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ORIGINAL ARTICLE

Phytochemical analysis and study on the Antioxidant Property of Peel Extracts of *Musa acuminata*

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ABSTRACT
Musa is one of the genera in the family Musaceae; that includes bananas and plantains and it is world's leading fruit crop. Being a tropical herb, banana has the ability to produce large amount of antioxidants and protect itself from oxidative stress caused by high temperature and strong sunshine.Production of Bananas also leads to production of large quantities of peels as waste. Plants species belonging to Musaceae are rich in antioxidant properties and so have the capability of scavenging free radicals, thereby protecting the cell. Therefore search for novel plant products with bioactive compounds rich in antioxidant activity is an area of wide spread research. Such studies on Musa acuminata fruit peel has not been conducted so extensively. Hence the present study aims to evaluate the phytochemical constitution of methanolic extracts of Musa acuminata and also the antioxidant property in vitro. The peel extracts was shown to have many secondary metabolites, especially phenolics and flavonoids. The extract also exhibited antioxidant property in vitro as shown by scavenging assays.
Key words: Antioxidant, Musa acuminate peel, Reducing power, Total phenolics, Total flavonoids, methanolic extract

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INTRODUCTION

Metabolism is the mother of all biological processes as result of which harmless, useful as well as harmful products are being produced. One such harmful byproduct is the category of free radicals which are continuously produced. Free radicals produced naturally or due to environmental stresses or other factors often create chances for life threatening diseases .Unstable molecules which has free electron or lack electrons, and which react with important molecules of life like DNA, fat and proteins are called free radicals. Normal physiology of the cell can be interrupted due these compounds. It can be formed as a part of natural metabolism and also due to environmental factors. Free electrons lack or have in excess of an electron in order to attain its stable configuration. They receive or give up electron to attain stability. Antioxidants are substances that have the ability to counteract free radicals. They are phytochemicals, vitamins and other nutrients which can impart protective action against free radical induced cell damage. Antioxidants inhibit free radical chain reaction by accepting or donating free electrons from it and they themselves without becoming free radicals. Thus they remove intermediate free radicals and they themselves get oxidized.

When the body is subjected to different types of stress, the sympathetic nervous system stimulates receptors known as beta-adrenergic receptors on the surface of heart muscle cells. This leads to several changes inside the cells, one of which is the phosphorylation of proteins, which in-turn causes contraction of the cells, thereby making it stronger and the heart beats with a greater force. Stimulation of the beta-adrenergic receptors also leads to increased production of free radicals in mitochondria of the cells and these then contribute to stronger contractions of the cells. When the cells were exposed to antioxidants, a major part of the effect of beta- adrenergic stimulation of the heart muscle cells disappeared. Thus in this

case, the free radicals are seen to play an important role, since they contribute to the heart being able to pump more blood in stress-filled situations[6].To counteract the effects of the harmful free radicals, many protective antioxidant defense mechanisms operate to detoxify and act as scavengers. In a healthy body, antioxidant activities occur in mainly at three lines of defense mechanisms, namely Preventive antioxidants-the first line of defense, Radical scavenging antioxidants-the second line of defense, and Repair and de novo enzymes -the third line of defense. Free radicals can be classified in to two: Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Reactive oxygen species is again divided into two – *oxygen centred radicals* and *oxygen centred non- radicals.* In which superoxide anion , hydroxyl radical , alkoxyl radical , and peroxyl radical comes under the category of oxygen centred radicals , whereas hydrogen peroxide and singlet oxygen are placed under oxygen centred non-radicals . Reactive nitrogen species consist of nitric oxide and nitric dioxide and peroxynitrite. If the production of free radicals excess and is not properly regulated, will causes variety of chronic and other degenerative diseases. The various diseases affected by free radicals are cancer, cardiovascular diseases, inflammatory diseases, neurological diseases, nephropathy and ocular diseases [26]. Most common diseases are atherosclerosis, cancer, Parkinson's disease, arthritis, Alzheimer's disease, aging and other age related problems [8, 9].

Musa spp. of Class Monocotyledonae, belonging to the Order Zingiberales, comprising banana which are the world's leading fruit crop. Being a typical tropical plant, banana is strongly protected by itself from the oxidative stress caused by strong sunshine and high temperature and by maintaining high production of antioxidant [17].

Since Musa spp. are highly affordable food source and the potential of fruit peel as a food source and in medicine it is undeniable. Several studies on a variety of *Musa* spp. has been conducted worldwide. But the potential antioxidant, antimicrobial and phytochemical studies on *Musa acuminata* fruit peel has not been encountered so far. Hence the objective of the present investigation is to screen qualitatively and quantitatively the secondary metabolites in *Musa acuminata* fruit peels and to determine its antioxidant potential *In vitro*.

MATERIAL AND METHODS

CHEMICALS

All the reagents used were of analytical grade and were purchased from SRL, Ranbaxy and Spectrochem, India.

Plant material and sample preparation

Musa acuminata fruit peels were collected from Thiruvananthapuram, Kerala. The collected material was dried under shade, powdered using a mechanical grinder and stored in air tight containers. The dried powder was extracted by random shaking method with 80%methanol in a conical flask and plugged with cotton. The extract was filtered through Whatman filter paper No. 1. The supernatant was collected and the solvent was evaporated and the dry extract was stored at 4°C in air tight bottles.

Qualitative phytochemical analysis

Evaluation of phytochemical chemical constituents of extracts was carried by qualitative chemical methods of Tiwari, *et al*., 2011. A small portion of the dry extract was subjected to tests for secondary metabolites by characteristic colour reactions.

1. Test for Carbohydrates: In order to find out the presence of carbohydrates, Benedict's test was done. The extract is dissolved in water and filter. Equal volume of Benedict's solution and extracts were mixed in a test tube and heated in boiling water bath for 10 min the changes in colour to yellow, green and red indicates the presence of reducing sugars.
2. Test for proteins: Biuret test was done to detect the presence of proteins. To 3 ml of extract 1 ml of 4% sodium hydroxide and 1ml of 1% copper sulphate were added. The change in colour of the solution to violet or pink indicates the presence of proteins.
3. Test for alkaloids: The extract is dissolved in dilute hydrochloric acid and filter. The filtrate was treated with saturated aqueous solution of picric acid (Hager's reagent). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.
4. Test for Sterols: A small quantity of extract is dissolved in chloroform, to 2 ml of extract 5 drops of concentrated sulphuric acid was added, shaken and allowed to stand. Formation of brown ring indicates the presence of phytosterols (Salkowaski test).
5. Test for phenolics: The presence of tannins/phenolics was tested by ferric chloride test. Treated the extract with 3-4 dropsof ferric chloride (5%) solution, formation of bluish black colour indicated the presence ofphenols/tannins.
6. Test for flavonoids: Presence of flavonoids was done by lead acetate test. The extract was treated

with few drops of lead acetate solution, formation of yellow precipitate indicated the presence of flavonoids**.** Yellow, orange, blue and violet colours indicate presence of anthocyanins, flavones and flavonones.

7. Test for Glycosides: Keller-Killani test was done to evaluate the presence of glycosides. To 2 ml of extract, glacial acetic acid, one drop 5 % ferric chloride and concentrated sulphuric acid were added. Appearance of reddish brown colour at the junction of the two liquid layers indicates the presence of cardiacglycosides.
8. Test for saponins: Presence of saponins was detected by foam test. A small quantity of the extract was shaken with 2 ml of water. Persistence of foam produced for ten minutes indicated the presence of saponins.

Quantitative Phytochemical Analysis

Determination of Total Phenolic Content **(TPC)**

Phenolics level of extract was determined using Folin-Ciocalteu method as described by Singleton, *et al*., 1999. 0.5 ml sample was mixed with 3 ml distilled water and 0.25 ml Folin-Ciocalteu reagent (1N). The mixture was allowed to stand at room temperature for 2 minutes and 0.75 ml sodium carbonate (20%) was added to the mixture. The volume was made upto 5 ml with distilled water. Absorbance was measured at 765 nm after standing for 2 hours. The content of phenolics was expressed as mg gallic acid equivalents.

Determination of Total Flavonoid Content **(TFC)**

Aluminium chloride colorimetric technique was used for flavonoids estimation [5]. 1 ml sample was mixed 0.5 ml aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM). Absorbance was measured at 415 nm after incubating 30 minutes at room temperature. The content of flavonoid was expressed as mg quercetin equivalents.

Reducing power assay

The reducing power of extract was determined as per the method of Oyaizu, 1986. Different concentrations of sample (1 ml) was mixed with 2.5 ml phosphate buffer (0.2 M; pH 6.6) and 2.5 ml potassium ferricyanide (0.1%), followed by incubation at 50^0C on a water bath for 20 min. After incubation, 2.5 ml TCA (10%) was added to terminate the reaction. The upper portion of the solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride solution (0.01%) was added. The reaction mixture was left for 10 minutes at room temperature and the absorbance was measured at 700 nm against blank. A higher absorbance of the reaction mixture indicates greater reducing power.

Determination of Total Antioxidant Capacity assay **(TAC)**

Total antioxidant capacity of the extract was evaluated by the method of Prieto [18]. 0.1 ml sample solution was mixed with 1 ml phospho-molybdenum reagent and incubated at 95^0C for 90 minutes. The tubes were cooled to room temperature and the absorbance was read at 695nm against a blank. TAC was expressed as mg gallic acid equivalents.

Free radical scavenging assays

The radical scavenging activity of the extract was studied with DPPH assay, ABTS assay, super oxide scavenging assay and hydroxyl radical scavenging assay. Each of these assays was performed with 6 different concentrations of the same extract. The percentage inhibition of the sample for respective substrates for every assay was calculated after determining the absorbance of the test sample with respect to the control using the formula:

$$PERCENTAGE\ INHIBITION = \frac{(AC - AT)}{AC} * 100\%$$

where AC is the absorbance of the control, and AT the absorbance of the sample tested.

An escalating graph was arrived by plotting the concentration of sample against percentage inhibition for every scavenging assays and the concentration of the test to impede 50% of the substrate was calculated and summarised in the tables.

All these 4 assays mentioned below were useful in helping us to conclude about the antioxidant properties of the entities present in *M. acuminata* peel extract.

DPPH Scavenging Assay

DPPH free radical, is stable at room temperature, and produces a violet colour, detected at 512 nm. In the presence of an antioxidant molecule it gets reduced and results in an uncoloured solution [16]. DPPH contains an odd electron which gets paired off in the presence of hydrogen donors (radical scavenging antioxidant) in the solution and the results in decolourisation which is proportional to the number of electrons captured from it. The structure and reduction of DPPH scavengers[26] (antioxidants) is given in figure 2. Reduction of DPPH yields non radical DPPH-H, which is colourless[12]. This reaction can be used to

test the ability to act as free radical scavengers and to evaluate the antioxidant content in our peel extract.

HYDROXYL RADICAL SCAVENGING ASSAY

Hydroxyl radical has very high reactivity, and has the ability to react with wide range of molecules in living cells; and hence scavenging of hydroxyl radical is important for evaluating antioxidant content in a sample [25].

ABTS SCAVENGING ASSAY

ABTS assay is a radical cation decolourisation test and is employed for assessing antioxidant properties of various substances spectrophotometrically [18]. It tests the capacity of the antioxidants to scavenge the long lived ABTS molecule [13]. Evaluation of antioxidant activity of single compounds and complex mixtures of various origins (body fluids, foods, beverages, plant extracts) are often investigated by ABTS radical. Potassium persulfate is used to generate ABTS radical cation in stable form for this assay. Generation of radical is interfered by antioxidant compounds therefore scavengers are added only added after radical formation [20]. The antioxidant sample is added to the reaction medium when stable absorbance is obtained,and the antioxidant activity measured in terms of decolorization indicates the presence of antioxidant compounds in it [10].

SUPEROXIDE SCAVENGING ASSAY

Super oxide is oxygen centered radical with selective reactivity. They perform an important role in formation of other reactive species such as singlet oxygen, hydrogen peroxide and hydroxyl groups responsible for oxidative damage in cells. The presence bioactive compounds is indicated by the decrease in optical density against the control, which is due to the result of reduction of superoxide by these compounds [14]. Superoxide scavenging assay is based on the ability of compounds in aqueous solution to inhibit formation of formazan by scavenging the superoxide molecules present in the riboflavin-light-NBT *in vitro* system. Superoxide generated by reduction of phytochemical flavin, reduced NBT and blue coloured formazan is formed [2].

STATISTICAL ANALYSIS

All the experiments were carried out in duplicates and data reported are mean ± standard error.

RESULTS AND DISCUSSION

Musa acuminata fruit peel was chosen for this study due to their ability to protect themselves from the oxidative stress caused by the environment and for it being the cheapest source of natural antioxidants. Almost 40% of the fruit weight is constituted by its peel which is the unintentionally wasted source of antioxidants. Hence it is an ideal sample to meet the requirements of the present antioxidant studies. The presence of multiple hydroxyl group allow phenolic compounds to exhibit redox properties and this make them act as antioxidants [23]. The fruits and vegetables are rich in different types of antioxidants. This makes it difficult to measure each antioxidant compound separately [11].

Preliminary phytochemical analysis

The phytochemical screening of crude methanolic extract of peel sample of *Musa acuminata* revealed the presence of some secondary metabolites such as phenolics, tannins, flavonoids, flavanones, steroids and cardiac glycosides as shown in Table 1.Phenolic compounds comprise a group of secondary metabolites which have favorable effects on the plant's host. Apart from this, phenolics demonstrate various biological properties that positively influence human health. Phenolic compounds exhibit redox properties due to its multiple hydroxyl groups and it allow them to act as antioxidants [23]. Generally, the antioxidant mechanisms of phenolic compounds are by neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals. By virtue of their ability to scavenge free radicals, the antioxidant activity of the plant products is associated to their bioactive compounds, antioxidant phenolics[4]. Phenols which are present in the form of secondary metabolites in the plants are known to have a wide range of therapeutic uses such as antimutagenic, antioxidant, anticarcinogenic and also decrease cardiovascular complications [27]. Most effective antioxidant constituent in plants is the polyphenols. So it is important to estimate the phenolic contents of extract in order to identify its antioxidant activity [22]. In the present study we estimated the total phenolic content of peel extract by Folin-Ciocalteu method using gallic acid as the standard. It is an oldest method in which the phenolic compounds react with the Folin-Ciocalteu reagent and formbluecolouredcompoundwithmaximumabsorbancenear750nm[24]. Total phenolic content in peel extract was found to be 12.5 ± 0.62 mg. Flavonoids are the most common and widely distributed group of plant phenolic compounds present in plants. It is characterized by a benzo-γ-pyrone structure. Total flavonoid content in different extracts was determined by aluminium chloride method using quercetin as the standard. Flavanoids due to the presence of phenolic hydroxyl groups are capable of scavenging reactive oxygen species and hence found to be strong antioxidants [3]. There are many

mechanisms like hydrogen donation, prevention of hydrogen abstraction, storage of free radical mediated chain reaction, elimination of peroxides and chelation of catalytic ions [7]. One of the most diverse and widespread group of natural compounds is flavonoids and they are the most important natural phenols. These compounds have a broad spectrum of biological and chemical activities which include the radical scavenging activities. Total flavonoid content in peel extract was found to be 10.25 ± 0.51 mg [21].
The extract showed significant level of phenolics and flavonoids (Table 2) and this may effectively eliminate free radicals and prevent oxidative stress.

Reducing power and total antioxidant capacity

An antioxidant molecule can contribute an electron to free radicals, which leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of ferrocyanide to ferricyanide in the presence of iron containing salts. The intense Prussian blue color of the complex was measured at 700 nm colourimetrically [27]. A higher absorbance value signifies a stronger reducing power of the extract. As shown in the figure 1, the extract shows a concentration dependent increase of absorbance indicates the potential of *Musa acuminata* peel extract to scavenge free radicals.
Total antioxidant capacity is the assay that evaluates both water-soluble and fat-soluble antioxidants. It is based on the reduction of Mo (VI) to Mo (V) by the antioxidants and subsequent formation of blue phosphate/Mo (V) complex at acidic pH [1]. TAC of *Musa acuminata* peel is found to be 70.24 ± 3.51 mg gallic acid equivalents(Table 2). The result is in accordance with total phenolics, flavonoids and reducing power of the extract.
Considering the phytochemical screening, total phenolics and flavonoids, reducing capacity and total antioxidant capacity, as the indices of antioxidant activity of the extract, the findings revealed the potential of *Musa acuminata* fruit peel as a source for natural antioxidants. It can be used as a natural antioxidant source to prevent diseases associated with free radicals. Studies are in progress for the bioassay guided fractionation and identification of the factors in charge for the activity.
Total antioxidant capacity was evaluated by the phosphomolybdenum method based on the reduction of Mo^{6+} to Mo^{5+} by the antioxidant compounds. The formation of this green Mo complex at a low pH is measured with a maximal absorbance at 695 nm. A higher absorbance value indicates that the extract has higher antioxidant capacity [11]. Generally reducing power is associated with the presence of reductants. By donating a hydrogen atom, this reductant, break free radical chain and thus shows antioxidant property. Figure1 shows the reducing power (as indicated by absorbance at 700 nm) of extract which increase with increase in concentration revealing the power of scavenging the free radicals by *Musa acuminata* peel extract [15].

SCAVENGING ASSAYS

Scavenging assays are investigations for the test extract based on its ability to neutralize free radicals which are used as the substrate for the test extract to act upon. It is difficult to quantify the antioxidants present in plant tissues due to the presence of several antioxidants present in it[9]. Therefore scavenging assays are useful to identify the presence of antioxidants. Redox properties of antioxidants enable reduction of certain compounds and this property is employed in scavenging assays [19].

DPPH Scavenging assay

As represented in Figure 2 the scavenging abilities of *Musa acuminata* peel extract was concentration dependent. DPPH scavenging activity (expressed as percentage inhibition) ranged from 18 to 70% with the gradation of concentration from 25 to 150 µg/mL.Then the concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC_{50}) was calculated. Lower IC_{50} value indicates higher antioxidant activity. In the case of activity against *Musa acuminata* peel extract the concentration required to scavenge 50% of initial concentration of DPPH was plotted out as 107.5 µg/mL from the given graph (Figure 2)and is specified in Table 3.

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging potential of different concentrations of *Musa acuminata* peel extract in methanol is represented in the figure 3. Here the hydroxyl radical scavenging activity is shown to be increasing with increasing concentration of the test extract. In the present investigation the percentage inhibition is found to range from 8 to 62% from 25 to 150 µg/ml of sample concentration. The concentration required to scavenge 50% of hydroxyl radical was determined from the graph as 135.2 µg/mL. These results indicated that the test extract had notable effect on scavenging hydroxylradical.

ABTS Scavenging assay

The methanol extracts of *Musa acuminata* was assayed and it exhibited a linear variation of inhibition percentage with added concentration of extract as represented in figure 4 The IC_{50} of ABTS assay was plotted out and determined as 102 µg/mL.

Superoxide Scavenging Assay

Musa acuminata peel extract was found to be scavenger of superoxide in this *in vitro* system as it inhibited the formation of blue formazan. The percentage inhibition was proportional to the concentration of the extract as shown in figure 5. The concentration of the test sample to impede 50% of superoxide was found to be 105 µg/mL and is represented in Table 3. From these results it is revealed that the test extract comprises optimum level of affluences to scavenge superoxide radical.

CONCLUSION

Banana is a highly favored fruit crop in the world and is cultivated in an area of 5.8million hectares and 564million fruits being produced. The noteworthy fact is that out of these fruits tons of peels are also produced which are usually inedible. So they discarded, and hence certain new ventures have been aiming to produce value added products from these wasted peels. *Musa acuminata* (natively known as red banana in Kerala) is considered not only to be delicious but also healthy fruit. So far there were no studies conducted on methanolic extract of the fruit peels of *Musa acuminata*. Studies on the phytochemical analysis, total phenolics and flavonoids, reducing capacity and total antioxidant capacity, has indicated the antioxidant activity of the extract and so the findings revealed the potential of *Musa acuminata* fruit peel as a source for natural antioxidants. Scavenging assays showed the *Musa acuminata* peel extract has high radical scavenging capacity making it a potential source of antioxidants. Thus it can be used as a natural antioxidant source to prevent diseases associated with free radicals.

Secondary metabolite	Result
Carbohydrates	Present
Proteins	Present
Alkaloids	Absent
Phytosterol	Present
Phenolics	Present
Tannins	Present
Flavonoids	Present
Flavanones	Present
Anthocyanins	Present
Glycosides	Present
Flavones	Absent
Saponins	Absent

Table 1. Phytochemical analysis of *Musa acuminata* peel Total phenolic content

TPC	12.5 ± 0.62 mg gallic acid equivalents
TFC	10.25 ± 0.51 mg quercetin equivalents
TAC	70.24 ± 3.51 mg gallic acid equivalents

TABLE 2. TPC, TFC and TAC of *Musa acuminata* peel

Scavenging assays	IC_{50} of extract (µg/mL)	IC_{50} of quercetin (µg/mL)
DPPH	107.5	15
ABTS	102	8.5
Hydroxyl radical	135.2	27.5
Superoxide	105	14

Table 3. IC_{50} of scavenging assay

IC_{50} is the concentration of test extract to scavenge 50% of the respective reagents.

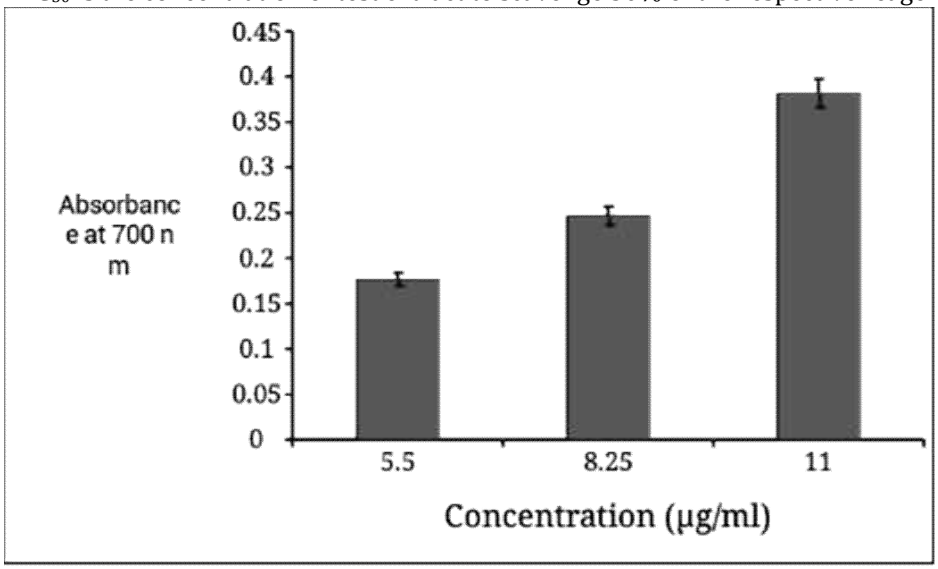
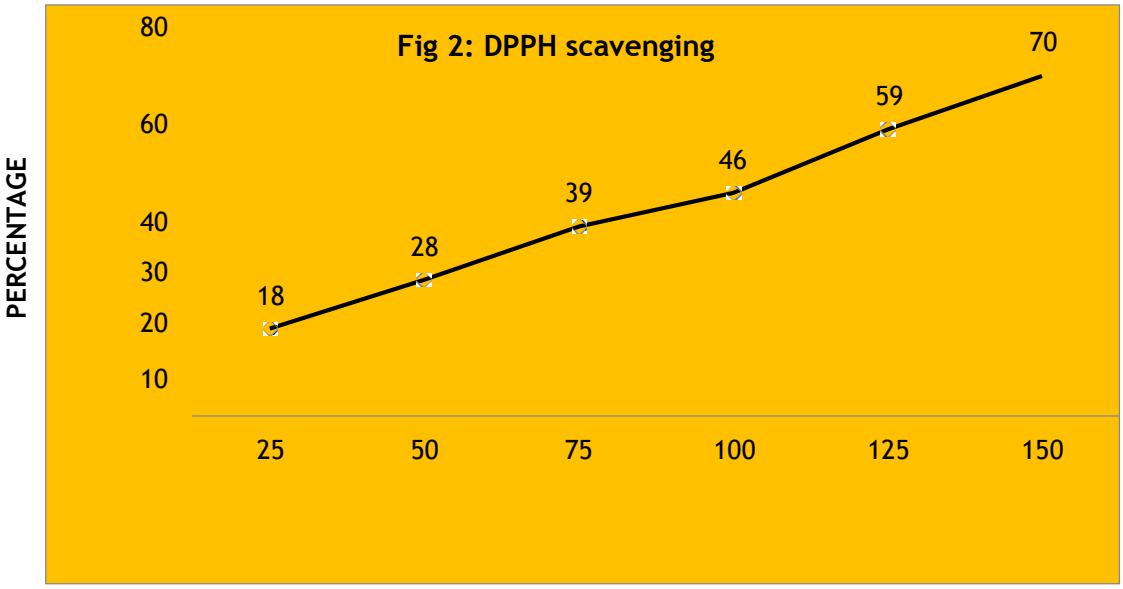
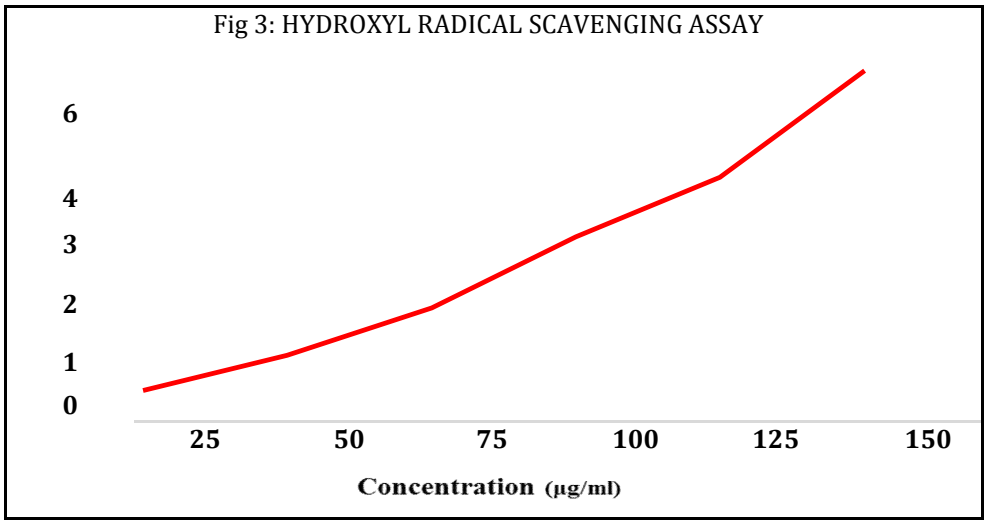


FIGURE 1. Reducing power of *Musa acuminata* peel.





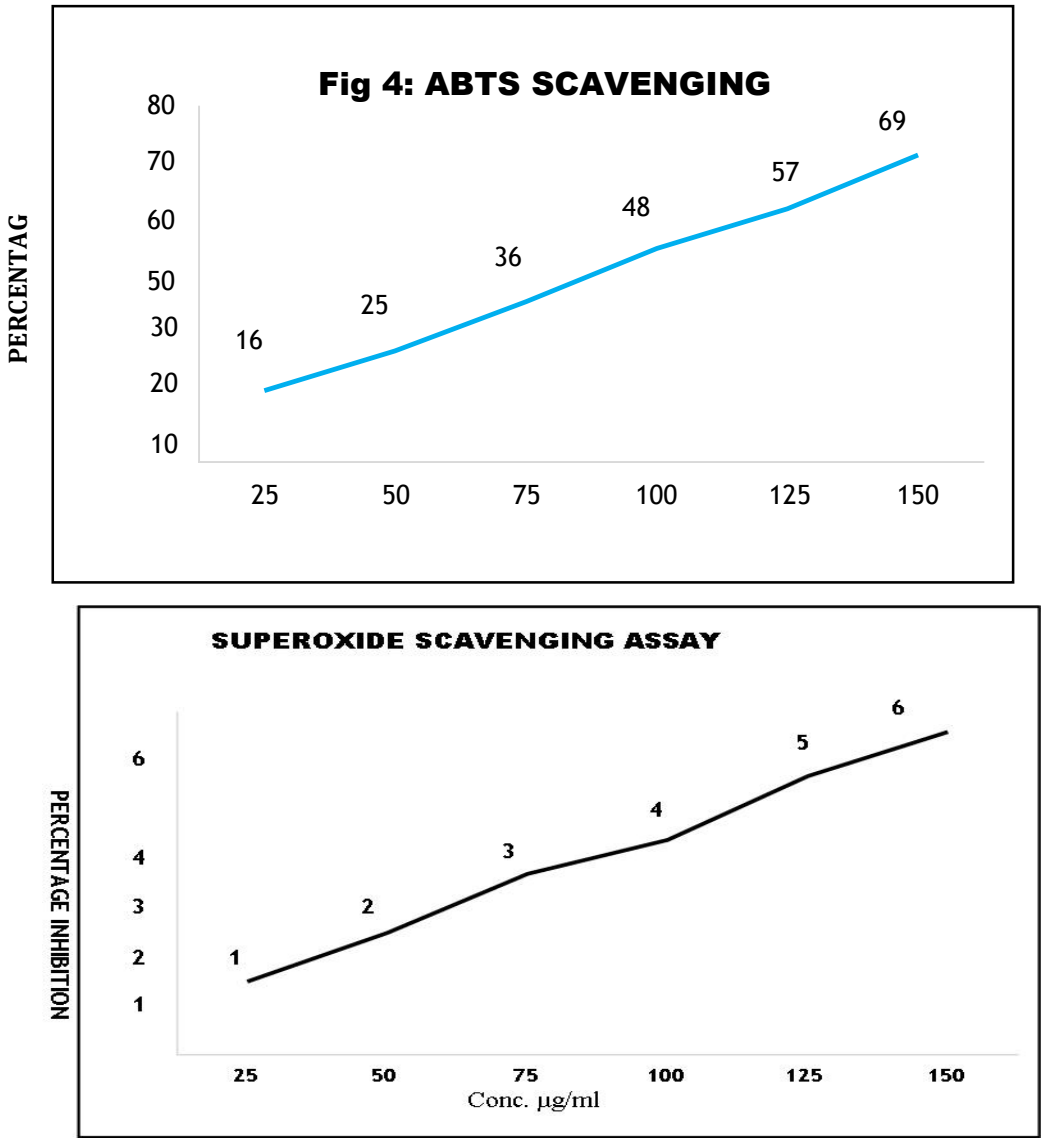


Fig 5: Superoxide Scavenging assay

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