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STUDIES ON THE PRELIMINARY PHYTOCHEMICAL SCREENING, *IN-VITRO* ANTIOXIDANT AND *IN-VIVO* TOXICITY OF *BANANA INFLORESCENCE*

M. C. Kamaraj *, Akshaya Ramakrishnan and Bhanu Deepthi V.

Centre for Product Development, Heavenly Fuel Pvt Ltd, Besant Nagar, Chennai - 600090, Tamil Nadu India.

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Banana inflorescence, Phytochemical constituents, Acute oral toxicity, Antioxidants, Albino rats

Correspondence to Author:
Dr. M. C. Kamaraj

Research Associate,
Centre for Product Development,
Heavenly Fuel Pvt Ltd, Besant Nagar,
Chennai - 600090, Tamil Nadu, India.

E-mail: koushigan@gmail.com

ABSTRACT: The current study aimed at investigating the phyto-chemical constituents, *in-vitro* antioxidant activity and *in-vivo* acute oral toxicity of *Banana inflorescence* (blossom) of *Musa acuminate*. All the experiments were conducted using standard procedures. The results of the phytochemical screening revealed the presence of different types of secondary metabolites like alkaloids, phenols, saponins, flavonoids, steroids, tannins, and glycosides. The acute toxicity study was conducted in Wister albino rats. The *Banana inflorescence* was administered as a single dose of 2000 mg/kg body weight and observed the behavioral changes and mortality rates. In this study, no visible behavioral changes and mortality observed up to 2000 mg/kg of the Wister albino rats. The antioxidant activity of *Banana inflorescence* was evaluated by DPPH free radical scavenging assay. Rutin was used as a reference compound. The IC_{50} value of *Banana inflorescence* was found to be 46.47372 % and the standard rutin was 27.69024%. We conclude that *Banana inflorescence* extract did not exhibit any toxic effects on mice. This extract also possesses many phytochemicals and significant antioxidant activity. Therefore, *Banana inflorescence* may be useful for therapeutic purposes.

INTRODUCTION: In developing countries, about 80% of the population uses natural products for various diseases [1]. Herbal drugs are generally considered as safe and have no side effects. Phytochemicals are responsible for the medicinal activity of plants [2]. These are non-nutritive chemicals that have sheltered humans from various diseases. Phytochemical constituents are the essential source for the organization of numerous pharmaceutical industries.

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In modern years, there has been major importance in identifying plant products with antioxidant properties, as free radicals are considered to play a major role in human diseases such as diabetes, cancer and inflammation [3]. The *in-vivo* toxicological effect of the extract of *Banana inflorescence* has not been reported. It is consequently deemed necessary to assess the acute oral toxicity of the extract of *Banana inflorescence* in the Wistar albino rat model.

This toxicity study would provide a baseline for further studies in developing this plant as herbal medicine. The *Banana inflorescence* of *Musa acuminate* is a by-product of bananas from the genus *Musa*, which belongs to the family of Musaceae. The *Banana inflorescence* has tremendous nutritional value, is a rich source of

dietary fiber and has some biologically active compounds like vitamin C, tannin, alpha-tocopherol and myoinositol phosphate [4]. Various species of *Musa* are reported as possessing diverse medicinal properties. Since, ancient times, *Banana inflorescence* has been used to treat excessive blood loss during the menstrual cycle [5]. It is very helpful to lower the muscle contractions due to the ability of blossoms to regulate the progesterone hormone that can, in turn, reduce painful bleeding. Besides, it also contains magnesium which can reduce anxiety during that period.

It is also believed to help women who are suffering from the polycystic ovarian syndrome. According to Bhavaprakasha Nighantu, the properties of *Banana inflorescence* are unctuous, sweet, astringent, heavy to digest, cold in potency, curing bleeding disorders, and Phthisis by reducing Vata-Pitta. *Banana inflorescence* has been used as a tonic for female disorders like menorrhagia, dysfunctional uterine bleeding, urinary calculi, etc. The modern era is observing a surge of gynecological diseases, especially menorrhagia, premenopausal bleeding, and Dysfunctional Uterine Bleeding (DUB). DUB is also abnormal uterine bleeding without a demonstrable organic cause [6]. DUB is one of the most commonly found gynecological diseases. It is divided into ovulatory and anovulatory. In adolescent and premenopausal age groups, 90% of DUB is anovulatory [7].

DUB can be either treated medically or surgically. The DUB treatment usually consists of measures to control acute bleeding, avert future bleeding episodes, and prevent serious long-term consequences of anovulation and endometrial cancer [8]. Management depends on the age and requirements of the patient. Contemporary lifestyle and dietary habits have impacted uterine health to a greater extent. In this scenario, the search for natural remedies is the need of the hour. Classical textual references to medicinal plants and traditional household herbal remedies are the most sought-after solution. It will be a great contribution to the management of DUB and similar bleeding disorders in women if the drug is time tested in terms of traditional knowledge along with scientific validation. Therefore, this study was conducted to scientifically validate the effect of *Banana inflorescence* on dysfunctional uterine bleeding. We have prepared *Banana inflorescence* by extracting the florets of the blossom. This study has examined preliminary phytochemical constituents, *in-vitro* antioxidant activity and *in-vivo* acute oral toxicity of *Banana inflorescence.*

MATERIALS AND METHODS:

Collection of Plant Material: *Musa acuminate* was collected from the papanasam surroundings of Thanjavur district, Tamil Nadu, India. The plant was identified by Dr. S. John Britto, Director, Rabinat Herbarium and center for Molecular systematic, St. Joseph's College, Trichy, and Tamil Nadu, India. The blossoms were separated from bracts, cleaned and sundried. Dried samples were finely powdered and kept in an airtight bottle under refrigeration till further analysis.

Chemicals: Folin – Ciocalteu reagent, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, DPPH (2,2-Diphenyl-1-picryl-hydrazyl), Rutin, Gallic acid, ferrous chloride, ferric chloride, Hydrochloric acid, sulphuric acid, sodium phosphate, ascorbic acid, were purchased from Hi-Media (Mumbai). All the chemicals and solvents used in this study were analytical grade.

Preliminary Phytochemical Screening: The preliminary phytochemical screening of powder extract of *Banana inflorescence* was done to find out the different phytochemical constituents such as alkaloids, phenolic compounds, flavonoids, saponins, tannins, glycosides, steroids and terpenoids using standard methods [9, 10].

Hager's Test for Alkaloids Determination [11]: About 50 mg solvent-free powder was stirred with 5 ml of dilute hydrochloric acid and filtered. To the filtrate, 2 ml of Hager's reagent (aqueous solution of picric acid) was added. A yellow precipitate appears that indicates the presence of alkaloids.

Ferric Chloride test for Phenolics Determination [12]: About 50 mg of the powder was dissolved in 5 ml of distilled water. To this, a few drops of 5% neutral ferric chloride solution was added. The presence of Phenolic compounds is indicated by the appearance of dark green colour.

Potassium Hydroxide Test for Tannins Determination [13]: The powder extract (500 mg) was added to 10 ml of freshly prepared 10%

potassium hydroxide (KOH) in a beaker and shaken to dissolve. A dirty precipitate formation indicates the presence of tannins in the sample.

Alkaline Reagent Test for Flavonoids Determination [14]: An aqueous solution of the powder was treated with a 10% ammonium hydroxide solution. The appearance of bulky white precipitate indicates the presence of flavonoids.

Liebermann-Burchard Reaction for Terpenoids Determination: About 50 mg of the powder was added to 1mL of chloroform, mixed and then added to acetic anhydride, followed by concentrated sulphuric acid from the sides of the tubes. The appearance of red and bluish-green colour indicates the presence of steroids and triterpenoids.

Frothing Test for Saponins Determination [15]: The powder extract (50 mg) was diluted with distilled water and made up to 10 ml. The suspension was shaken in a graduated cylinder for 15 min; an increase in the layer of foam indicates the presence of saponins.

Borntrager's Test for Glycosides Determination: About 50mg of powder was hydrolyzed with concentrated hydrochloric acid for 2 h in a water bath and filtered. To 2 ml of filtrate, 3 ml of chloroform was added and shaken. The chloroform layer was separated and a 10% ammonia solution was added. The formation of pink colour indicates the presence of glycosides.

Salkowski Test for Steroids Determination: 2 ml of chloroform and 1ml concentrated sulphuric acid were added to 10 drops of the powder mixed with isopropyl alcohol, slowly until double phase formation. The presence of a dish-brown colour in the middle layer marks the presence of a steroidal ring.

Determination of Total Phenolics: The quantitative estimation of phenolics in the extract of *Banana inflorescence* was determined based on the standardized method [16]. About 0.5 ml of 1N Folin-Ciocalteu reagent was added to the 2.5 ml of 20% sodium carbonate solution and then the volume was made up to 10 ml with water. Followed by 40 min dark incubation, the absorbance was recorded at 725 nm against blank for the estimation of phenolics. The results were based on the calibration curve: $y = 0.029x - 0.065$, $R^2 = 0.955$, where x was the absorbance and y was the Gallic acid equivalents (mg/g) and the results were expressed in terms of milligrams of Gallic acid equivalents (GAE) per gram of extract.

Determination of Total Flavonoids: The total flavonoid in the extracts is estimated by the general procedure [17]. To each 300 µl of Banana blossom powder extracts 2 ml of distilled water was added, followed by150 µl of NaNO2. The contents of the tubes were subjected to incubation for 6 min at room temperature. After incubation 150 µl of AlCl3 (10%) was added and incubated again for 6 minutes. Then 2 ml of 4% NaOH was added, vortexed well, and kept at room temperature for another 15 min. The pink color's absorbance, thereby developed, was read spectrophotometrically at 510 nm. The results were based on the calibration curve: $y = 0.002x + 0.006$, $R^2 = 0.992$, where x was the absorbance and y was the rutin equivalents (mg/g) and the results were expressed in terms of milligrams of rutin equivalents per gram of extract.

***In-vitro* Antioxidant Assays:**

DPPH Radical Scavenging Activity: The radical scavenging activity of the extract was determined by the standardized method of DPPH radical scavenging activity [18]. A methanol solution of the sample extract at various concentrations was added to 5 ml of 0.1 mm methanolic solution of DPPH and allowed to stand for 20 min at 27°C. The absorbance of the solution was read spectrophotometrically at 517 nm. Methanol was served as blank, and a solution without powder extract of *Banana blossom* served as the negative control. The methanol, DPPH and standard rutin mixture served as a positive control. The IC50 value of the extract expressed the radical scavenging ability of the extract.

***In-vivo* Toxicity Assay:** The experiments were carried out on 8-9 weeks old healthy Wistar albino female rats weighing between 137 to 154g were used. The experimental procedures followed in this study were according to internationally acknowledged principles on laboratory animal use and care. The experimental procedures relating to the animals were duly approved by the University

Animal Ethical Committee [19]. Ethical clearance was obtained from the Institutional Animal Ethical Committee [JSSCP/IAEC/OT/Pharmacology/33/2019-20]. All procedures were in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services document and guidelines established by the Institutional Animal Ethical Committee of the University.

Determination of Acute Oral Toxicity: Wister albino rats were kept in the department in well-aerated polycarbonate cages under standard conditions 23.4-28.8° 12 h light and 12 h dark cycle. The rats were housed in cages at random, selected ones were tagged and marked on the cages for identification. They were permitted to adapt to laboratory conditions for a week before starting the experiment. Drinking water and food (*Ad-libitum)* were provided during the experimental period. According to the procedures reviewed by the Organization for Economic Co-operation and Development [OECD]. The dose of 2000 mg/kg body weight of *Banana inflorescence* was administered orally to the rats in the treatment group. The dose limits were selected based on acute oral toxicity studies in rats, in conformity with the organization supporting Economic Co-operation and Development [OECD] guidelines 423. The acute toxicity test was carried out in rats by repeated 2000 mg/kg body weight doses. Food was provided to the rats approximately an hour after treatment. The rats were observed for any sign of toxicity effect for the first 3 h, after the treatment period, up to 15 days. Visual observations on mortality, behavioral pattern, and changes in physical appearance, wound, ache, and signs of illness were monitored up to 15 days at 24 h interval [20].

RESULTS AND DISCUSSION:

Qualitative Phytochemical Screening: The *Banana inflorescence* has a great nutritional value, similar to banana fruits. They are excellent sources of vitamins, minerals, a good source of fiber and protein. In the current study, the presence of alkaloids, phenolic compounds, flavonoids, tannins, saponins, steroids, and terpenoids in the *Banana inflorescence* was confirmed and depicted in **Table 1.** The strong phytochemicals have health benefits and provide protection from the risk of cancer, cardiovascular disease, cholesterol, diabetes and hypertension *etc.* [21].

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF *BANANA INFLORESCENCE*

S. no.	Phytochemical constituents	Presence or absence
1	Alkaloids	++
2	Phenolic compounds	++
3	Tannins	+
4	Flavonoids	++
5	Terpenoids	+
6	Steroids	+
7	Glycosides	+
8	Flavanol glycosides	+
9	Cardiac glycosides	-
10	Saponins	+
11	Phytosterol	+
12	Fixed oils and fats	+
13	Carbohydrates	++
14	Proteins	++
15	Amino acids	+

(+): presence of chemicals, (-): absence of chemicals or not detectable concentration, (+) < (++) < (+++): based on the intensity of characteristic.

Alkaloids are naturally occurring chemical compounds in plant parts that normally have pharmacological effects. Phenolic compounds have potent antioxidant properties as oxygen scavengers, peroxide decomposers, free radical inhibitors and metal chelating agents. It also possesses anti-bacterial, anti-viral, anti-tumour and cardioprotective properties [22]. Flavonoids are capable of scavenging oxygen-derived free radicals and also possess anti-allergic, anti-viral and anti-inflammatory properties [23]. Terpenoids are plant-based compounds and it is widely used in the food

industry and also in pharmaceutical and chemical industries and recently used in evolving biofuel products [24]. Saponins are bioactive compounds over biological and pharmacological properties that naturally arise in plants as triterpenes [25]. Glycosides are a polyphenolic group usually in plants with several anti-inflammatory properties [26]. Steroids have antibacterial properties and medicinal and pharmaceutical activities and boost the immune response [27]. The phytochemicals in the *Banana inflorescence* identified in this study may support the contribution of this flower in the prevention of various diseases, including cancer and cardiovascular diseases.

Determination of Total Phenolics and Flavonoid Contents: The quantitative phytochemical screening of total phenolics and flavonoids content was analyzed in the extract of *Banana inflorescence* shown in **Table 2**. The total phenolic content of *Banana inflorescence* extract showed a content value of 247.06%. The total flavonoid content of *Banana inflorescence* extract showed a content value of 45.83%. Total phenolic content was found to be higher than flavonoid in *Banana inflorescence*. Phenolic compounds play a significant role in antioxidant activity as well as an important biological function of the plant [28].

TABLE 2: QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *BANANA INFLORESCENCE*

Quantitative phytochemical analysis	
Phytoconstituents	**Percentage (%)**
Phenols	247.06
Flavonoids	45.83

***In-vitro* Antioxidant Assay:** Antioxidants are the organic constituents are highly used over natural sources, which are also a mixture of phytocompounds. The intense generation of oxidative stress by prooxidants damages the cellular molecules such as proteins, nucleic acids, and lipids which may cause tissue interruption [29]. In such conditions, antioxidants perform a vital role in eliminating oxidative stress by scavenging the radicals causing oxidative damage. Therefore, the antioxidants found in the powder extract of *Banana inflorescence* can play a vital role in scavenging the free radicals. This study aimed to evaluate the antioxidant capacity of Banana powder extracts of Banana inflorescence to identify its ability to scavenge the free radicals. The results are reported in **Table 3**. The DPPH scavenging assay was used to study the free radical scavenging activity of the extract by calculating the IC_{50} (half maximal inhibitory concentration) values of the extract. The lower IC_{50} value of *Banana inflorescence* reflects higher DPPH radical scavenging activity. It can be concluded that the *Banana inflorescence* extract showed more potent *in-vitro* antioxidant activity in a dose-dependent manner with a higher percentage of inhibition at 50 mcg/ml. The IC_{50} value of *Banana inflorescence* extract was found to be 46.47% antioxidant activity of this *Banana inflorescence* might be due to the presence of higher amounts of phenolic compounds. Generally, the phenolic compounds are considered primary antioxidants.

TABLE 3: DETERMINATION OF DPPH SCAVENGING ACTIVITY

Extract	DPPH radical scavenging activity IC_{50} value (µg/ml)
Banana inflorescence	46.47372 %
Rutin (control)	27.69024 %

***In-vivo* Toxicity Study:** Acute oral toxicity test was performed at 2000 mg/kg body weight post orally as a single dose for 15 days.

The control and Banana inflorescence extract groups' body weight was increased progressively throughout the experimental period as shown in **Table 4**. No toxic signs and necropsy findings were observed in **Table 5**. At the end of the observation period, the rats were sacrificed using diethyl ether anesthesia and subjected to detailed necropsy.

TABLE 4: BODYWEIGHT, BODY WEIGHT CHANGES AND PRE-TERMINAL DEATHS

Dose (mg/kg b.wt.)	Rat No.	Sex	Bodyweight (g)					No. dead / No. tested
			Initial	Day 8	Weight change (day 8 – Initial)	Day 15	Weight change (day 15 – Initial)	
2000	R007	Female	140	146	6	149	9	0/6
	R008	Female	144	148	4	155	11	
	R009	Female	141	145	4	151	10	
	R010	Female	154	158	4	165	11	
	R011	Female	137	144	7	153	16	
	R012	Female	143	147	4	151	8	

TABLE 5: EFFECT OF *BANANA INFLORESCENCE* ON PHYSIOLOGICAL CHARACTERISTICS OF WISTER ALBINO RATS

Rat No.	Fasting body weight	Dose-volume (ml)	Day of observation																		Necropsy findings
			Day 1					2	3	4	5	6	7	8	9	10	11	12	13	14	15	
			30 min	1 h	2 h	3 h	4 h															
R007	134	1.3	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R008	139	1.4	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R009	136	1.4	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R010	149	1.5	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R011	131	1.3	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R012	136	1.4	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD

CONCLUSION: In conclusion, our results showed the presence of various phytochemicals in the powder extract of *Banana inflorescence*, which may be responsible for the pharmacological properties of the extract. Phytochemical constituent of *Banana inflorescence* of Musa acuminate revealed the presences of alkaloids, phenols, saponins, flavonoids, steroids, terpenoids and carbohydrates are in the extract. The *Banana inflorescence* has the highest phenolic content with a high antioxidant potential, which can be a potential source of natural antioxidants. There were no significant differences observed in the bodyweight of the rats treated with *Banana inflorescence*. Medicinal plants should have low toxicity because of their long-term use in humans. Various medicinal plants have been reported to exhibit toxicity. Preliminary toxicological evaluation is necessary for authentication of the safety of herbal medicine. Therefore, this study was conducted to assess the toxicity of *Banana inflorescence* extracts in animal models. No animal was found dead, and there were no toxic signs observed during and after the experimental period. Further *in-vitro* antioxidant studies and long-term toxicity studies using *Banana inflorescence* may therefore be warranted before this extract can be developed as a pharmaceutical product.

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