590 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

\[
\% \text{ of Superoxide (O}_2^{-}\text{) radical inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

Nitric Oxide (NO) radical scavenging activity

Nitric Oxide (NO) radical scavenging activity was carried out by the method\textsuperscript{19} and the reaction mixture contains different concentrations (20-120 µg/mL) of ethanol Bract extract of *Musa paradisiaca* and 1 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH-7.4) was mixed with the ethanol Bract extract. The mixture was incubated at 25°C for 150 minutes. From the incubated mixture about 0.5 mL was taken out and 0.5 mL of Griess reagent (1% sulphanilamide (w/v), 2% Orthophosphoric acid (v/v) and 0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride) was added and incubated at room temperature for 30 minutes and the absorbance was measured at 546 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

\[
\% \text{ of Nitric oxide (NO) radical inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

ABTS$^{-}\,$ (2,2-azinbis (3-ethylbenzo thiazoline-6-sulfonic acid) radical cation scavenging activity

The ethanol Bract extract of *Musa paradisiaca* from the stock solution was taken in various concentrations and this assay was performed according to the method\textsuperscript{20}. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. Fresh ABTS solution was prepared for each experiment. The ethanol Bract extract of *Musa paradisiaca* in varying concentrations (5-30 µg/mL) were allowed to react with 500 µL of the ABTS solution for 15 minutes in dark condition and the absorbance was measured at 734 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

\[
\% \text{ of ABTS}^{-}\,$ radical cation inhibition = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

Phosphomolybdenum reduction activity

The antioxidant capacity of the ethanol Bract extract of *Musa paradisiaca* was assessed as described\textsuperscript{11}. The ethanol Bract extract of *Musa paradisiaca* with varying concentrations ranging (20-120 µg/mL) was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 µM). The reaction mixture was incubated in water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

\[
\% \text{ of Phosphomolybdenum reduction} = \left( \frac{\text{Sample} - \text{Control}}{\text{Sample}} \right) \times 100
\]

Ferric (Fe$^{3+}$) reducing power activity

The reducing power of ethanol Bract extract of *Musa paradisiaca* was determined by slightly modified method\textsuperscript{22}. One mL of ethanol Bract extract of *Musa paradisiaca* of different concentrations (20-120 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH-6.6) and potassium ferriyanide [K$_3$Fe (CN)$_6$] (1 mL, 1% w/v). The mixtures were then incubated at 50°C for 20 min in water bath. 500 µL of trichloroaetic acid (10 % w/v) was added to each mixture, followed by 100 µL of Ferric chloride (0.01% w/v) was added and the absorbance was measured at 700 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

\[
\% \text{ of Fe}^{3+}\,$ reduction = \left( \frac{\text{Sample} - \text{Control}}{\text{Sample}} \right) \times 100
\]

Statistical analysis

All the experiments were conducted in triplicates. All data were reported as the mean±standard deviation (SD) of three replicates.

Gas Chromatography–Mass Spectrometry (GC–MS) Profiling

In GC-MS analysis, the ethanol Bract extract of *Musa paradisiaca* was injected into a HP-5 column (30 m X 0.25 mm Id with 0.25 µm film thickness), Agilent technologies 6890 N J EOL GC Mate II GC-MS model. Following conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; and mass range of 50-600 mass units\textsuperscript{23}.

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.
RESULTS AND DISCUSSION

Determination of total phenols and flavonoids

Flavonoids and phenolics are the most important bioactive natural product of secondary metabolites and act as an antioxidant and anti-ageing substances, capable of scavenging free radicals and reducing the risk of cancer. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Other examples include flavones, rutin, naringin, hesperidin and chlorogenic.

Flavonoids possess ideal structure for free radicals scavenging activity and have been found to be more effective antioxidants in vitro than tocopherols and ascorbates. They are efficient reducing agents that can stabilize the polyphenols derived radicals and delocalise the unpaired electrons. Flavonoids are powerful metal chelators and scavengers of free radicals and also act as anti-inflammatory, anti-ulcer, antimuturm and anticancer agents. They interact with cellular signal pathways that control cell cycle, differentiation and apoptosis. The total phenol content was 218.3±0.43 GAE/mg of extract and the total flavonoid content was 47.37±0.17 QE/mg of extract. These results provide a comprehensive profile of the antioxidant activity of ethanol bract extract of Musa paradisiaca with respect to their phenols and flavonoids content.

In vitro antioxidant activities of ethanol bract extract of Musa paradisiaca

DPPH• radical, Superoxide (O2−) radical and Nitric Oxide (NO) radical scavenging activities of ethanol bract extract of Musa paradisiaca

Evaluation of antioxidant activity by DPPH method is the best screening option for herbal based drugs. DPPH• (1,1-Diphenyl-2-picrylhydrazyl) is a stable nitrogen centered free radical which has an unpaired valence electron at one atom of nitrogen bridge. The ability of ethanol bract extract of Musa paradisiaca to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The ethanol bract extract of Musa paradisiaca demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picryl hydrazine and the reducing capacity increased with increasing concentration of the extract. The maximum DPPH radical scavenging activity of ethanol bract extract of Musa paradisiaca was 52.05±0.38% at 120 µg/mL concentration (Table 1). The IC50 value for the ethanol bract extract of Musa paradisiaca was found to be 68.47 µg/mL concentration respectively and was compared with standard (Ascorbic acid, IC50 = 12.94 µg/mL concentration).

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT2−) to blue formazan, which is measured at 590 nm using UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide (O2−) radical scavenging activity of ethanol bract extract of Musa paradisiaca was 57.71±0.45% at 120 µg/mL concentration (Table 1) and the IC50 value for the ethanol bract extract of Musa paradisiaca was found to be 71.37 µg/mL concentration respectively. It was compared with the standard of ascorbic acid (IC50 = 15.18 µg/mL concentration).

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc and is involved in the regulation of various physiological processes. NO is generated in biological tissues by specific nitric oxide synthase (NOSs), which metabolizes arginine to citralline with the formation of NO via a five electron oxidative reaction. Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO2, N2O4, N2O3, NO3− and NO2 are very reactive. The maximum Nitric Oxide (NO) radical scavenging activity of ethanol bract extract of Musa paradisiaca was 49.63±0.32% at 120 µg/mL concentration (Table 1) and the IC50 value for the ethanol bract extract of Musa paradisiaca was found to be 93.94 µg/mL concentration respectively. It was compared with the standard of ascorbic acid (IC50 = 13.76 µg/mL concentration).

Table 1: DPPH• radical, Superoxide (O2−) radical and Nitric Oxide (NO) radical scavenging activities of ethanol bract extract of Musa paradisiaca

| S. No | Concentration (µg/mL) | Percentage of inhibition* |
|-------|-----------------------|--------------------------|
|       |                       | DPPH• radical            | Superoxide (O2−) radical | Nitric Oxide (NO) radical |
| 1     | 20                    | 29.34±0.13               | 19.73±0.10               | 16.2±0.33                |
| 2     | 40                    | 37.42±0.27               | 38.15±0.39               | 27.95±0.46               |
| 3     | 60                    | 43.81±0.35               | 42.03±0.22               | 34.19±0.19               |
| 4     | 80                    | 45.16±0.11               | 47.55±0.16               | 42.58±0.24               |
| 5     | 100                   | 50.47±0.42               | 53.38±0.21               | 45.1±0.18                |
| 6     | 120                   | 52.05±0.38               | 57.71±0.45               | 49.63±0.32               |

(*Average value of 3 replicates)
ABTS•⁺ radical cation scavenging activity of ethanol bract extract of Musa paradisiaca

ABTS•⁺ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and ABTS•⁺ radical cation gets reduced in the presence of ethanol bract extract of Musa paradisiaca and the remaining radical cation concentration was then quantified at 734 nm. It can be prepared using K₃S₂O₈ as an oxidant. The blue-green colour of ABTS solution is formed by the loss of an electron by the nitrogen atom quenches the hydrogen atom (3-ethylbenzothiazolin-6-sulfonic acid). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom of ABTS (2, 2-azinobis (3-ethylbenzothiazolin-6-sulfonic acid)). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom). The maximum ABTS•⁺ radical cation scavenging activity of ethanol bract extract of Musa paradisiaca was 84.28±0.13% at 30 µg/mL concentration (Table 2) and the IC₅₀ value for the ethanol bract extract of Musa paradisiaca was found to be as 10.04 µg/mL concentration respectively, which was compared with standard ascorbic acid (IC₅₀ = 4.83 µg/mL concentration).

Table 2: ABTS•⁺ radical cation scavenging activity of ethanol bract extract of Musa paradisiaca

| S. No | Concentration (µg/mL) | Percentage of inhibition* |
|-------|-----------------------|---------------------------|
|       |                       | ABTS•⁺ radical cation      |
| 1     | 5                     | 41.31±0.20                |
| 2     | 10                    | 49.76±0.41                |
| 3     | 15                    | 54.89±0.18                |
| 4     | 20                    | 73.35±0.35                |
| 5     | 25                    | 81.17±0.26                |
| 6     | 30                    | 84.28±0.13                |

(*Average value of 3 replicates)

Both DPPH• radical scavenging activity and ABTS•⁺ radical cation scavenging activity are classified as single electron transfer reactions. They are used to assess the antioxidant reducing capacity of the ethanol bract extract of Musa paradisiaca to scavenge both the radical indicators by reduction process directly through electron transfer or by radical quenching process. The correlation between antioxidant properties by six different methods and the total phenolic content indicates the ability of antioxidants to scavenge the free radicals.

Phosphomolybdenum reduction and Ferric (Fe³⁺) reducing power activities of ethanol bract extract of Musa paradisiaca

The total antioxidant activity of ethanol bract extract of Musa paradisiaca was measured spectrophotometrically by phosphomolybdenum reduction method, which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum phosphomolybdenum reduction of ethanol bract extract of Musa paradisiaca was 45.38±0.40% at 120 µg/mL concentration with the RC₅₀ value of 121.44 µg/mL concentration respectively (Table 3). It was compared with the standard ascorbic acid (RC₅₀ = 9.80 µg/mL).

The reducing power of Fe³⁺ to Fe²⁺ by ethanol bract extract of Musa paradisiaca was studied and showed reduction ability in a dose dependent manner. The maximum reduction of ethanol bract extract of Musa paradisiaca was 57.06±0.32% at 120 µg/mL concentration (Table 3). Fe (II) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. The RC₅₀ value for the ethanol bract extract of Musa paradisiaca as found to be 85.43 µg/mL concentration respectively and was compared with the standard (21.69 µg/mL concentration) Ascorbic acid.

Table 3: Phosphomolybdenum reduction and Ferric (Fe³⁺) reducing power activities of ethanol bract extract of Musa paradisiaca

| S. No | Concentration (µg/mL) | Percentage of reduction* |
|-------|-----------------------|--------------------------|
|       |                       | Mo⁶⁺ reduction | Fe³⁺ reduction |
| 1     | 20                    | 25.46±0.24 | 19.38±0.10 |
| 2     | 40                    | 30.93±0.46 | 27.11±0.36 |
| 3     | 60                    | 36.04±0.38 | 34.44±0.47 |
| 4     | 80                    | 38.72±0.19 | 46.82±0.15 |
| 5     | 100                   | 41.17±0.21 | 51.56±0.29 |
| 6     | 120                   | 45.38±0.40 | 57.06±0.32 |

(*Average value of 3 replicates)

Gas Chromatography–Mass Spectrometry (GC–MS) Profiling

GC-MS is an analytical challenge and also a unique tool for reliable characterization of complex mixtures which has very high sensitivity and specificity. The active principles with their Retention time (RT), Molecular formula and Molecular weight (MW) (Figure 2 and Graph 1) were recorded along with pharmacological activities (Table 4).
Figure 2(a): Mass Spectrum of ethanol bract extract of *Musa paradisiaca*
Figure 2(b): Mass Spectrum of ethanol bract extract of *Musa paradisiaca*

Graph 1: GC-MS Chromatogram of ethanol bract extract of *Musa paradisiaca*
CONCLUSION

Bananas are one of the most widely consumed fruits in the world because of its taste, nutritional value and potential health benefits. It is ranked fourth among the world’s food crops in monetary value. Antioxidants play important role in preventing certain diseases, like heart disease and cancer therefore the best way to get antioxidants is by eating a variety of healthy food, especially vegetables, fruits, and nuts. A plant-based diet is believed to protect against chronic oxidative stress-related diseases. Much of the research work has not been carried out on the bracts of *Musa paradisiaca* in regard with antioxidant activities. Further, the active compounds analyzed shall be evaluated for anti-cancer activity, proving as a medicine for scavenging the free radicals along with the potentiality to kill the cancerous cells.

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