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HYDROGEN PEROXIDE SCAVENGING ASSAY AND ANTIOXIDANT ACTIVITY OF FLESHY *MUSA ACCUMINATA* "RED DACCA"

Asiyabi M.[1]*, Dr. K. B. Ilango[1], Gowtham K. V.[2], Mugesh T.[3], Pon Manisha C.[4], Senjudar S.[5] and Yeaswanth Raj V.[6]

[1]Assitant Professor, Department of Pharmacology, Shree Venkateshwara College of Paramedical Sciences, Gobi - Tamilnadu – 638455.

[1]Professor, Head of the Department of Pharmaceutics, Shree Venkateshwara College of Paramedical Sciences, Gobi - Tamilnadu – 638455.

[2,3,4,5,6]B. Pharm Final Year Students, Shree Venkateshwara College of Paramedical Sciences, Gobi - Tamilnadu – 638455.

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***Corresponding Author**
Prof. Asiyabi M.
Assitant Professor, Department of Pharmacology, Shree Venkateshwara College of Paramedical Sciences, Gobi - Tamilnadu - 638455.

ABSTRACT

The purpose of this study was to look at the *in-vitro* chemical components and bioactivity of red banana pulp extract as antioxidant agents. New and Expensive sources of essential chemicals are yet to be found. Antioxidants have an important role in biological system and their use is implicated in the prevention of various diseases. When the normal level of antioxidants defense mechanism is not sufficient for the shown that proper intake of antioxidant will help quenches all these inevitably free radicals in the body thus, improving the health by lowering the risk of various disease.

KEYWORDS: Antioxidant, red banana pulp, eradication of free radicals, lowering the risk of disease.

INTRODUCTION

Fruits have been shown to lower the incidence of serious chronic degenerative illnesses since they are a rich source of vitamins, carbohydrates and a variety of phytochemicals. One of the most popular fruits consumed globally is the banana, which is a member of the *Musaceae* family and species *Musa*. There are around 70 species in the genus *Musa*and there are between 300 and 500 distinct subspecies. Over 116 million tonnes of bananas are produced worldwide each year, with India producing the most, at about 30 million tonnes annually.[1]

Both the peel and the flesh of the banana fruit are abundant in beneficial photochemical, such as polyphenols, flavonoids, fatty acids, carotenoids, phytosterols and amines. Polyphenols, fatty acid and phytosterols are particularly prevalent in bananas include catechin, epicatechin, gallic acid, cinnamic acid, chlorogenic acid and protocatechuic acid where as phytosterols such stigma sterol, free radicals may be continually created by our bodies, which can lead to the development of significant illnesses as heart disease, cancer, arthritis, inflammation and ageing. Antioxidants are substances that can scavenge free and radicals prevent the harm they can do.[2]

The flesh of uncooked red bananas ranges in colour from cream to bright pink. Bananas are an excellent source of minerals, vitamins, flavonoids, carbohydrates and phenolic compounds from a nutritional standpoint. It is simple for people of all social strata to obtain bananas. A banana can be used to treat pain, inflammation, coli-tis-related intestinal lesions and snakebites. Free radicals may be continuously created in our bodies, which can lead to significant illness including heart disease, cancer, arthritis, inflammation and ageing. Antioxidants are substances that may scavenge free radicals and prevent the damage they bring to cells. The antioxidants and phytochemicals found in banana, such as vitamin C, vitamin E, flavonoids and beta carotene have the ability to scavenge free radical in many banana flesh enzymes.[3]

PLANT PROFILE

A species of wild banana native to Southeast Asia is called *Musa acuminate*. Many Musa plant parts have been utilised topically and orally in traditional medicine and some research has supported this potential for healing. Studies have shown that *Musa accuminata's* phenolic compounds play a significant role in its wide variety of pharmacological characteristics. All plant components, including the roots, stem, pseudo stem, leaves, fruits and flowers have long been used in local and traditional medicine throughout America, Asia, Oceania, India and Africa.

TAXONOMY

- Kingdom : Plantae
- Subkingdom : Viridiplantae
- Infrakingdom : Streptohyta
- Super kingdom : Embryophyta
- Division : Trachephyta

- Subdivision : Spermatophytina
- Class : Mangoliopsida
- Super order : Liliacae
- Order : Zingiderales
- Family : Musaceae
- Genus : Musa
- Species : Acuminate

MATERIALS AND METHODS

Phytochmeicla Studies

The preliminary phytochemicals screening was done for the extract of ***RED DACCA.*** The screening tests were performed for various phytoconstituents like phenols, tannins, carotenoids and flavonoids.[19]

A. TEST FOR PHENOLS

1. Bramer's test: To 3 ml of extract add dilute ferric chloride solution. The appearances of dark blue or greenish black colour indicates the presence of phenol.
2. Lead acetate test: To the extract add 10% lead acetate solution. Shake well. The appearance of white colour precipitate shows the presences of phenol.

B. TEST FOR TANNINS

1. Bramer's test: To 3ml of extract add ferric chloride solution. The appearances of dark blue or greenish black colour indicates the presence of tannins.
2. Lead acetate test: To the extract add 10% lead acetate solution. Shake well. The appearance of white colour precipitate shows the presences of tannins.

C. TEST FOR FLAVONOIDS

1. Sulphuric acid test: Add H_2SO_4 in sample

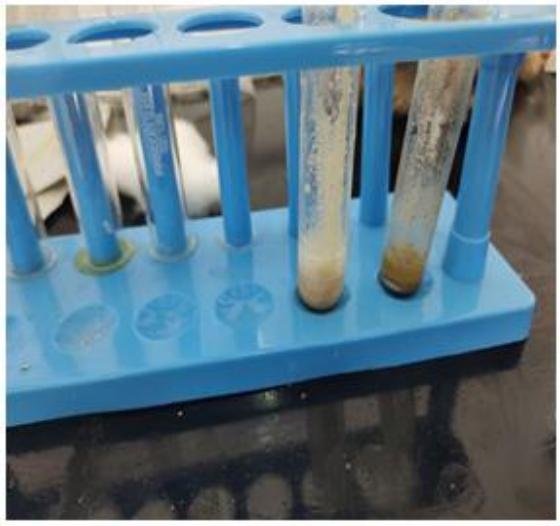
- Flavonoids and Flavonoids: Deep yellow colour
- Chalcones and Aurones: Red or red-bluish
- Flavonones: Orange to red colours

D. TEST FOR CAROTENOIDS

1. Concentrated sulphuric acid test: 1gm of extract adds 10ml of chloroform ($CHCL_3$) in test tube. Shake vigorously and the mixtures were filtered and add 85% of sulphuric acid presence of green or blue colour.

1. TEST FOR PHENOL
(Bramer's test) – appearances of greenish black colour

2. TEST FOR TANNINS
(Lead acetate test) – appearances of white precipitate



3. TEST FOR FLAVONOIDS (Sulphuric acid test) – appearances of orange to red colour

RESULT OF PHYTOCHEMICAL STUDIES

S.NO	CHEMICAL CONSTITUENT	CHEMICAL TEST	PRESENT/ ABSENT
1	PHENOL	BRAMER'S TEST LEAD ACETATE TEST	+ +
2	TANINS	BRAMER'S TEST LEAD ACETATE TEST	+ +
3	FLAVONOIDS	SULPHURIC ACID TEST	+
4	CAROTENOIDS	CONCENTRATED SULPHURIC ACID TEST	+

NOTE: (+) PRESENT

(-) ABSENT

***IN-VITRO* STUDIES**

1. HYDROGEN PEROXIDE SCAVENGING (H_2O_2) ASSAY

Human beings are exposed H_2O_2 indirectly via to the environment nearly about 0.8mg/kg/day with intake mostly from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapour or mist and through eye or skin contact. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH) that can initiate peroxidation and cause DNA damage in the body.[20]

The ability of plant extracts to scavenge hydrogen peroxide can be estimated. A solution of hydrogen peroxide (40 mm) is prepared in phosphate buffer (50mm pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20-60 Ig/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 mins against blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = [(A_i \times A_t)/A_i] \times 100$$

Where,

A_i is the absorbance of control

A_t is the absorbance of test.

Hydrogen peroxide is a weak oxidizing agent and can in active a few enzymes directly, usually by oxidation of essential thiol (-5H) groups. Hydrogen peroxide can across cell membrane rapidly once inside the cell, H_2O_2 can probably react with Fe^{3+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effect. It is therefore, biologically advantageous for cells to control the amount of hydrogen peroxide that

is allow to accumulate. The ability of a compound to scavenge H_2O_2 is a good predictor of its potential antioxidant function. The hydrogen peroxide (H_2O_2) scavenging assay was determining using a UV-VIS spectrophotometer.[21]

RESULT

The absorbance value for control is 3.9 and the absorbance value for test is 1.9. Then the scavenged percentage hydrogen peroxide is

$$\% \text{ Scavenged } (H_2O_2) = [(A_i - A_t)/A_i] \times 100$$

$= [(3.9 – 1.90] \times 100$

$= [(2/3.9)] \times 100$

$= 0.51 \times 100$

% Scavenged (H_2O_2) = 51%

Hydrogen Peroxide Scavenging Assay Using Ultra Violet Visible Spectroscopy

RESULT AND DISCUSSION

TOTAL PHENOLIC CONTENT

Phenols are photochemical obtained from shamanic acid and pentose phosphate via the phenylpropanoid metabolism. This phenolic compound has a hydroxyl substituent on the benzene ring.[22] Phenolic compounds are flavonoids, phenolic acids, flavonoid complexes,

and anthocyanins 1. Phenolic compounds primarily have anti-aging, anti-inflammatory, antioxidant, and anti-cancer benefits. The total content of phenolic compounds in banana extracts (pulp) was determined using a calibration of hydrogen peroxide (reference compound).

TOTAL FLAVONOIDS

Flavonoids are polyphenolic compounds with a benzo-y-pyrone structure synthesized in the phenylpropanoid pathways. They are part of the diet and have a positive effect on health, mainly antioxidants in vitro. Flavonoids have antibacterial, antiviral properties; protect the heart from cancer and other diseases.[23]

Total flavonoid content was measured in Catechin Equivalents (CAE). In general, the pulp contains a higher level of flavonoids than the peel.

TOTAL CAROTINOIDS

Carotenoids are soluble in polar solvents, including edible fats and oils. Because carotenoids are fat-soluble, they are commonly extracted from plant sources using organic solvents such as chloroform, hexane, acetone, and petroleum ether. Samples may contain large amounts of water, an organic solvent that is miscible with water to produce safe extracts. This can be avoided by using food grade solvents such as ethanol. Therefore, ethanol was used as a solvent to obtain a carotenoid concentrate from food crops under semi-production conditions. The addition of banana flour at%, 10%, 20%, 30% and 40% showed an increase in ethanol solvent content.[24] For ethanol and methanol, the solvent has a lower carotenoid content than acetone. Acetone solvents can dissolve more carotenoid compounds than ethanol and methanol solvents. There is a hydroxyl group in the Zeaxantis component, showing a more polar comparison of this compound to its other analogues such as alpha-carotenoid and beta-carotene.

DETERMINATION OF ANTIOXIDANT INGREDIENTS

The antioxidant activity of plant materials is strongly related to the levels of phenolic and flavonoid compounds they contain. Phenols are secondary plant metabolites that are very important in the chelation of redox metal ions, including lipid free radical chains, and prevent the conversion of hydrogen peroxide to reactive oxygen radicals, as is well recognized. The most common water-soluble antioxidants found in plants and foods are phenolic compounds.[25] Flavonoids are classified as one of the important groups of antioxidant

components commonly found in fruits and vegetables. Vitamin E is the most important fat-soluble antioxidant in the cellular defense system and comes exclusively from food.

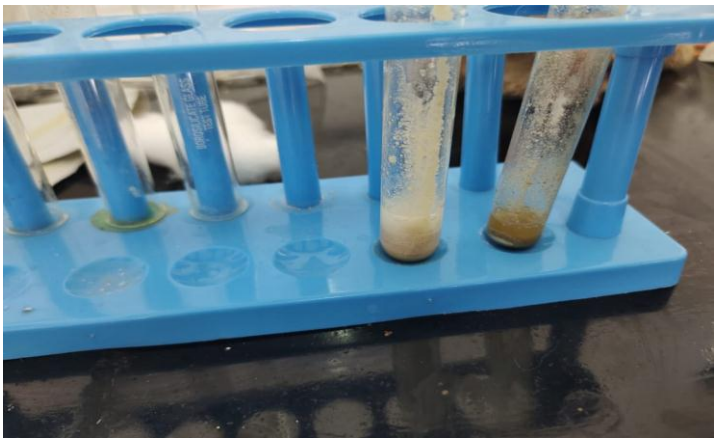
For the antioxidant components contained in ethanolic banana extracts. A pulp sample from Musa acuminata showed a higher phenol content (13.45 = 0.35 mg/g) A than samples from the red banana variety. The results confirmed that banana pulp is a good source of phenols and flavonoids. Phenolic acids and flavonoids are among the most important phytochemicals responsible for the antioxidant capacity of fruit and vegetables. It was found that the vitamin E content of yellow banana flesh was significantly lower than that of the red banana variety. The value found in banana pulp in this study was greater than that of other tropical plants. The results showed that the total phenolic content is higher than the flavonoid content in the ethanolic extract of the banana pulp samples. Therefore, the higher radical scavenging activity of the ethanolic extract of the banana pulp samples may be due to the higher levels of phenolic compoundsin these samples.

Aqueous extracts of yellow and red bananas were low in antioxidants. There is a significant difference in antioxidant components between the two banana pulp extracts. However; the aqueous extracts of the yellow banana varieties contain a much higher amount of antioxidant components than the red banana varieties. This indicates that ethanol is a better solvent for extracting the antioxidant component from red banana pulp than the aqueous extract.

PHYTOCHEMICAL TEST

Different banana solvent extracts prepared in this work showed the presence of the tested phytochemicals such as flavonoids, phenols, glycosides, etc. Different studies have shown the presence of different phytochemicals in different parts of the bananaextracts from different solvents. Several flavonoids and related compounds (leucocyanidin, quercetin and its 3-O-galactoside, 3-O-glucoside and 3-O-rhamnosyl were glycosides) have been isolated from unripe red banana pulp. Serotonin, noradinerin, tryptophan, indole compounds, tannins, starch, iron, crystallizing and non-crystallizing sugars, vitamin C.Group B vitamins, albinoids, fats and mineral salts have been found in the meat of Musa acuminata. Preliminary phytochemical studies have shown that the flesh of Musa accuminata contains some secondary metabolites such as glycosides, alkaloids, saponins and essential oils. Flavonoids and tannins. Preliminary phytochemical studies on selected ethanolic and methanolic extracts of Musa acuminata have revealed the presence of some secondary metabolites. The ethanolic extract contained alkaloids, steroids, tannins, xanthoproteins and glycosides while the

methanolic extract had alkaloids, saponins, xanthoproteins and glycosides. In another work, acetone, ethyl acetate, ethanol, methanol and water are used in the maceration process. Phytochemical studies of these extracts have shown the presence of various phytonutrients such as carbohydrates.



HYDROGEN PEROXIDE SCAVENGING CAPACITY

Ability to scavenge water and ethanolic extracts of Musa accuminata in hydrogen peroxides compared to the standard. Musa acuminata extracts could avenge hydrogen peroxide depending on amount. 100 µg aqueous and ethanolic extracts of Musa acuminata showed hydrogen peroxide scavenging activity ranging from 15.44-30.13%. The results show that the hydrogen peroxide scavenging activity value of 100g Musa acuminata extracts decrease the absorbance values.[26] The reduced absorbance of the reaction mixture was indicative of increased hydrogen peroxide selling activity, indicating an increased level of antioxidant activity.

HYDROGEN PEROXIDE SCAVENGING ACTIVITY USING UV-VIS SPECTRPSCOPY

CONCLUSION

This article is focused on *in-vitro*method of antioxidant evaluation. The uses of banana inflorescences unripe materials for obtaining extracts with antioxidant properties were proved to be a viable alternative with the possibility of industrial application, Most of the cases the antioxidative compound were generally higher in the flesh than in the peel and in the greener than in the ripe components. The highest level of phenolic compounds and flavonoids as the best antioxidant activity, were found under the following extraction conditions; Temperature of 60ºC, ethanol concentration 90% and stirring extraction without the ultrasound, this conditions respect the advantages of reduce costs at the industrial level. There was a relationship between the content of phenolics and flavonoids and *in-vitro* antioxidant potential of extracts of banana inflorescences the total antioxidant activity of Musa accuminata extracts and the standard compounds was determined by the hydrogen peroxide scavenging assay. The antioxidant capacity of samples was determined by the decrease in absorbance readings. Decreased absorbance of the reaction mixture indicated increased hydrogen peroxide scavenging activity, meaning increased level of antioxidant activity.

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