Phytochemical Screening and *In-Vitro* Antioxidant Activity of Different Solvent Extracts of *Musa Rasthali* Fruit

N. FREDERICK[1], J. V. MANI[2]

[1]Department of Chemistry, SHIATS, Allahabad, Uttar pradesh, India.
[2]Department of Chemistry, SHIATS, Allahabad, Uttar pradesh, India.
* **E-mail:** niharikafrederick12@gmail.com

Abstract

The present study investigated the 'Phytochemical Screening and In-vitro antioxidant Activity of Different Solvent Extracts of Musa rasthali fruit'. Extracts were prepared by successive extraction using soxhlet apparatus. Hexane, ethyl acetate and methanol were used as a solvent for successive extraction. Selection of solvents was done on the increasing order of polarity. Phytochemicals like carbohydrates, reducing sugar, alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, cycloglycosides, total phenols, quinines, anthraquinones and steroids were analysed qualitatively whereas total alkaloids, total saponins, tannins, total phenolic contents, total flavonoid contents and total tannin contents were analysed quantitatively. In-vitro antioxidant activity of the fruit extracts of Musa rasthali (Rasthali banana) were expressed as percentage inhibition of DPPH free radicals and IC_{50} values (µg/ml). Spectrophotometric method were used for quantitative analysis and DPPH free radical scavenging activity. The total alkaloids for different solvent fruit extracts of Musa rasthali were expressed in percentage ranged from 22 to 60 %. The total saponins for different solvent fruit extracts of Musa rasthali were expressed in percentage ranged from 26.66 to 51.66 %. The total tannins for different solvent fruit extracts of Musa rasthali were expressed in mg/ml ranged from 0.0045 to 0.0142 mg/ml. The total phenolic contents for different solvent fruit extracts of Musa rasthali ranged from 0.42 to 5.61 µg/ml of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid contents of different solvent fruit extracts of Musa rasthali varied from 6.11 to 24.50 µg/ml, expressed as quercetin equivalents. The total tannin contents of different solvent fruit extracts of Musa rasthali ranged from -1.99 to 4.27 µg/ml of dry weight of extract, expressed as tannic acid equivalents. Percentage inhibition values ranged from 57.04 to 92.29 %. The IC_{50} values in µg/ml of different solvent fruit extracts of Musa rasthali ranged from 9-19 µg/ml respectively. The lower IC_{50} values reflects more antioxidant activity with better protective action. From qualitative analysis, the methanolic extract showed highest amount of phytochemicals than ethyl acetate and hexane extracts of M. rasthali. Among the different fruit extracts of Musa rasthali (Rasthali banana), the methanolic fruit extract of M. rasthali showed the highest phenolic, tannin and flavonoid contents and strong free radical scavenging activity. The high contents of phenolic compounds contributes largely to the antioxidant activity.

Keywords: *Musa rasthali*, Phytochemical screening, Antioxidant activity.

Introduction

The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product, organic chemistry and plant biochemistry and is closely related to both. It is concerned with enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, metabolism, natural distribution and biological functions **(Harborne, 1998)**. Medicinal plants are those plants which contain natural chemical compounds gifted from Almighty God. Medicinal plants also consist of components of therapeutic values and have been used as remedies for human diseases since long. They probably constitute a single larger functional group of the plants globally. According to an estimate, 120 or so plant based drugs prescribed for use through the world come from just 95 plant species **(Lewington, 1990).** Phytochemicals simply means plant chemicals. They are naturally occurring components in fruits, vegetables, legumes and grains. They give plants its colour, flavour, smell and natural defense system (disease resistance). According to (**Liu, 2004)** phytochemicals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major degenerative diseases. Plant metabolites produces products that aids in the growth and developments of plants but are not required for the plants to survive. There are two types of plant metabolites: Primary and Secondary. Secondary plant metabolites facilitate primary metabolites in plants. The primary metabolites consist of chemical reactions that allow the plants to live. In order for the plants to stay healthy, secondary metabolites plays a pinnacle role in keeping all the plants systems working properly. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy was used for the treatment of bacterial infections **(Gracelin *et al.*, 2013).** The qualitative and quantitative distribution of these metabolites differs from plant to plant and part to part **(Kumar *et al.*, 2012).** Plants have basic nutritional importance by their content of proteins, carbohydrates, fats, oils minerals, vitamins and water responsible for growth and development in man and animals. Much more than these, researchers have come up with the fact that some plant chemicals which have been regarded as anti-nutritional or anti-nutrients have potentials in helping to reduce the risk of several deadly diseases in men **(Williamson *et al.*, 1997; Stahl *et al.*, 1998; Agte *et al.*, 2000; Chung *et al.*, 1998)**. Reports show that these phytochemicals reduce LDL i.e.the cholesterol involved in depositing fat in the arteries, prevent blood clotting which can reduce the risk for a heart attack or a stroke. ***Musa* species *(Banana)***, belong to family - Musaceae is the second most important fruit crop in India next to *mango*. Its year round availactivity, affordactivity, varietal range, taste, nutritive and medicinal value makes it the favourite fruit among all classes of people. It has also good export potential. Hi-tech cultivation of the crop is an economically viable enterprise leading to increase in productivity, improvement in produce quality and early crop maturity with the produce commanding premium price. In India, *Musa* species *(Banana)* ranks first in production and third in area among fruit crops. Commercially, *Musa (banana)* are classified as dessert types and culinary types. The dessert types have classic yellow *Musa (banana)* used mainly for cooking and are non - plantains. Important cultivars include ***Musa cavendish*** only. The culinary types have starchy fruits, plantains and are used in the mature unripe form as vegetables. Important cultivars include ***Cavendish banana* (*Musa cavendish), Robusta, Monthan, Poovan, Nendran, Red banana (Musa acuminate), Nyali, Safed Velchi, Basrai, Ardhapuri, Rasthali banana (Musa rasthali), Karpurvalli, Karthali*** and ***Grand Naine*** **(Ehiowemwenguan *et al.*, 2014).** Antioxidants have been widely used as food additives to provide

protection against oxidative degradation of foods. In response to the increased popularity and greater demand for medicinal plants a number of conservation groups are recommending that wild medicnal plants to brought into recultivation **(Aqil *et al.*, 2006).** It is now clear that the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effect on human body. The main features of an antioxidant has activity to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from wide variety of sources. The alternatives to reduce the presence of reactive oxygen in higher organisms have suggested the consumption of fruits rich in bioactive compound such as anthocyanins **(Salinas *et al.*, 2009).** Such natural antioxidant could prevent the formation of the above reactive species related disorders in human beings without the use of synthetic compounds, which may be harmful and carcinogenic to the lungs and liver. Antioxidants can prevent the free radical generation by reacting with them, chelating catalytic metals and also by playing as oxygen scavengers. Although the development of some synthetic antioxidants in the past few years has flourished, they are not yet widely used as therapeutic agents due to their toxicity. As a result, the development of natural antioxidant has now drawn the attention of scientific community and different kinds of plant material have already been reported as 'natural antioxidant' **(Thirugnanasampandan *et al.*, 2009).** The reason for choosing fruits of *Musa rasthali (Rasthali banana)* was its medicinal applications. This is partly because *Musa rasthali (Rasthali banana)* species aid in the body's retention of Calcium, Nitrogen and Potassium; all of which work is to build healthy and regenerated tissues. *Musa rasthali (Rasthali banana)* fruit can be used to fight intestinal disorders and promote healing. Other medicinal benefits of *Musa rasthali* includes aiding in constipation and diarrhea relief, treatment of arthritis, reducing high blood pressure, risk of stokes and in treatment of anemia. In recent years, the material of *Musa rasthali (Rasthali banana)* fruit have gained popularity as they are rich source in phytochemical contents.

Experimentation

The attempt has been made to identify and estimate phytochemicals, qualitatively and quantitatively in different solvent fruit extracts of *Musa rasthali* respectively and to evaluate the *in-vitro* antioxidant activity.

Material and Methods

The present work entitled as **"Phytochemical Screening and *In-vitro* Antioxidant Activity of Different Solvent Fruit Extracts of *Musa rasthali*"** was carried out in Department of Chemistry, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-University), Allahabad-211007, U.P (INDIA).

Collection of plant material:

The plant material of *Musa rasthali (Rasthali banana)* was collected and authenticated by Dr. V.M Prasad, Head, Department of Horticulture, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-University), Allahabad-211007, U.P (INDIA).

Preparation of Plant Materials:

The plant material of *Musa rasthali (Rasthali banana)* fruit was washed thoroughly using tap water and wiped using a clean cloth. The fruits of the *Musa rasthali (Rasthali banana)* fruit were cut into slices. Next, the cut material was allowed for shaded drying. The dried fruit material was powdered using electric blending machine and kept in a tight-capped bottles.

Preparation of Plant Extracts:

The extracts preparation was carried out by Soxhlet extraction procedure. Selection of solvent was done on the basis of increasing polarity using hexane, ethyl acetate and methanol. Soxhlet apparatus was used to extract the crude compound from the *Musa rasthali (Rasthali banana)* fruit using hexane, ethyl acetate and methanol as its extraction solvent. Twenty gram of each *Musa rasthali (Rasthali banana)* extract powder was placed inside a thimble made from thick filter paper, used to get purest form of extract. 300ml of extraction solvent was added into a distillation flask. The solvent was heated to begin the distillation process and the cycle was allowed to stand for three days.

The extract was filtered using whatmann filter paper and the extract was concentrated on a water bath and was stored in a sterile container at refrigerator for 4°C until further use.

Methods:

Preliminary Phytochemical Screening was studied:

Qualitative Phytochemicals Analysis:

Preliminary qualitative phytochemcials screening for bioactive compounds was carried out by the methods as described by (Kokate et al., 2010; Harborne, 1998; Egwaikhide and Gimba, 2007; Savithramma et al., 2011; Evans and Trease, 1999; Sofowara, 1993) :

Detection of carbohydrates:

Molish's test: To 2 ml of the extract, 2 ml of Molish's reagent and 2 ml of conc. Sulphuric acid was added. Formation of a reddish ring indicated the presence of carbohydrate.

Detection of reducing sugar:

Fehling test: Boiled 2 ml of Fehling's solution was added to 2 ml of the extract and boiled for 5 minutes. Formation of a first yellow precipitate then brick red precipitate indicated the presence of reducing sugar.

Detection of alkaloids:

A. Mayer's test: A little of the extract was stirred with Mayer's reagent (potassium mercuric iodide). Formation of cream coloured precipitate indicated the presence of alkaloids.

B. Dragendroff's test: Evaporate the extracts. To the residue, add dil. Hydrochloric acid, shake it well and filter it. Take 2 ml filtrate ,add to it few drops of dragendroff's reagent, precipitate was seen.

Detection of saponins:

Foam test: About 2 ml of the extract was diluted with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

Detection of tannins:

A. Lead acetate test: To 2 ml of the extract, few drops of 1% lead acetate was added and the formation of yellowish precipitate indicated the presence of tannins.

B. Acetic acid solution test: Take small quantity of aqueous extract, add acetic acid solution, red coloured solution was found.

Detection of flavonoids:

Flavonoids test: To a small quantity of the extract dil. Sulphuric acid was added. The appearance of orange colour indicated the presence of flavonoids.

Detection of terpenoids:

Terpenoid test: To 2 ml of extract, 2 ml of conc. Acetic acid and conc. Sulphuric acid was added. Formation of bluish green ring indicated the presence of terpenoids.

Detection of coumarins:

Coumarins test: 3 ml of 10% sodium hydroxide was added to 2 ml of extract. Formation of yellow colour indicated the presence of coumarins.

Detection of cycloglycosides:

A. Keller-killiani's test: To one of the extract, 2 ml of 3.5% ferric chloride solution was added and allowed to stand for one minutes. 1 ml of conc. Sulphuric acid was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface indicated the presence cardiac glycoside.

B. Borntrsger's test: Take 1 ml extract, add dil. Sulphuric acid, heat and filter it, cool the filtrate. Add 1 ml of benzene or chloroform, shake well and separate it to organic solvent. Add 1 ml of ammonia, formation of ammonical layer was not turned pink or red.

Detection of total phenols:

Ferric chloride test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of deep blue colour indicated the presence of phenol.

Detection of quinones:

Quninones test: The extract with 5 ml of conc. Hydrochloric acid resulted in yellow precipitate, indicating the presence of quinones.

Detection of anthraquinones:

Anthraquinones test: To 2 ml of extract, 2 ml of 10% ammonium hydroxide was added. Formation of pink colour indicated the presence of anthraquinones.

Detection of steroid:

A. Salkowski's test: 5mg of extract was dissolved in 2 ml of chloroform and equal volume of conc. Sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids compound, in the extract.

B. Sulphur powder test: Take 1 ml of extract, add few amount of sulpur powder, powder sinked to bottom of the test tube.

Quantitative Phytochemicals Anaylsis:

Preliminary quantitative phytochemcials screening for bioactive compounds was carried out by the methods (Evans and Trease, 1999) described below are:

Determination of Total Alkaloids:

0.5g of extract was extracted with 5ml of methanol: (1:1v/v) mixture and solvent evaporated. The resultant residue was mixed with 20ml of dil. sulphuric acid and partitioned with ether to remove unwanted materials. The aqueous then extracted with excess chloroform to obtain the alkaloid fraction. The chloroform extraction was repeated several times and the bulk of extract was concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to the initial weight of the sample powder (Evans and Trease, 1999).

Alkaloid (%) = [Weight of Alkaloid residue/ Volume taken] X 100

Determination of Total Saponins:

1g of powdered extract was placed in a 500ml flask containing 6ml of 50% alcohol. The mixture was boiled under reflux for (30 minutes) and was immediately filtered while hot through a coarse filter paper. 0.04g of charcoal was added, the content was boiled and filtered while hot. The extract was cooled (some saponins may be separated) and an equal volume of acetone was added to complete the precipitation of saponins. The separated saponins was collected by decantation and dissolve in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter (Evans and Trease, 1999). The filtrate was allowed to cool to room temperature there by resulting in precipitation of saponins. The separated saponins was collected by decantation and suspended in about 20ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride and the saponins was left to dry. It was weighed with reference to the weight of extract used:

Saponins (%) = [Weight of Saponins residue/ Volume taken] X 100

Determination of Tannins:

0.1g extracts was put into a 100ml conical flask and 50ml of distilled water was added. The flask was gently heated to boiling for 1 hour, and the filtrate was collected in a 50ml volumetric flask. The residue was washed several times and the combined solution made to the volume with distilled water. To 10ml of sample solution in a 50ml volumetric flask, 2.5ml of Folin-Denis reagent and 10ml of sodium bicarbonate solution was added and made to volume with distilled water. The samples in the flask was allowed to stand for 20 minutes after which optical density was measured using spectrophotometer at 725nm (Evans and Trease, 1999).

Tannin (mg/ml) = [Absorbance of test/ Absorbance of standard] X Conc. of standard

Total Phenolic Contents:

The total phenolic content in the extracts and the potencies was measured using Folin-Ciocalteu reagent method. The samples (0.4 ml) (1µg/ml extracts) of various concentrations (0.4 to 1.2 ml) was transferred into test tubes. To this solution, distilled water (1.0 ml) and Folin-Ciocalteu reagent (1.0 ml) was added, and the tubes shaken thoroughly. After 1 min, sodium carbonate solution (1.6 ml, 7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. A linear dose response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm using UV-Visible spectrophotometer. The total phenolic compounds concentration in the extract was expressed as µg of gallic acid equivalent per 10mg of dry weight of extract (µg GAE/100ml) (Evans and Trease, 1999).

Total Flavonoid Contents:

The total flavonoids content of the extracts and the potencies was determined according to colorimetric method. In brief, the sample solution (0.5 ml) of various concentrations (0.5 to 1.3 ml) was mixed with distilled water (2 ml) and subsequently with 5% sodium nitrite solution (0.15 ml). After 6 min of incubation, 10% aluminium chloride solution (0.15 ml) was added and then allowed to stand for 6 min, followed by additon of 4% sodium hydroxide solution (2 ml) to the mixture. Consequently, water was added to the sample to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture's absorbance was determined at 510 nm using UV-Visible spectrophotometer. The total flavonoids content was expressed in µg of quercetin equivalent (QE) per milligram of extract (µg QE/10ml) (Evans and Trease, 1999). This method was repeated for all samples.

Total Tannin Contents:

The total tannin content of the given sample was estimated by the following standard procedure. The sample extract(1 ml) of various concentrations (1.0 to 1.8 ml) was mixed with Folin-Ciocalteau's reagent (0.5 ml), followed by the addition of saturated sodium carbonate solution solution (1ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible spectrophotometer. Increasing concentrations of standard tannic acid was prepared. The tannin content was expressed as µg tannic acid equivalent per 10 milligram of the sample (µg TAE/100ml) (Evans and Trease, 1999).

In - Vitro Antioxidant Activity Assay:

The DPPH free-radical scavenging activity assay elucidated by **(Ogunlana and Ogunlana, 2008)** was followed. The hydrogen atom or electron donating activity of the different extract was measured from the bleaching of the purple coloured methanol solution of DPPH. The stable 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical was used for determination of free radical scavenging activity of the extracts. The different concentrations (10-60µg/ml) each of the extract of Banana species was added with 1ml volume of DPPH (4mg in 100ml 95% methanol) methanol solution. DPPH solution was used as control and methanol as blank. Gallic acid was used as standard of concentrations. When 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical reacts with antioxidant, DPPH was reduced from deep violet to light yellow after 30 minutes of incubation in dark at room temperature. The absorbance was measured at 517nm. The percentage free radical scavenging activity of different fruit extract of Musa (banana) species was calculated.

Results and Discussion

Qualitative phytochemical screening: Chemical tests was carried out for bioactive compounds by the methods as described by **(Kokate *et al.*, 2010); (Harborne, 1998).**

S.no.	Reagents/tests	Hexane extract	Ethyl acetate extract	Methanol Extract
1.	Carbohydrates			
	Molish's test	+	+	+

2.	Reducing sugar			
	Fehling's test	-	+	+
3.	Alkaloids			
A.	Mayer's test	+	+	+
B.	Dragendroff's test	+	+	+
4.	Saponins			
	Foam test	+	+	+
5.	Tannins			
A.	Lead-acetate test	-	+	+
B.	Acetic acid solution test	-	+	+
6.	Flavonoids			
	Flavonoid test	-	+	+
7.	Terpenoids			
	Terpenoid test	-	+	+
8.	Coumarins			
	Coumarin test	-	-	-
9.	Cycloglycosides			
A.	Keller-killiani's test	+	+	+
B.	Borntrager's test	+	+	+
10.	Total phenols			
	Ferric-chloride test	-	+	+
11.	Quinones			
	Quinone test	+	+	+
12.	Anthraquinones			

	Anthraquinone test	-	-	-
13.	Steroids			
A.	Salkowski's test	-	-	-
B.	Sulpur powder test	-	-	-

Present = + and absent = -

Table 1: Qualitative phytochemical screening of *Musa rasthali (Rasthali banana)* fruit extracts

The **table.1** showed the qualitative phytochemical analysis of carbohydrates reducing sugar alkaloids, saponins. Flavonoids, tannins, coumarins, terpenoids, cycloglycosides, total phenols, steroids, quinones, anthraquinones.**Table.1** showed the phytochemicals like carbohydrates, alkaloids, saponins, cycloglycosides and quinones are present whereas steroids, coumarins and anthraquinones was absent in different fruit extracts of *musa rasthali (rasthali banana)*. Reducing sugar, flavonoids, terpenoids, total phenols and tannins was present in methanolic and ethyl acetate extracts while it was absent in hexane extract. The highest amount of phytochemicals was present in methanolic extract than ethyl acetate and hexane extracts. The results allowed summing up that *musa rasthali* is a good natural source of phytonutrients of biological significance. From the **table.1** showed that the methanolic extract contains more number of phytochemicals than ethyl acetate and hexane extracts. Many compounds that occur in plant tissues are labile and almost inevitably may undergo change during extraction. Polarity of the solvent used for extraction may influence the group of bioactive compounds obtained from the plant material **(Gunavathy *et al*., 2014)**. It has been observed that, organic solvent especially methanol is found to be more desirable to extract the phytoconstituents **(Okaw, 2001)**. The presence of wide range of phytoconstituents particularly the flavonoids and the phenols exhibit multiple biological activities and can be used in the treatment of various disorders **(Atraqchi *et al*., 2014)**.

Quantitative phytochemical screening:

Chemical test was carried out for bioactive compounds by the methods as described by **(Evans and Trease, 1999)**.

***Musa* species**	**Hexane extract (%)**	**Ethyl acetate extract (%)**	**Methanol extract (%)**
Musa rasthali (Rasthali banana)	**22**	**40**	**60**

Table 2: Total alkaloids in % of *Musa rasthali* (*Rasthali banana*) fruit extracts

The **Table.2**, showed the percentage of total alkaloids for:- *Musa rasthali* were 60%, 40% and 22% for methanol, ethyl acetate and hexane extracts respectively. From the **Table.2**, it was cleared that the percentage of total alkaloids in different fruit extracts of *Musa rasthali (Rasthali banana)* was highest in methanolic extract and lowest in hexane extract. This difference in the percentage was due to the polarity of solvents. Methanol is highly polar solvent followed by ethyl acetate and hexane solvents. It was reported by **(Apriasari *et al*., 2014)** that methanolic extracts of *Mauli banana (Musa* species*)* had the highest concentration of total alkaloids.Selection of solvents was done on the nature of polarity of

solvents used. The use of three solvents for extraction of total alkaloids revealed that the phytochemical composition of the extract varies with the solvent used. Thus, methanol is the best solvent used for the extraction procedure in fruit of *Musa rasthali (Rasthali banana)*. Alkaloids are a diverse group of low-molecular-weight, nitrogen containing compounds found in about 20% of plant species. Plant-derived alkaloids currently in clinical use include the analgesics, anticancer agents, muscle relaxant, antiarrythmic, antibiotic, and sedative **(John *et al.*, 2014)**.

***Musa* species**	**Hexane extract (%)**	**Ethyl acetate extract (%)**	**Methanol extract (%)**
Musa rasthali (Rasthali banana)	**26.66**	**35.00**	**51.66**

Table 3: Total saponins in % of different *Musa rasthali* (*Rasthali banana*) fruit extracts

The **Table.3**, showed the percentage of total saponins for:- *Musa rasthali* were 51.66%, 35.00% and 26.66% for methanol, ethyl acetate and hexane extracts respectively. From the **Table.3,** it showed maximum percentage of total saponins was present in methanolic extract and minimum percentage was present in hexane extract in different fruit extract of *Musa rasthali (Rasthali banana)*. It was reported by **(Apriasari *et al.*, 2014)** that methanol extracts of *Mauli banana (Musa* species*)* had the highest concentration of total saponins. Selection of solvents was done on the nature of polarity of solvents used. The use of three solvents for extraction of total saponins revealed that the phytochemical composition of the extract varies with the solvent used. Thus, methanol is the best solvents used for the extraction procedure of total saponins of *Musa rasthali (Rasthali banana)*. Saponins are natural detergents found in many plants. Saponins have detergent or surfactant properties because they contain both water-soluble and fat-soluble components **(Cheeke, P.R., 2000)**. Some studies suggest used of saponins in medical treatment such as epilepsy, excessive salivation, chlorosis and migraines **(Sumanthy *et al.*, 2011)**.

***Musa* species**	**Hexane extract (mg/ml)**	**Ethyl acetate extract (mg/ml)**	**Methanol extract (mg/ml)**
Musa rasthali (Rasthali banana)	**0.0045**	**0.0049**	**0.0142**

Table 4: Tannins in mg/ml of different *Musa rasthali* (*Rasthali banana*) fruit extracts

The **Table.4**, showed the tannins for:- *Musa rasthali* were 0.0142mg/ml, 0.0049mg/ml and 0.0045mg/ml for methanol, ethyl acetate and hexane extracts respectively. From the **Table.4**, it showed that the tannins was highest in methanolic fruit extract and minimum in hexane fruit extract in different *Musa rasthali (Rasthali banana)*. Selection of solvents was done on the polarity of solvents used. It was reported by **(Apriasari *et al.*, 2014)** that methanolic extracts of *Mauli banana (Musa* species*)* had the highest concentration of tannins because selection of solvents was done on the basis of polarity. The use of three solvents for extraction of tannins revealed that the phytochemical composition of the extract varies with the solvent used. Thus, methanol is the best solvent used for the extraction procedure of tannins in fruits of *Musa rasthali (Rasthali banana)*. Tannins are believed to have some general antimicrobial and antioxidant activities. Tannins are known as polymeric phenolic substances because of its capability of precipitating gelatin from solution which is known as astringency **(Sumanthy *et al.*, 2011)**. Tannins do not act as pro-oxidants and in fact react very rapidly to quench the hydroxyl radical. *In*

vivo studies have shown that tannin protein complexes in the gastrointestinal tract provide persistent antioxidant activity **(Parimala and Shoba, 2013)**.

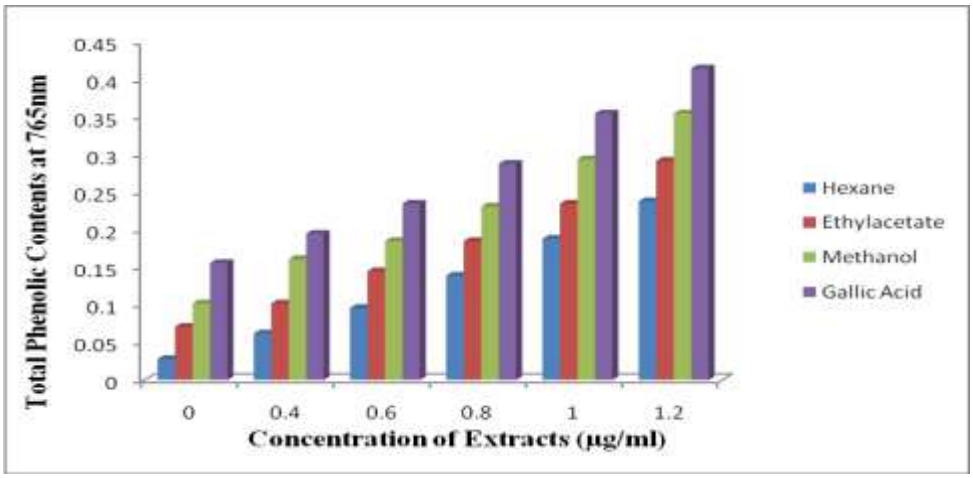

Figure 1: Total phenolic contents of different fruit extracts of *Musa rasthali (Rasthali banana)*

Total phenolic contents of *Musa rasthali (Rasthali banana)* fruit extracts was carried out using Folin-Ciocalteu reagent (FCR) in terms of gallic acid equivalent per 100ml of dry weight of extract(µg GAE/100ml) **(Evans and Trease, 1999)**. The total phenolic contents was obtained at different concentrations (0 to 1.2 µg/ml) of different fruit extracts are hexane, ethyl acetate and methanol respectively. From the **Figure.1**, the total phenolic content of different fruit extracts for methanol (5.61µg/ml), ethyl acetate (3.21µg/ml) and hexane (0.42µg/ml) whereas of standard gallic acid was (8.64µg/ml) respectively. The total phenolic contents was highest in gallic acid than the fruit extracts of *Musa rasthali (Rasthali banana)* species. From the **Figure.1**, it was concluded that the total phenolic contents in the fruit extracts of *Musa rasthali (Rasthali banana)* was high in methanolic extract than ethyl acetate and hexane fruit extracts. Selection of solvents was done on the nature of polarity used. In **Figure.1**, when methanolic extracts of *Musa rasthali (Rasthali banana)* was compared with the standard gallic acid then the total phenolic contents of methanolic fruit extracts of *Musa rasthali (Rasthali banana)* was less than the gallic acid. According to **(Sulaiman *et al.*, 2011)**, they said that heat treatment applied during soxhlet extraction also may increase the extraction of phenolic compounds from fruit materials of *Musa rasthali (Rasthali banana)*. Solvents used for extraction also significantly affected the total phenolic concentration. It was reported by **(Singhal and Ratra, 2013)** that the high concentration of phenols was measured in methanolic extracts of *Musa acuminate* peel. The extracts obtained using more polar solvents contain large concentration of phenols than the low polar solvents contain small concentration of phenols.

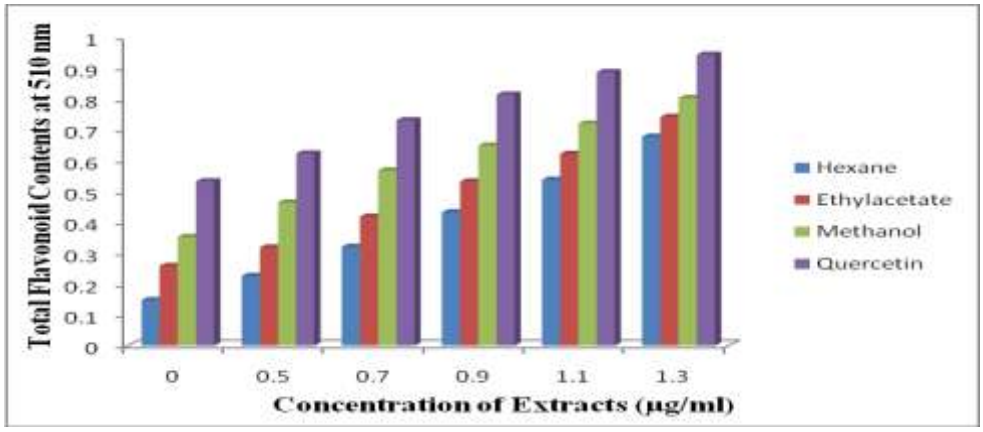

Figure 2: Total flavonoid contents of different fruit extracts of *Musa rasthali (Rasthali banana)*

Total flavonoid contents of *Musa rasthali (Rasthali banana)* fruit extracts was carried out using Colorimetric method in terms of quercetin equivalent per 100mg of dry weight of extract (µg QE/100ml) **(Evans and Trease, 1999)**. The total flavonoid contents was obtained at different concentrations (0 to 1.3 µg/ml) of different fruit extracts are hexane, ethyl acetate and methanol respectively. From the **Figure.2**, the total flavonoid content for fruit extracts of methanol (24.50µg/ml), ethyl acetate (14.65µg/ml) and hexane (6.11µg/ml) whereas of standard quercetin (37.90µg/ml) respectively. The total flavonoid contents was highest in quercetin than different fruit extracts of *Musa rasthali (Rasthali banana)*. From the **Figure.2**, it was concluded that total flavonoid contents in the fruit extracts of *Musa rasthali (Rasthali banana)* was high in methanolic extract than ethyl acetate and hexane extracts. Selection of solvents was done on the nature of polarity used. In **Figure.2** showed that the total flavonoid contents in methanolic extracts of *Musa rasthali (Rasthali banana)* was less than standard quercetin. It was reported by **(Singhal and Ratra, 2013)** that the high concentration of flavonoids was measured in methanolic extracts of *Musa acuminate* peel. The extracts obtained using more polar solvents contain large concentration of flavonoids than the low polar solvents contain small concentration of flavonoids.

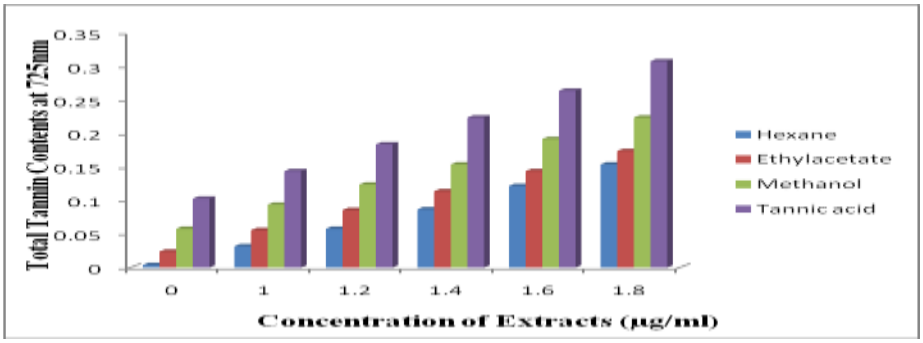

Figure 3: Total tannin contents of different fruit extracts of *Musa rasthali (Rasthali banana)*

Total tannin contents of *Musa rasthali (Rasthali banana)* fruit extracts was carried out using Folin-Ciocalteu reagent (FCR) method in terms of tannic acid equivalent per 100mg of dry weight of extract (µg TAE/100ml) **(Evans and Trease, 1999)**. The total tannin contents was obtained at different concentrations (0 to 1.8 µg/ml) of different fruit extracts are hexane, ethyl acetate and methanol respectively. From the **Figure.3**, the total tannin content for fruit extracts of methanol (4.27µg/ml), ethyl

acetate (0.60µg/ml) and hexane (-1.99µg/ml) whereas of standard tannic acid was (8.87µg/ml) respectively. From the **Figure.3**, it was concluded that total tannin contents in the fruit extracts of *Musa rasthali (Rasthali banana)* was high in methanolic extract than ethyl acetate and hexane extracts. Selection of solvents was done on the nature of polarity used. **Figure.3** also showed that methanolic extracts has less total tannin contents than standard tannic acid. It was reported by **(Apriasari *et al*., 2014)** that methanolic extracts of *Mauli banana (Musa* species*)* had the highest concentration of total tannins contents. Selection of solvents was done on the increasing nature of polarity (methanol > ethyl acetate > hexane).

In vitro - antioxidant activity assay:

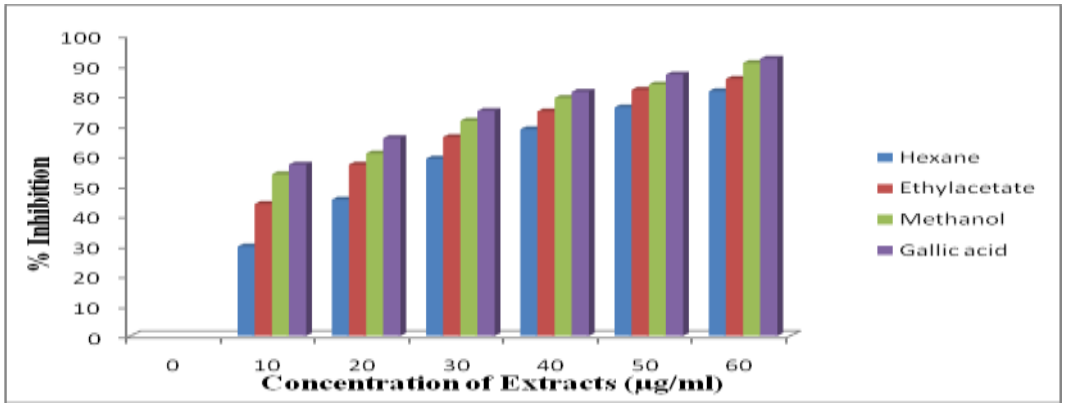

Figure 4: DPPH free radical scavenging activity of different fruit extracts of *Musa rasthali (Rasthali banana)* against standard gallic acid

Figure.4, showed the DPPH free radical scavenging activity of *Musa rasthali (Rasthali banana)* in different fruit extracts with various concentration (10-60µg/ml). The extraction procedure does affect the antioxidant activity of the extracts **(Sulaiman, 2011)**. The extraction of antioxidant substances of different chemical structure was achieved using solvents of different polarity **(Canadanovic *et al.*, 2008)**. The extract which shows highest antioxidant activity **(Figure.4)** has the highest phenolic contents. Phenols are important constituents of plants because of their scavenging activity on free radicals due to their hydroxyl groups. Therefore, the phenolic contents of plants may contribute directly to their antioxidant action **(Tosun *et al.*, 2009)**. **Figure.4** also showed that the methanolic fruit extract gives highest free radical scavenging activity followed by ethyl acetate and hexane extracts of *Musa rasthali (Rasthali banana)*. The free radical scavenging activity increases as concentration of various fruit extracts increases. When it was compared with standard gallic acid, it was found that methanolic extract show lower % inhibition. This type of work was earlier reported by **(Vadnere *et al*., 2012)**. It was also reported by **(Singhal and Ratra, 2013)** that the methanolic extract show maximum scavenging activity of *Musa acuminate* peel which were almost significant to that of standard.

50% Inhibition Concentration Value (IC_{50}):

Musa Species	Hexane extract (µg/ml)	Ethyl acetate extract (µg/ml)	Methanol extract (µg/ml)	Gallic acid (µg/ml)
Musa rasthali (Rasthali banana)	**19**	**9**	**9**	**4**

Table 5: *In-vitro* 50% inhibition concentration (IC_{50}) of different solvent fruit extracts of *Musa rasthali* (*Rasthali banana*)

From the **Table.5**, it shows the 50% inhibition concentration (IC_{50}) of different solvent fruit extracts of *Musa rasthali (Rasthali banana)* calculated from DPPH free radical scavenging activity for *Musa rasthali (Rasthali banana)* of hexane extract was 19 µg/ml, ethyl acetate was 11 µg/ml and methanolic extracts was 9 µg/ml respectively and for gallic acid was 4 µg/ml. Among the three different solvent fruit extracts of *Musa rasthali* (*Rasthali banana*), methanolic extract shows better IC_{50} value than ethyl acetate and hexane extracts. The "Inhibition concentration" also called as IC_{50} value was defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour) **(Molyneux, P 2004).**

From the **Table.5**, it also showed that 50% inhibition concentration (IC_{50}) of methanolic extracts of *Musa rasthali* (*Rasthali banana*) was greater than the standard gallic acid on the basis of following trends i.e., Hexane extract > Ethyl acetate extract > Methanolic extract > Standard gallic acid. This type of similar result was earlier reported by **(Rao *et al*., 2012)**. It was earlier reported by **(Molyneux, P (2004),** that higher the antioxidant activity, the lower is the value of IC_{50}. Lower IC_{50} value reflects better protective action which was earlier reported by (**Patel, 2011**).

Conclusion:

From qualitative analysis, among the different solvent fruit extracts of *Musa rasthali (Rasthali banana)* showed highest amount of phytochemicals in methanolic extract than ethyl acetate and hexane extracts. From quantitative analysis, the different solvent extracts of *Musa rasthali* (*Rasthali banana*): the % of total alkaloids, % of total saponins, total tannins in mg/ml, total phenolic contents, total tannin contents and total flavonoid contents was found highest in methanolic fruit extract than ethyl acetate and hexane fruit extracts. It was noticed that the high contents of phenolic compounds in the extracts were obtained using solvents of high polarity; the methanolic extract manifested greater power of extraction for phenolic compounds. When the free radical scavenging activity for the different fruit extracts of *Musa rasthali (Rasthali banana)* was compared with the standard gallic acid, it was less than standard gallic acid which is 92.29%. At higher concentration i.e., 60 µg/ml, the percentage inhibition of free radical scavenging activity became highest. When the concenteration increases, the percentage inhibition also increases. The IC_{50} value of methanolic extract compared with standard gallic acid was found to be lowest than the methanolic extract. The lower the IC_{50} value, the higher the antioxidant activity. The IC_{50} value of different fruit extracts of *Musa rasthali (Rasthali banana)* compared with standard gallic acid suggested that these extracts have potent antioxidant activity.

The high contents of phenolic compounds, flavonoids compounds showed a linear correlation with antioxidant compounds indicated that these compounds may contribute to the strong antioxidant activity.

This preliminary phytochemical screening, both qualitative and quantitative & antioxidant activity has revealed its phytochemical composition varies with the solvent used. Fruit of *Musa rasthali (Rasthali banana)* are of low cost, easily available in the whole year and can be consumed by rich and poor people. The beneficial medicinal effects from fruit of *Musa rasthali (Rasthali banana)* species resulted from the combination of secondary metabolites present in these fruits. It was concluded that fruit of *Musa rasthali (Rasthali banana)* species was regarded as natural plant sources of antioxidants of high importance.

Further studies are recommended on the fruit of *Musa rasthali (Rasthali banana)* species to identify, isolate and characterize and elucidate the structure of bioactive compounds. This study suggested the great value for fruit of *Musa rasthali* (*Rasthali banana*) species for its use in pharmacological properties which will help in the development of new bioproducts. The three different species of *Musa rasthali* have better antioxidant properties which could be a very useful reactive oxygen species that are formed during oxidative stress.

Acknowledgements

i). The author are very Thankful to Department of Chemistry, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-University), Allahabad-211007, U.P (INDIA).

ii) I am very thankful to Dr. V.M. Prasad, Head, Department of Horticulture, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-University), Allahabad-211007, U.P (INDIA) for providing fruits of *Rasthali banana* and *Red banana*.

iii) It's my pleasure to express my thanks to Dr. Amit Chattree (Member), Associate Dean & Head of Department, Department of Chemistry for giving an opportunity to do my dissertation work in Chemistry Department.

iv) I express my heartfelt and sincere thanks to Mrs. Jyoti V.Mani (Advisor) for noble guidance, valuable suggestion and constant support throughout the research work.

v) I express my sincere thanks to Dr. Manisha Prasad (Co-Advisor), who has given valuable guidance and help during my research work.

vi) I am grateful to Dr. E.P. Lal (Chairman), for her blessing and words of encouragement during my research work.

References:

[1] Apriasari, M.L., Iskandar. And Suhartono, E. "Bioactive compound and antioxidant activity of methanol extract *Mauli Banana's* (*Musa* sp) stem", *International Journal of Bioscience, Biochemistry and Bioinformatics,* 4(2):110-115, 2014.

[2] Aqil, F., Ahmed, I. And Mehmood, Z. "Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants", *Turkish Journal of Biol*ogy, 30:177-183, 2006.

[3] Atraqchi, N. And Wafaa, M. "Preliminary phytochemical screening and in vitro evaluation of antioxidant activity of Iraqi species of *Silybum marianum* seeds", *International Journal of Pharmacy*, 5(5):378-383, 2014.

[4] Canadanovic, B.J., Cetkovic, G., Dilas, S., Tumbad, V., Bogdanovic, G., Mandic, A., Markow, S., Cvetkovic, D. And Canadanovic, V. "Radical scavenging, antibacterial and

antiproliferative activities of *Melissa officinalis* L. Extracts", *Journal of Medicinal Food,* 11:133-143.

[5] Cheeke, P.R. "Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition", *Journal of Animal S*ciences, 77:1-10,2000.

[6] Egwaikhide, P. A. And Gimba, C.E. "Analysis of the phytochemical content and anti-microbial activity of *Plectranthus glandulosis* whole plant", *Middle-East Journal of Scientific Research,* 2(3-4):135-138, 2007.

[7] Evans, W. C. And Trease. Trease and Evans Pharmacognos, 14th Edition W. B. Saunders Company Limited, New York, 134-156, 1999.

[8] Gunavathy, N., Padmavathy, S. And Murugavel, S.C. "Phytochemical evaluation of *Musa acuminate* bract using screening of FTIR and UV-VIS spectroscopic analysis", *Journal of International Academic Research for Multidisciplinary*, 2(1):212-220, 2014.

[9] Harborne, J.B. Phytochemical methods: a guide to modern techniques of plant analysis, third ed. Chapman and hall, London, 302,1998.

[10] John, B., Sulaiman, C.T., George, S.S. and Reddy, V.R.K. "Spectrophotometric estimation of total alkaloids in selected *Justicia* species", *International Journal of Pharmacy and Pharmaceutical Sciences,*6(5):647-648, 2014.

[11] Kokate, C. K, Purohit, A. P. And Gokhale, S. B. *Pharmacognosy,* Nirali prakashan, Pune, 6.15-6.36, 2010.

[12] Lewington, A. Plants for people: the natural history museum,London, UK, 232, (1990).

[13] Liu, R.H. "Potential synergy of phytochemicals in cancer prevention: mechanism of action", *Journal of Nutrition,* 134:34795-34855, 2004.

[14] Min, G. And Chun-Zhao, L. "Comparison of techniques for the extraction of flavonoids from cultured cells of *Saussurea medusa* Maxim", *World Journal of Microbiology and Biotechnology*, 21:1461-1463, 2005.

[15] Mohsen, M.S. and Ammar, S.M.A. "Total phenolic contents and antioxidant activity of corn tassel extracts", *Food Chemistry*, 112: 595-598, 2008.

[16] Molyneux, P. "The use of the stable free radical diphenylpicryhydrazyl (DPPH) for estimating antioxidant activity", *Songklanakarin Journal of Science and Technology,* 26(2):211-219, 2004.

[17] Ogunlana, O.E. and Ogunlana, O.O. "In-vitro assessment of the free radical scavenging activity of *Psidium guajava*", *Journal of Agriclture and Biological Sciences,* 4(6):666-671, 2008.

[18] Okaw, D.E. "Evaluation of chemical composition of indigenous spice and flavouring agent", *Nigeria Journal of Sustain Agriculture & Environment*, 6(1):30-37, 2001.

[19] Parimala, M. And Shoba, F.G. "Phytochemical analysis and *in-vitro* antioxidant acitivity of hydroalcoholic seed extract of *Nymphaea nouchali Burm*. F", *Asian Pacific Journal of Tropical Biomedication,* 3(11): 887-895, 2013.

[20] Patel, R.M. and Patel, N.J. "*In vitro* antioxidant activity of coumarin compounds by DPPH, super oxide and nitric oxide free radical scavenging methods", *Journal of Advanced Pharmacy Education & Research,* 1:52-68, 2011.

[21] Rao, G.B., Rao, Y.V., Pavani, S. And Dasari, V.S.P. "Qualitative and quantitative phytochemical screening and in-vitro antioxidant and antimicrobial activities of *Elephantopus scaber* Linn.", *Recent Research in Science and Technology,* 4(4):15-20, 2012.

[22] Salinas, M.Y., Almaguer, V.G., Pena, V.G. and Rios, S.R. "Ellagic acid and anthocyanin profiles in fruits of *Raspberry* (*Rubusidaeus* L.) In different ripening stages", *Revista Chapingo Serie Horticulture,* 15:97-101, 2009.

[23] Savithramma, N., Rao, L.M. and Suhrulatha, D. "Screening of Medicinal Plants for secondary metabolites", *Middle-East Journal of Scientific Research,* 8(3): 579-584, 2011.

[24] Singhal, M. And Ratra, P. "Antioxidant activity, total flavonoid contents and total phenolic contents of *Musa acuminate* peel extracts", *Global Journal of Pharmacology,* 7(2):118-122, 2013.

[25] Sofowora, A. "Medicinal Plants and Medicine in Africa", *John Willey Spectrum Book Ibadan Nigeria,* 120-123, 1993.

[26] Stankovic, M.S. "Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. Extracts", *Kragujevac Journal of Science,* 33:63-70, 2011.

[27] Sulaiman, S.F., Nor Adlin Md. Yusoff, N.A., Eldeen, I.M., Seow, E.M., Sajak, A.A.B., Supriatno. and Ooi, K.L. "Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian *banana's* (*Musa* sp.)", *Journal of Food Composition and Analysis,* 24:1-10, 2011.

[28] Sumathy, V., Jothy Lachumyl, S., Zakaria, Z. And Sasidharan, S. "*In-vitro* bioactivity and phytochemical screening of *Musa acuminate* flower", *Pharmacologyonline,* 2:118-127, 2011.

[29] Thirugnanasampandan, R., Mutharaian, V.N. and Narmatha, B.V. "In-vitro propagation and free radical studies of *Smilax zeylanica* Vent.", *African Journal of Biotechnology,* 8:395-400, 2009.

[30] Tosun, M., Ercisli, S., Sengul, M., Ozer, H. and Polat, T. "Antioxidant properties and total phenolic content of eight Salvia species from Turkey", *Biological Journals,* 41:175-181, 2009.

[31] Vadnere, G.P., Patil, A.V., Wagh, S.S and Jain, S.K. "In vitro free radical scavenging and antioxidant activity of *Cecer arietinum", International Journal of Pharmaceutical Technological Research* CODEN (USA), 4:343-350, 2012

[32] Williamson, G., Dupont, M.S., Heaney, R.K., Roger, G. And Rhodes, M.J. "Induction of slutathione S transferase activity in hepg2 cells by extracts of fruits and vegetables", *Food Chemistry,* 2:157-160, 1997.

[33] Zhou, K. And Yu, L. "Effects of extraction solvent on wheat bran antioxidant activity estimation", *Lebensmittel – Wissenschaft Technologic, Food Science & Technology by Elsevier,* 37:717-721, 2004.