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In vitro studies on the biological activities of flowers of banana (*Musa Paradisiaca* L.)

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ABSTRACT

Reactive oxygen species including include superoxide anion radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen are capable of damaging DNA, proteins, carbohydrates and lipids in aerobic organisms. Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological function. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells. Obesity is a significant risk factor for increased morbidity and mortality from cardiovascular disease and diabetes and it is also associated with many other medical conditions including cancer, liver and kidney diseases. Recently, studies have been intensified towards finding out a natural source of drug without any side effects. In this view, in the present study we have evaluated the antioxidant, anti-inflammatory and anti-obesity activities of banana flower extract. From the results obtained, it can be concluded that the flower extract of banana revealed promising antioxidant, anti-inflammatory and anti-obese effects due to significant total phenol content.

Key words: Banana flower, Aqueous extract, Antioxidant, Anti-inflammatory, Lipase.

INTRODUCTION

Inflammation is part of the body's immediate response to infection or injury. It is characterized by redness, swelling, heat, and pain. These occur as a result of increased blood flow; increased permeability across blood capillaries, which permits large molecules (e.g., complement, antibodies, and cytokines) to leave the bloodstream and cross the endothelial wall; and increased movement of leukocytes from the bloodstream into the surrounding tissue. Inflammation functions to begin the immunologic process of elimination of invading pathogens, toxins and to repair damaged tissue. These responses must be ordered and controlled. The movement of cells into the inflammatory or infected site is induced by the up-regulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on the surface of endothelial cells, which allows leukocyte binding and subsequent diapedesis.

Oxygen, the most critical element for life, is highly lethal to strict anaerobes and is the main source of free radicals. By definition a free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. When cell uses oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. These species play a dual role as both toxic and beneficial compounds. At low or moderate levels, ROS and RNS

exert beneficial effects on cellular responses and immune function. At high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures [1]. Oxidative stress plays a major part in the development of chronic and degenerative diseases such as diabetes, hypertension, atherosclerosis, hypercholesterolemia and macular degeneration, loss of immune function, skin disorders, neuro-degeneration and aging.

The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments [2]. In India, around 20,000 medicinal plants have been recorded**;** however, traditional communities are using only 7,000 - 7,500 plants for curing different diseases [3]. The World Health Organization (WHO) estimates that 80% of the people in developing countries depend on traditional medicine for their primary health care, and about 85% of traditional medicines involve the use of plant extracts. On a global scale, approximately one third of the top selling medicinal products of either therapeutic or preventive interest against various disorders/diseases in human are natural products or their derivatives. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs [4].

In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans. Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka and can be traced back to 6000 BC. Ayurvedic medicines are largely based on herbal and herbomineral preparations and have specific diagnostic and therapeutic principles [5]. The World Health Organization has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines [6]. Consumption of phytochemical-rich foods such as fruits, vegetables, spices and some beverages (red wine, green and black tea etc) are associated with prevention of diseases mediated by oxidative stress and inflammation such as certain cancers, atherosclerosis and neurodegenerative diseases [7]. Plant based products play a major role in curing many diseases associated with inflammation. The conventional drugs available in the market to treat inflammation produce various side-effects. Due to these side-effects, there is need for the search of newer drugs with less or no side-effects. There are hundreds of phytoconstituents reported to have many pharmacological activities although most of these reports are of academic interest and very few find entry in clinical trials. In view of this, in the present study we have evaluated the antioxidant and anti-inflammatory activityies of flowers of *Musa paradisiaca*.

Banana (*Musa paradisiaca)* is a familiar tropical and largest herbaceous flowering plant. From its native South-Western Pacific home, the banana plant spread to India by about 600 BC and later on it spread all over the tropical world. *Musa paradisiaca* is a herbaceous plant (up to 9 m long) with a robust treelike pseudostem, a crown of large elongated oval deep-green leaves (up to 365 cm in length and 61 cm in width), with a prominent midrib, Each plant produces a single inflorescence like drooping spike, and large bracts opening in succession, ovate, 15-20 cm long, concave, dark red in color and somewhat fleshy (Figure 1). Fruits are oblong, fleshy,5-7cm long in wild form and longer in the cultivated varieties. Fruits, stems and flowers are regularly used in south Indian cuisine. All parts of this plant have medicinal properties. The banana flower is rich in vitamins, flavonoids and protein. The flower has been used in traditional medicine to treat bronchitis, constipation and ulcer problems. It eases menstrual cramps. Consuming one cooked banana flower with one cup of curd or yogurt is one of the most efficient ways of treating excessive bleeding during menstruation. The cooked banana flower and curd combination increases the level of progesterone in the body and thereby reduces bleeding associated with menorrhagia.

Figure: 1 Flower of *Musa paradisiaca*

The fruit of *M. paradisiaca* and *M. sapientum* is traditionally used in diarrhoea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uremia, nephritis, gout, hypertension, cardiac disease [8, 9]. *M. spaientum* is also used in the treatment of excess menstruation with *Canna indica* L. var. *speciosa* [10]. Banana leaves (ashes) are used in eczema [11], as cool dressings for blister and burns [8]. Flowers are used in dysentery and menorrhagia. Stem juice of fruited plant is used for treating diarrhoea, dysentery, cholera, otalgia, haemoptysis [8]. The root is used as anthelmintic [9], blood disorders, venereal diseases [8]. The plant is also used in inflammation, pain and snakebite [12]. At present, due to modern food practices the interest to consume banana flowers is found to be declining. Since inflammation and free radical damage are the root cause for many human ailments, the proposed study is designed in such a way to provide scientific evidence to prove the anti-inflammatory, antioxidant and lipid lowering potentials of banana flowers *in vitro*

MATERIALS AND METHODS

Collection of plant material

The flowers of *Musa paradisiaca* were collected from Mannargudi, Tamilnadu. Plant materials were identified and authenticated by the botanist Dr. K. Kandavel, S.T.E.T Women's College Mannargudi, Tamilnadu. The collected materials were cleaned, bracts were removed, flowers lets were shade dried and coarsely powdered.

Preparation of the extract

The shade dried plant materials were subjected to pulverization to get coarse powder. The powdered materials were subjected to aqueous extraction separately with water. These extract were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40º-50º). The aqueous extracts of medicinal plants were put in air tight containers separately and stored in refrigerator till the time of use.

Estimation of total phenol content

The total phenolic concentration of aqueous extract was estimated according to the modified method of Singleton et al. [13]. Extract (10 µl) was taken in a 96 well microplate and 25 µl of Folin reagent and 230 µl of 4.4% of Na_2CO_3 were added and incubated for 30 min in dark place. Then the absorbance was measured at 750 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). A calibration curve was prepared using standard gallic acid (100 – 1000 mg/L, R^2 = 0.9978) and used to express the results as gallic acid equivalents (GAE).

Assessment of antioxidant activity

The antioxidant activity was analyzed using DPPH free radical scavenging assay[14]. Extracts (10 µl) were taken in the 96 well microplate and 200 µl of DPPH solution (2.5 mg/100 ml) and incubated for 30 min in dark place. Then the absorbance was measured at 515 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). The radical scavenging activity of tested samples was calculated using the formula (Antioxidant activity = Abs control – Abs test / Abs control x 100) and expressed on percentage basis.

Lipid peroxidation inhibition assay

The MDA levels of plant extracts treated liver homogenate was analyzed according to the method of Yen and Hsieh [15]. Thin liver slice sample (0.2 g) was weighed and treated with 2 ml of PBS and 0.5 ml of extract for 2 h. Then the sample was homogenized with 2 ml of PBS and the liver homogenate (0.5 ml) was mixed with 1 ml of TBA and heated in a boiling water bath for 30 min and cooled to room temperature. The samples are subsequently centrifuged at 3000 rpm for 10 min and the absorbance of the supernatant was read at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane as a precursor of MDA and the TBARS values were then calculated using the standard curve and expressed as mg MDA equivalents per 100 g of sample.

Assessment of anti-inflammatory activity

The anti-inflammatory activity was evaluated using RBC membrane stabilization method [16]. Blood sample (2 ml) was collected from volunteer in a heparinized tube and washed with PBS twice and centifuged at 3000 rpm for 10 min (Centrifuge Make: Eppendorf, Model 5810-R). Then RBC was suspended in phosphate buffer and taken in a tube (0.5 ml) with 0.5 ml of extract and 0.5 ml hypotonic solution and incubated for 30 min at room temperature. Then the contents were centrifuged at 1500 rpm for 10 min and the supernatant was collected and the absorbance was read at 560 nm using Micro plate reader (Make: Biotek, Model: Epoch). Based on the absorbance of extract and control, the membrane stabilization effect was calculated and expressed on percentage basis.

Pancreatic lipase inhibitory activity

The lipase inhibition activity of plant extract was determined as per the method proposed by Kim et al. [17]. In this assay, the porcine pancreatic lipase activity was measured using p-nitrophenyl butyrate (NPB) as a substrate. Lipase solution (1 mg/mL) was prepared in a 0.1 mM potassium phosphate buffer (pH 6.0). To determine the lipase

inhibitory activity, 1 ml of extract were pre-incubated with 1 ml of lipase for 10 min at 37°C. The reaction was then started by adding 0.1 mL NPB substrate. After incubation at 37°C for 15 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer and the percentage of inhibitory activity was calculated.

RESULTS AND DISCUSSION

Total phenol content

Phenolics include simple phenols, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins. These compounds are among the most widely occurring secondary metabolites in the plant kingdom, acting mainly as phytoalexins, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light, among others [18].

In the present study total phenol content of aqueous extract of *M. paradisiaca* flowers was estimated using Folin's-Ciocalteau method. The total phenol content was found to be 144 mg GAE / 100 g of sample (Figure 2). Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry [19]. The Folin-Ciocalteu method is described in several pharmacopoeias [20]. The reaction forms a blue chromophore constituted by aphosphotungstic phosphomolybdenum complex [19, 21], where the maximum absorption of the chromospheres depends on the alkaline solution and the concentration of phenolic compounds. Many studies have discussed the use of Folin-Ciocalteau reagent to determine polyphenols.

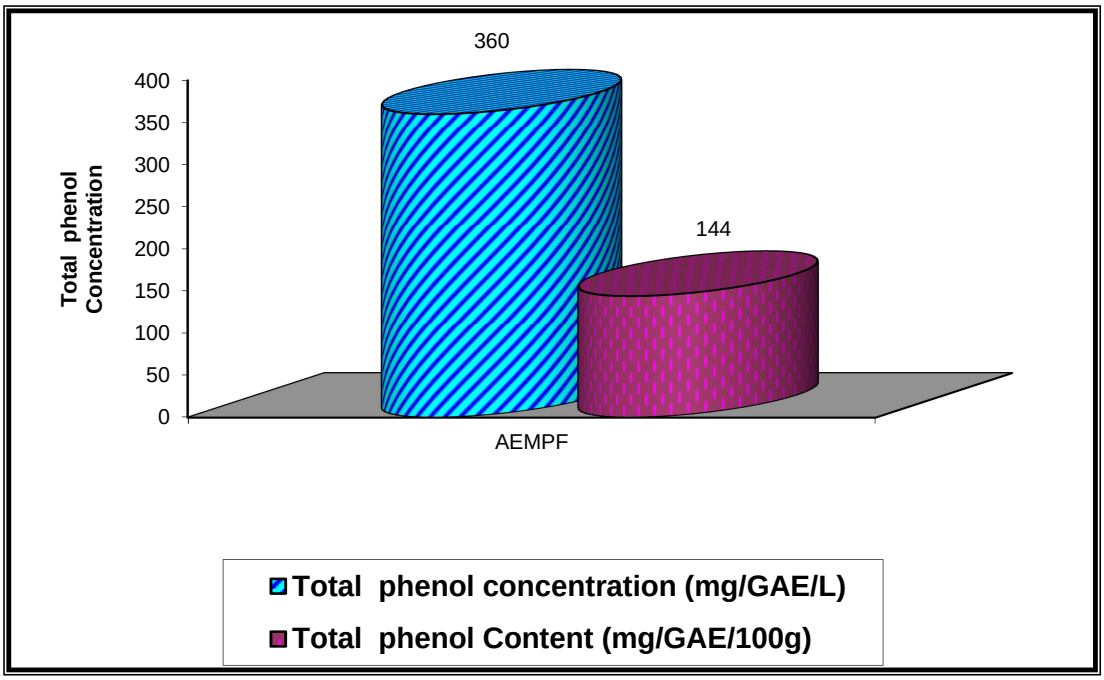

Figure 2: Total phenol content of aqueous extract of *Musa paradisiaca* L. flowers

Antioxidant activity

Free radicals are chemical species which contain one or more unpaired electrons. They are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [22]. Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins and nucleic acids [23]. Free radicals damage contributes to the etiology of many chronic health problems such as inflammatory disease, cataract and cancer. Antioxidants prevent free radicals induced tissue damage by preventing the formation of radicals, scavenging them or by promoting their decomposition [24].

The initiation, promotion and progression of cancer, as well as the side-effects of radiations and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease and neurodegenerative diseases such as Parkinson's disease [25]. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases.

They exert their action either by scavenging the ROS or protecting the antioxidant defense mechanisms [26]. Antioxidant capacity is widely used parameters for assessing the bioavailability of food stuffs as medicinal plant. The antioxidant properties of plant extract should be evaluated to ensure the effectiveness of such antioxidant materials [27].

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, hypertension, arthritis, ischemia, gastritis, central nervous system injury, reperfusion injury of many tissues, cancer, alzheimer's disease, parkinsonism, diabetes mellitus and AIDS [28]. Antioxidants have been reported to prevent oxidative damage by free radicals and ROS and may prevent the occurrence of disease, cancer and aging. There is considerable evidence that antioxidants could help to prevent these diseases because they have the capacity to quench free radicals [29]. Although some synthetic antioxidants, such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), exhibit potent free radical scavenging effects, they have been demonstrated to exert toxicological effects as compared with natural antioxidants [30]. Antioxidant compounds obtained from natural sources such as grains, oilseeds, beans, leaf waxes, bark, roots, spices, fruits and vegetables have been investigated [31]. Nowadays food scientists and nutrition specialists agree that food antioxidants, consumed daily contribute to the conservation of good health [32].

The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ*, or externally supplied through food or other supplements. Exogenous and endogenous antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS and RNS, therefore can enhance the immune defense and reducing the risk of cancer and degenerative diseases [33]. The role of antioxidants is to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention. Antioxidant enzymes are directly involved in the neutralization of ROS and RNS, e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) [34].

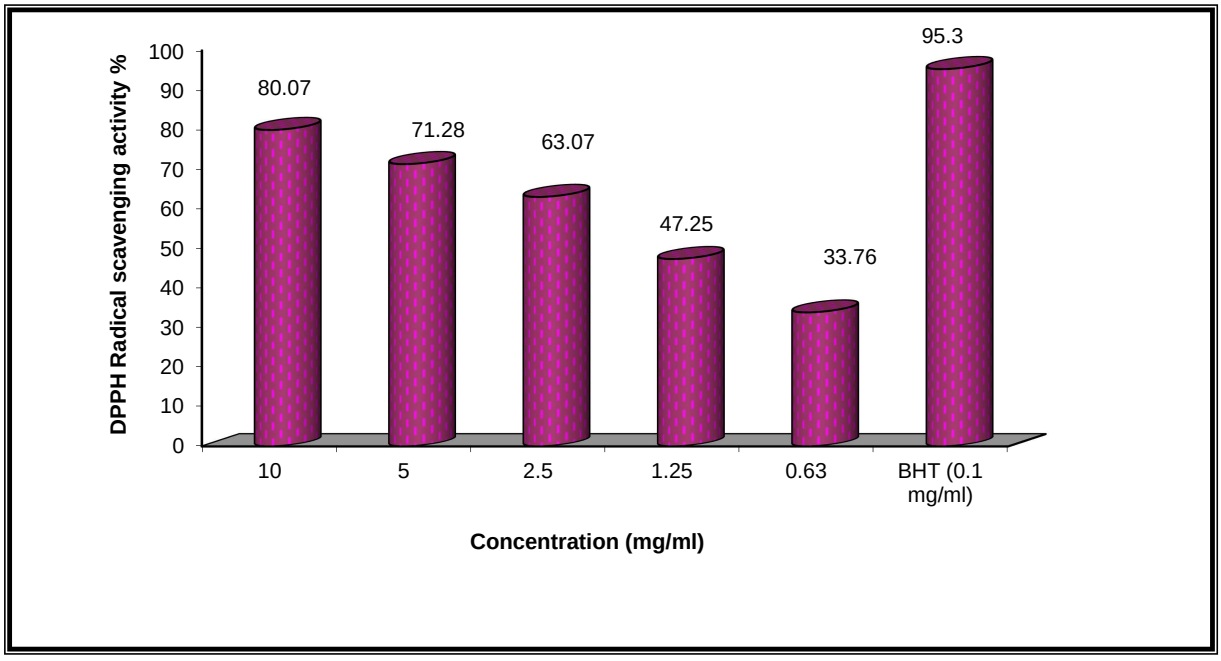

Figure 3: *DPPH* radical scavenging activity of the aqueous extract of *Musa paradisiacal* L flowers

In the present study, the aqueous extract of *M. paradisiaca* flowers has been evaluated for its antioxidant activity using DPPH scavenging activity. The extract exhibited radical scavenging activity of 80.07% at 10 mg/ml (Figure 3). The radical scavenging activity of different extracts was tested using methanolic solution of the stable free radical DPPH. Unlike laboratory generated free radical such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless product (2, 2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine resulting in a decrease in absorbance at 518 nm [35] reveals the free radical scavenging activity of the plant extract and the results are expressed as percentage of the ratio of decrease in absorbance at 518 nm to the

absorbance of DPPH solution activity. Radical scavengers may protect tissues from free radicals, thereby preventing diseases such as cancer [36].

Table 1: Estimation of percentage inhibition of lipid peroxidation

S. No.	Sample	MDA Concentration (nM)	MDA Content(nM/100 g Tissue)	Lipids Peroxidation inhibition (%)
1.	Control	3.08 ± 0.11	1.86 ± 0.07	-
2.	*M. paradisiaca* extract	1.70 ± 0.08	0.63 ± 0.03	34
3.	BHT(Positive control)	0.28 ± 0.06	0.17 ± 0.03	10

In the present study, the aqueous extract of *Musa paradisiaca* flowers has been evaluated for its antioxidant activity using lipid peroxide scavenging activity. The extract exhibited peroxide scavenging activity of 34% at 10 mg/ml (Table 1) and the potential of the extract to scavenge peroxide radicals is found to be more than the standard antioxidant BHT. Free radical induced lipid peroxidation has been associated with a number of disease processes [37]. The lipid peroxidation of the cell membrane has been associated with a number of the cell membrane has been associated with a number of pathologic phenomena such as cancer, diabetes mellitus and inflammatory diseases.

***In vitro* Anti inflammatory Activity**

Although inflammation is a normal response, when it occurs in a sustained and an uncontrolled or inappropriate manner, excessive damage to host tissues and disease can ensue. Such uncontrolled or inappropriate inflammatory responses are characterized by hyper-expression of endothelial and leukocyte adhesion molecules, appearance of soluble forms of adhesion molecules in the circulation, sequestration of leukocytes to sites where they are not usually found, production of inflammatory mediators, and damage to host tissues.High concentrations of TNF-α, IL-1, and IL-6 are particularly destructive and are implicated in some of the pathologic responses that occur in endotoxic shock, in acute respiratory distress syndrome, and in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.

The earliest cells to appear at inflamed sites are granulocytes, with monocytes, macrophages, and lymphocytes appearing later. Granulocytes, monocytes, and macrophages are involved in pathogen killing, in clearing up cellular and tissue debris, and also in tissue repair. The activity of these cells is induced by certain triggers. One important exogenous trigger is bacterial endotoxin (also known as lipopolysaccharide, LPS), a component of the cell wall of Gram-negative bacteria, which can directly activate monocytes and macrophages, inducing them to form cytokines, such as tumor necrosis factor-α (TNF-α); interleukin 1 (IL-1), IL-6, and IL-8; eicosanoids, such as prostaglandin (PG) E2; nitric oxide (NO); matrix metalloproteinases (MMP's); and other mediators. Endotoxin also induces adhesion molecule expression on the surface of endothelial cells and leukocytes.

The cytokines produced by monocytes and macrophages also serve to regulate the whole-body response to infection and injury. Thus, inflammation and the inflammatory response are part of the normal, innate immune response. Inflammatory mediators also provide a link between innate and acquired immune responses.The actions of inflammatory cytokines, which initiate a cascade of inflammatory mediators, thus amplifying the initial inflammatory signal, are opposed by anti-inflammatory cytokines such as IL-10 and by receptor antagonists such as IL-1 receptor antagonist. Chronic overproduction of TNF-α and IL-1 can cause adipose tissue and muscle wasting and loss of bone mass and may account for alterations in body composition and tissue loss seen in inflammatory diseases, as well as its clear and obvious association with classic inflammatory diseases. Inflammation is now recognized to play an important role in the pathology of other diseases, such as cardiovascular disease and neurodegenerative diseases of aging. Additionally, the realization that adipose tissue is a source of inflammatory cytokines has given rise to the notion that obesity, the metabolic syndrome,and type 2 diabetes have an inflammatory component.

The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost. In the present study, HRBC membrane stabilization model was selected for *in vitro* assessment of anti-inflammatory property of *Musa paradisiaca*. Erythrocytes have been used as a modern system by a number of scientists to investigate the interaction of drugs with membranes [38]. Drugs, like anesthetics, tranquilizers and non-steroidal anti-inflammatories, stabilize erythrocytes against hypotonicity induced (stress) hemolysis. Therefore, they prevent the release of haemoglobin as a result of their membrane stabilizing activity [39]. The membrane stabilizing activity of red blood cells (RBC) that are exhibited by some drugs is used as *in vitro* method for assessing the anti-inflammatory activity of various components.

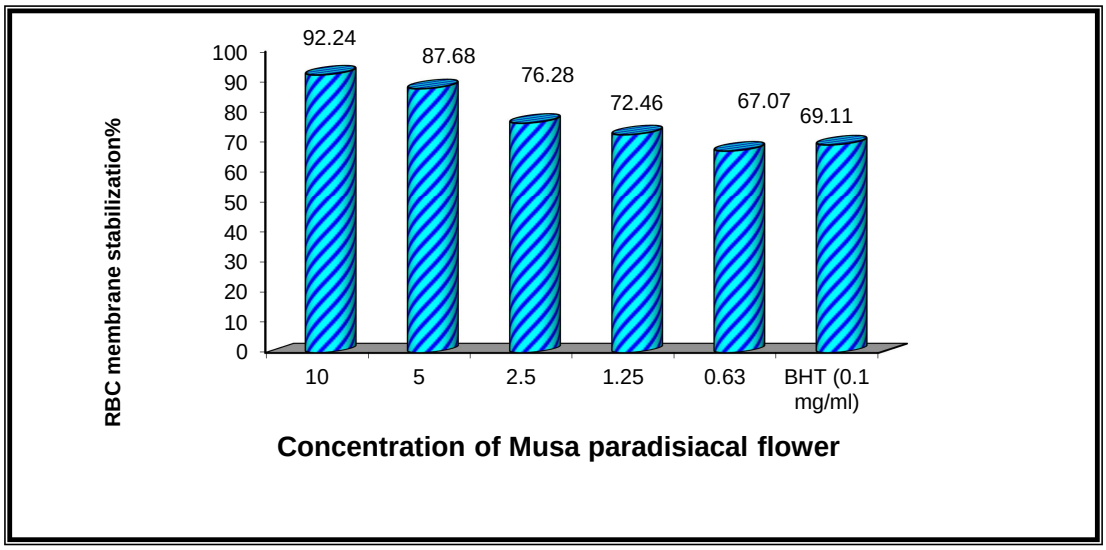

Figure 4: Anti inflammatory Activity (RBC Assay) of the aqueous extract of *Musa paradisiaca* L. flowers

In the present study, 5 different concentrations of the aqueous extract of *Musa paradisiaca* has been evaluated for the HRBC membrane stabilization activity.10mg/ml of the extract has been found to stabilize the RBC membrane up to 92.24% (Figure 4). The extract exhibited membrane stabilization activity in a dose dependent manner. The erythrocyte membrane is analogous to the lysosomal membrane [40] and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzyme and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [41]. In this view, the activity of the extract is comparable to the action of NSAID, The membrane stabilizing activity of the extract is compared with standard analgesic Aspirin (69.11%).

Lipase inhibition

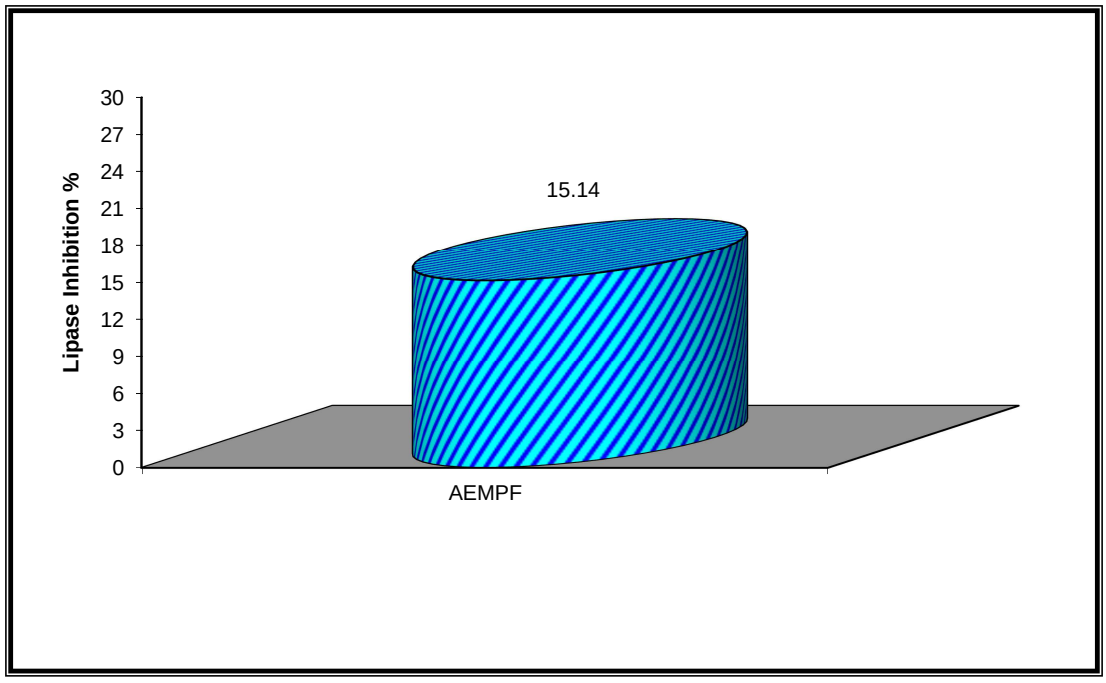

Figure 5: Estimation of percentage inhibition of pancreatic lipase

Pancreatic lipase inhibition is the most widely studied mechanism for the identification of potential anti-obesity agents. Only one blockbuster drug, orlistat, approved by FDA and available for the obesity treatment apart from the centrally acting anti-obesity drugs, is acting through the pancreatic lipase inhibition. Discovery of orlistat was done from the naturally occurring molecule lipstatin. The success of naturally occurring compounds for treatment of obesity has influenced the research for the identification of newer pancreatic lipase inhibitors that lack unpleasant side effects.

In the present study, aqueous extract of banana flower exhibited lipase inhibitory activity up to 15.14%at a concentration of 10 mg/ml (Figure 5). From the data of the result obtained, it could be concluded that the flowers of *Musa paradisiaca* have antioxidant, anti-inflammatory and lipid lowering activities. Till now, many plant extracts and isolated compounds were identified for the pancreatic lipase inhibition. Other than that, many microbial products and isolated compounds, basic protein protamines [42], ε-polylysine [43], polysaccharides like chitosan [44], dietary fibers from wheat bran and cholestyramine [45], soya proteins [46], and synthetic compounds etc. have been studied for inhibitory potential against pancreatic lipase. However, plant and microbial origin isolated molecules were widely studied and reported for the pancreatic lipase inhibition.

CONCULSION

Plants have contributed lot of medicinal compounds being used today to treat diseases like cancer, jaundice, diabetes, inflammation etc. They are the vital sources of wide variety of phytochemicals from which novel therapeutic agents can be discovered. In this view, attempts were made in the present study to evaluate the antioxidant, anti-inflammatory and lipase inhibition activities of aqueous extract of *Musa paradisiaca* flowers *in vitro*. The present *in-vitro* study is a preliminary evaluation of anti-inflammatory and anti oxidant activities of *Musa paradisiaca* and provided scientific validation for the use of this plant in folk medicine. Further research work to analyze *in-vivo* anti-inflammatory and anti oxidant activities of *Musa paradisiaca* on animal models and to isolate the phytoconstituents responsible for such activities are necessary to potentiate the usage of the plant.

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REFERENCES

[1] T Bahorun, MA Soobratte, V Luximom-Ramma, OL Aruoma, *Int. J. Med. Update*, **2006**, 2, 25-41.
[2] B Ballabh, OP Chaurasia, *J. Ethnopharmacol*, **2007**, 112, 341-349.
[3] R Perumalsamy, P Peter Natesan, P Gopalakrishnakone, *Bioinf*, **2008**, 3, 100-110.
[4] S Anusuya, *Biochem. Pharmacol*, **2013**, 2, 190.
[5] B Patwardhan, B Hopper, *J. Alt. Comp. Med*, **1992**, 19, 9-10.
[6] WHO, Traditional Medicine Strategy (2002–2005). WHO/EDM/TRM/2002.1. Geneva, Switzerland, 2002.
[7] B Halliwell, *Trend. Pharmacol. Sci*, **2011**, 32, 125-30.
[8] S Ghani, Medicinal Plants of Bangladesh: Chemical Constituents and Uses. 2nd Ed. The Asiatic Society of Bangladesh, Dhaka, Bangladesh, **2003**, p. 315.
[9] CP Khare, Indian Medicinal Plants, Springer Science, New York, USA, **2007**, p. 426.
[10] P Partha, ABME Hossain, *Bangladesh J. Plant Taxon*, **2007**, 14, 129-145.
[11] RI Okoli, O Aigbe, JO Ohaju-Obodo, JK Mensah, *Pak. J. Nutr*, **2007**, 6, 490-496.
[12] F Coe, GJ Anderson, *Econ. Bot.*, **1990**, 53, 363-383.
[13] VL Singleton, R Orthofer, RM Lamuela-Raventos, *Method. Enzymol*, **1998**, 299, 152-178.
[14] C Sanchez-Moreno, JA Larrauri, FA Saura-Calixto, *J. Sci. Food Agric*, **1998**, 76, 270-276.
[15] GC Yen, CL Hsieh, *J. Agric. Food Chem*, **1998**, 46, 3952-3957.
[16] S Sakat, AR Juvekar, MN Gambhire, *Int. J. Pharm. Pharmacol. Sci*, **2010**, 2, 146-155.
[17] YS Kim, YM Lee, H Kim, J Kim, DK Jang, JH Kim, JS Kim, *J. Ethnopharmacol*, **2010**, 130, 621-624.
[18] OR Gottlieb, MR Borin, *Mem. Inst. Oswaldo. Cruz*, **2000**, 95, 115-120.
[19] P Schofield, DM Mbugua, AN Pell, *Anim. Feed Sci. Tech*, **2001**, 91, 21-40.
[20] Council of Europe, Determination of tannins in herbal drugs. In *European Pharmacopoeia*, 6th Ed. European Directorate for the Quality of Medicines: Strasbourg, France, **2007**, p. A286.
[21] IG Gulcin Sat, S Beydemir, M Elmastas, OI Kufrevioglu, *Food Chem*, **2004**, 87, 393-400.
[22] J Thambiraj, S Paulsamy, R Sevukaperumal, *Asian Pac. J. Trop. Biomed*, **2012**, S127-S130.
[23] JM McCord, *Am. J. Med*, **2000**, 108, 652-659.
[24] V Lobo, A Patil, A Phatak, N Chandra, *Pharmacog. Rev*, **2010**, 4, 118-126.

[25] AL Rao, M Bharani, V Pallavi, *Adv. Pharmacol. Toxicol*, **2006**, 7, 29-38.
[26] M Umamaheshwari, TK Chatterjee, *Afr. J. Trad. Comp. Alter Med*, **2008**, 5, 61-73.
[27] M Sathuvan, V Anadhan, R Thangam, P Perumal, R Ramasamy, M Kandasamy, *Asian Pac. J. Trop. Biomed*, **2012**, S741-S749.
[28] JT Kumpulainen, JT Salonen, Natural antioxidants and anticarcinogens in Nutrition, Health and Disease, The royal society of chemistry, UK, **1999**, pp. 178-187.
[29] AT Diolock, *Free Rad. Res*, **1997**, 27, 511-532.
[30] M Saito, H Sakagami, S Fujisawa, *Anticancer Res*, **2003**, 23, 4693-4701.
[31] HM Chen, K Muramoto, FS Yamavchi, K Nokihara, *J. Agric. Food Chem*, **1996**, 44, 2619-2623.
[32] B Halliwell, JMC Gutteridge, Free radicals in biology and medicine, Claredon press, Oxford, England, **1989**, pp. 550-610.
[33] M Valko, CJ Rhodes, J Moncol, M Izakovic, M Mazur, *Chem. Biol. Interact*, **2006**, 160, 1-40.
[34] P Pacher, JS Beckman, S Liaudet, *Physiol. Rev*, **2007**, 1, 315-424.
[35] T Yamaguchi, H Takamura, T Matoba, J Terao, *Biosci. Biotechnol. Biochem*, **2002**, 62, 1201-1204.
[36] T Nakayama, MT Yamada, S Kawakishi, *Biochem Pharmacol*, **1998**, 45, 265-267.
[37] D Giugliano, A Ceriello, G Paolisso, *Diabetes Care*, **1996**, 19, 257-267.
[38] OO Oyedepo, AJ Femurewas, *J. Pharm*, **1995**, 33, 65-69.
[39] P Seeman, *Pharmacol Rev*, **1972**, 4, 583-655.
[40] S Shenoy, K Shewetha, K Prabhu, R Maradi, KL Bairy, T Shanbhag, *Asian Pac. J. Trop. Med*, **2010**, 3, 32.
[41] R Vadivu, KS Lakshmi, *Bangaladesh J. Pharmacol*, **2008**, 3, 121-124.
[42] T Tsujita, Y Matsuura, H Okuda, *J. Lipid Res*, **1996**, 37, 1481-1487.
[43] T Tsujita, H Takaichi, T Takaku, S Aoyama, J Hiraki, *J. Lipid Res*, **2006**, 47, 1852-8.
[44] M Sumiyoshi, Y Kimura, *J. Pharm. Pharmacol*, **2006**, 58, 201-7.
[45] D Lairon, H Lafont, JL Vigne, G Nalbone, J Leonardi, JC Hauton, *Am. J. Clin. Nutr*, **1985**, 42, 629-38.
[46] DM Roy, BO Schneeman, *J. Nutr*, **1981**, 111, 878-885.