In vitro Biological Activities and Fatty Acid Composition Analysis of *Musa paradisiaca* L. Inflorescence

Nusrat Tabassum Shristy, Md. Omar Sha Rafi, Sauda Sultana Mimi, Vaskar Chowdhury, Md. Hasanur Rahman and Tofail Ahmad Chowdhury

Department of Chemistry, Faculty of Science, University of Dhaka, Dhaka-1000, Bangladesh

(Received: February 20, 2024; Accepted: June 2, 2024; Published (web): June 13, 2024)

ABSTRACT. *Musa paradisiaca* L. is native to tropical climate. Inflorescences of this herb are used as a treatment of digestive distresses. This study involved analysis of the fatty acid contents and some *in-vitro* pharmacological activities of the inflorescence. Modified Kupchan method was used for extraction in solvents of varying polarity. Fatty acid content analysis was conducted on n-hexane extract; Palmitoleic acid was found to be the highest in proportion in both bound (34.40%) and free (37.80%) forms. n-hexane and dichloromethane extracts exhibited cytotoxicity against carcinoma cell (HeLa cell line). Ethyl acetate extract possessed significant amount of free radical (DPPH) scavenging capability (IC_{50} = 66.92±0.3065 µg/ml). The n-Hexane extract was found to be rich in phenolics (26.40 ± 0.033 mg GE/g) and flavonoids (83.40± 0.099 mg QE/g). Dichloromethane fraction exhibited the highest zone of inhibition (15 mm) against the bacterium *Shigella dysenteriae*. Ethyl acetate fraction showed blood clot lysis activity of 29.35% (standard streptokinase 63.05%). Presence of potent bioactivities imply the prospects of this sample as a source of lead compounds for effective formulations against chronic digestive diseases.

Key words: *Musa paradisiaca*, antioxidant, phenolics, free radical scavenging, antimicrobial, thrombolytic

INTRODUCTION

Plants have been used as nutrient supplements and remedy to various diseases since ancient time. Plant based drugs are generally considered to be cheaper, easily available, eco-friendly; have higher adaptability, compatibility and less adverse effects than the synthetic drugs.[1] Some of the secondary metabolites from plants are used for their antimicrobial traits[2], whilst others are natural antioxidants[3]; some are found to be active against several carcinomas.[4] Recent domination of carcinomas and multi-drug resistant bacterial infections have made the analysis and isolation of the phytochemicals and their bioactivity determination a demanding area of modern research.

Correspondence to: Md. Hasanur Rahman
E-mail: hasanur@du.ac.bd

Dhaka Univ. J. Pharm. Sci. **23**(1): 69-76, 2024 (June)
DOI: https://doi.org/10.3329/dujps.v23i1.74096

Musa paradisiaca L. (Bengali name: Atia Kola) is a perennial herb from the plant family Musaceae. It is a wild native variety found in almost all districts of Bangladesh. All the cultivated banana plants are around 5 m tall[5]; each plant consists of rhizome, pseudostem, leaf, shoot, inflorescence and fruit. The fruit is an excellent source of nutritional diet containing high amounts of carbohydrate, sugar, vitamins, iron and potassium.[6] Green fruits, flower and sucker of banana exhibit antihyperglycemic activities.[7-9] Peel extracts have antihypertensive and antioxidant properties[10], pulp and skin possess antimicrobial activity[11], stem extracts are found to be useful in the treatment of some renal diseases.[12] People in some of the Southeast Asian countries consume the inflorescence as a food item.[13] Also, in Bangladesh, its juice is taken orally for the cure of blood dysentery; flower extracts are used for the remedy of diabetes, dysentery in India.[14] Inflorescences have been reported to possess anti-inflammatory[15], antidiabetic[16], antioxidant[17] and cardioprotective activities.[18]

The studies related to the biological activities of *M. paradisiaca* inflorescence were not given much concern previously. Hence, current study involves, determination of cytotoxic activities, total phenolic and flavonoid content, antioxidant and antimicrobial and thrombolytic activities propounding the beaming prospects of this agricultural waste to be converted into a value-added product fulfilling the needs of human health.

MATERIALS AND METHODS

Collection and processing of the plant sample. *M. paradisiaca* inflorescences were collected from Narayangonj Sadar, Bangladesh and properly identified by Department of Botany; University of Dhaka (DUSH-10814). Inflorescences were thoroughly washed with water to remove additional contaminants. The flowers, bracts and peduncles were sliced into small pieces and kept for room temperature drying for several days. These were oven dried at 40°C. The dried pieces were ground to powder using a grinder (Cyclotec 200 meshes) and stowed in an airtight container for extraction and further phytochemical investigations.

Extraction. Preliminary extraction of the powdered sample was carried out by cold extraction method. About 200 g of the finely powdered sample was soaked in a flat-bottom airtight container using 1L of analytical grade methanol. The container was kept in a dry place for 13 days with occasional shaking. The solution was then filtered (cotton and Whatman filter paper) and concentrated (Buchi Rotavapor R-3). The crude methanol extract of the sample was further fractionated with different solvents by using the modified Kupchan method.[19] About 5g of the crude extract was dissolved in a 90 ml: 10 ml of methanol-water mixture. The solution was taken in a separatory funnel and fractionated using solvents of increasing polarity, such as n-hexane (n-hex), dichloromethane (DCM) and ethyl acetate (EA). These fractions were evaporated to condensed semi-solid mass by using rotary evaporator at 40°C and kept in airtight container for further analysis.

Fatty acid content analysis. About ~0.5g of the n-hexane extract of the sample was taken for analysis of fatty acid composition using Gas-Liquid Chromatography. Both bound and free form of acids (BFAs and FFAs) were isolated from the inflorescences. These were further converted into methyl esters and analyzed by GLC (Shimadzu 9A, column-BP-50, Detector-FID, 170ºC-1min/4ºC - 270ºC-30 min).[20]

Cytotoxicity assay. Cytotoxic effects of different extracts of the sample were tested in Centre for Advanced Research in Sciences (CARS), University of Dhaka. Each fraction (n-hex, DCM, EA, MeOH and AQ) was dissolved in 2.5% DMSO solution and filtered. These filtered solutions were used for the determination of cytotoxicity against HeLa (a human cervical carcinoma cell), Vero (a kidney epithelial cell extracted from African green monkey) and BHK-21 (a baby hamster kidney fibroblast cell) cell lines using a Biological Bio Safety Cabinet (Model: NU-400E, Nuaire, USA), CO_2 incubator (Nuaire, USA), trinocular microscope with camera (Optika, Italy), hemocytometer etc. The evaluation process involves the procedure described in literature.[21]

Antioxidant activity. Antioxidant activity of the sample extracts was evaluated by their scavenging activities on the stable 2,2 - diphenyl-1-picrylhydrazyl radical (DPPH radical) following the method of Brand-Williams.[22] 2.0 ml of the methanolic solution of each extract were mixed with 2.0 ml of the DPPH methanol solution. The mixtures were kept at dark for 30 minutes and the absorbance of each solution was measured against a blank at 517 nm using a double beam UV-Vis spectrophotometer. The antioxidant potential was determined by the bleaching of the purple-colored solution of DPPH radical by the sample extracts as compared to that of the standard butylated hydroxytoluene (BHT). The following equation was used to calculate the % inhibition of the sample:

$$\% \text{ Inhibition} = (1- A_{sample} / A_{control}) \times 100$$

Where, $A_{control}$ and A_{sample} indicate the absorbance of the DPPH methanol solution and the

reaction mixture respectively. A plot comparing percent inhibition to extract concentration gave the effective dose needed to neutralize 50% of the DPPH radical solution, *i.e.*, the IC_{50} values for each extract.

Total phenolic content. Total phenolic content of different extracts was determined using Folin-Ciocalteu's reagent, following a modified version of Singleton and Rossi method.[23] 1.0 mg/ml of each extract was mixed with 5ml of Folin-Ciocalteu's reagent (1:10 v/v distilled water) and 4 ml (75g/l) sodium bicarbonate solution. The solutions were then allowed to stand for 30 minutes at 40°C for color development. The absorbance of each solution was measured using a double beam spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian) against a blank solution, at 765 nm. Gallic acid solutions of different concentrations (6.25, 12.5, 25, 50, 100, 200, 400 µl) were taken as standards and a calibration curve was plotted. Total phenolic content of each extract was expressed as mg gallic acid equivalents per gram of dry extract (mg GE/g).

Total flavonoid content. Total flavonoid content of the sample was determined by aluminium chloride colorimetric method.[24] 2.5 ml $AlCl_3$ reagent. (2% $AlCl_3$ and 1 M NaOAc) was added to 5 ml of each extract solutions (1 mg/ml). The mixtures were vortexed and allowed to settle down for 30 minutes at room temperature. The absorbance of each solution was measured against a blank at 430 nm using a UV-Vis spectrophotometer. Total flavonoid content of each fraction was determined and expressed as mg quercetin equivalents per gram of dry extract. The equation procured from the standard quercetin calibration curve was used for calculation.

Antimicrobial activity. The activity of the sample extracts against a number of pathogenic bacteria and fungi were tested by method of disc diffusion.[25] A total of 5 gram-positive (*Bacillus sereus, B. megaterium, B. subtilis, Staphylococcus aureus, Sarcina lutea),* 8 gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphii, S. typhi, Shigella boydii, S. dysenteriae, Vibrio mimicus, V. parahemolyticus*) and 3 fungal strains (*Aspergillus niger*, *Candida albicans*, *Sacharomyces cerevisiae*) were collected from Microbiology laboratory, Department of Pharmacy, State University of Bangladesh. About 8 mg of the crude methanol extract and its fractions (hexane, dichloromethane, ethyl acetate and aqueous fractions) were converted into their corresponding solutions and the sterilized filter paper discs (6 mm) were soaked with these. The amount and dose of sample was 400 µg/disc. Ciprofloxacin (30 µg/disc) disc was used as standard for comparison of the activity with that of the extracts.

Thrombolytic activity. The activity to break down the blood clot by the extracts was evaluated by standard method using standard streptokinase.[26] Standard solution (15,00,000 I.U) in an Eppendorf tube was mixed with 5 ml sterilized water. 100 µl of this suspension was employed as stock. The crude extract and other fractions (10 mg each) were taken in separate vials each containing 1ml of distilled water. Human volunteer with no previous recorded pathological conditions provided the blood samples. Previously weighed sterile Eppendorf tubes containing 1 ml of blood in each were kept in incubation at 37°C for 45 minutes for clot formation. Following the removal of blood serum, each tube was again weighed.

Clot weight = weight of clot containing tube – weight of tube alone

100 µl aqueous solution of the sample extracts were taken separately to each of the tubes containing clotted blood followed by incubation at 37°C for 90 mins. The tubes were weighed again to determine the variations in weight due to clot disruption. The percentage of clot lysis was calculated from the following equation:

% Clot lysis= (Weight of the lysed clot) / (Weight of clot before lysis) x100

Statistical analysis. The experimental results of antioxidant activities, phenolic, flavonoid contents and thrombolytic activities were expressed as mean ± SD of three replicates. Data were statistically analysed by using GraphPad Software, USA. The

results with P value less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Extractive value. The extractive values of *M. paradisiaca* inflorescence in different solvents with varying polarity were measured using the modified Kupchan method of liquid-liquid partitioning. The extractive values of the sample in n-hex, DCM, EA, AQ and MeOH were found to be 28.36%, 29.42%, 23.63%, 13.89% and 4.68% respectively. The results indicate that the sample contains most of its extractable compounds in comparatively less polar fractions like hexane or DCM. These compounds can be esters of different fatty acids, various essential oils or other non-polar secondary metabolites. Moderate extractive value of EA and AQ fractions denote the probability of the presence of some polar constituents like flavonoids, terpenoids, phenolics etc.

Fatty acid composition analysis. The bound fatty acids (BFA) and free fatty acids (FFA) from the n-hexane extract of the sample were transformed into corresponding methyl esters. Total BFA and FFA of the sample were found from the evaporation. The amount and percentages of BFA and FFA found in this process is recorded in Table 1.

Table 1. A list of the amount and percentages of BFA and FFA in sample.

Amount of n-hexane extract taken (g)	Amount of BFA found (g)	Percentage of BFA in the sample (g/100g)	Amount of FFA found (g)	Percentage of FFA in the sample (g/100g)
0.593	0.2662	0.44	0.2421	0.4

*BFA= Bound Fatty Acids; FFA= Free Fatty Acids.

Most of the fatty acids in the sample are found to be bound as the glyceryl esters, phospholipids, glycolipids and other forms. The BFA and FFA of the sample were further analyzed by GC for the determination of the compositions and relative percentages of different fatty acids and the results are shown in Figure 1.

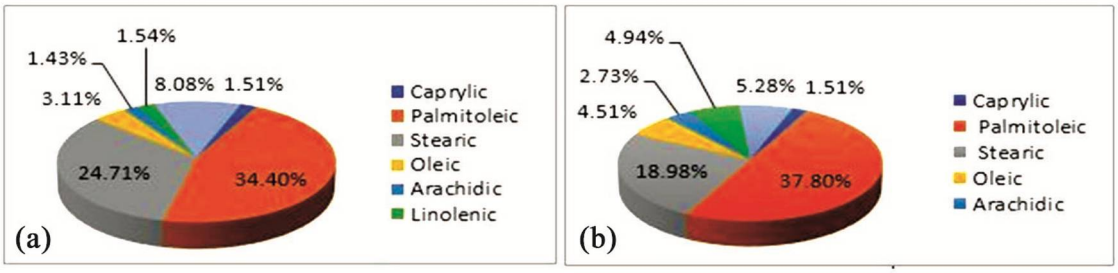

Figure 1. Composition and relative percentages of BFA (a) and FFA (b) in the sample.

The highest proportion of the fatty acid found in *M. paradisiaca* inflorescence was palmitoleic acid. It has shown anti-inflammatory activities and insulin sensitivity in different experiments.[27] The inflorescence sample also contain small amount of linolenic acid which is an omega-3 fatty acid. These acids are necessary in reducing the bad cholesterols in human body thus preventing the chances of chronic heart diseases.

Composition analysis of the sample in Figure 2 shows that, BFA contains high percentages of saturated acids whereas FFA contains more unsaturated acids. Greater amounts of unsaturated acids in FFA are reasonable as the double bonds in unsaturated acids are more susceptible to hydrolysis or dissociation by external conditions (enzymes, light, bacteria etc.). Again, the total percentage of unsaturated acid in the sample is higher than that of

the saturated ones. Higher degree of unsaturation can lead to quick rancidity of the oil.

Cytotoxicity assay. The cytotoxic effects of the sample extracts (n-hexane, dichloromethane, methanol, ethyl acetate and aqueous) were tested against HeLa, Vero cell and BHK-21 using 2.5% DMSO as solvent. The results are shown in Table 2.

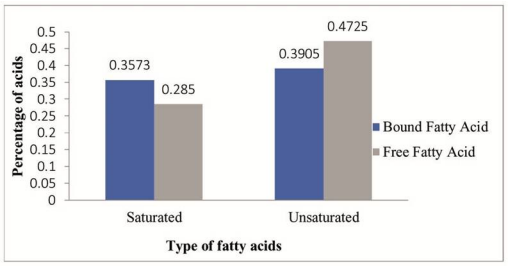

Figure 2. Composition of saturated and unsaturated fatty acids in the sample.

Table 2. Cytotoxicity analysis of different extracts of *M. paradisiaca*.

Sample Name		Solvent (+)	Solvent (-)	HEX	DCM	MeOH	AQ	EA
Survival of cells	HeLa	>95%	100%	<5%	10-20%	>95%	>95%	>95%
	Vero	>95%	100%	>95%	>95%	>95%	>95%	>95%
	BHK-21	>95%	100%	>95%	>95%	>95%	>95%	>95%

Remarks: Cell cytotoxicity was observed for sample n-hexane and dichloromethane on HeLa cell line.

The inflorescence sample exhibited cytotoxicity against HeLa cell line for the hexane and DCM extract. The ability of the sample extracts of being potentially toxic against a carcinoma cell line (HeLa) and non-toxic against other normal cells suggest the possibility of the presence of latent anticancer agents in the sample.

Antioxidant activity. The free radical scavenging activity of the *M. paradisiaca* inflorescence extracts was tested by measuring its ability to neutralize the stable 1,1 - diphenyl-2-picrylhydrazyl radical (DPPH radical) in comparison to the standard butylated hydroxytoluene (BHT) solutions. The IC_{50} values of different extracts of the sample are shown in Figure 3.

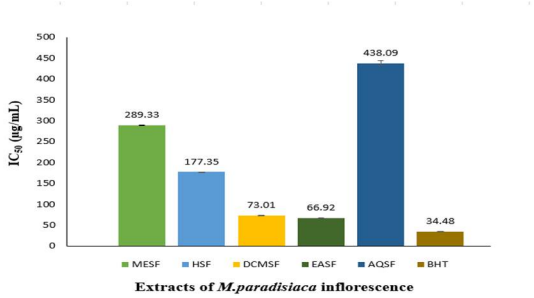

Figure 3. Free radical scavenging activities of different extracts of *M. paradisiaca*.

IC_{50} values of the extracts suggest that the ethyl acetate and DCM extracts possess significant radical scavenging capability (66.92 ± 0.3065 µg/ml and 73.01 ± 0.3635 µg/ml respectively) compared to the standard. Free radicals and other reactive species can damage cell membranes, proteins, DNA etc. through oxidative stress leading to several chronic diseases.[28] Antioxidants can scavenge and neutralize these free radicals thus preventing damages to active cells.

Total phenolic content. The amount of phenolics present in different extracts of the sample are demonstrated in Table 3. The hexane extract of the sample possessed the highest phenolic content (26.40± 0.033 mg GE/g of dry extract). Other extracts also demonstrated significant phenolic contents.

Total flavonoid content. Flavonoids have strong antioxidant properties due to their ability to neutralize free radicals. Total flavonoid content of the sample extracts was assessed by the aluminum chloride colorimetric method using quercetin as standard. The results are indicated in Table 3. The hexane extract contained highest amounts of flavonoids (83.40± 0.099 mg QE/g of dry extract).

Table 3. Total phenolic and flavonoid contents of different extracts (expressed as mean±SD (n=3)) of *M. paradisiaca* inflorescence.

Extracts of *M. paradisiaca*	n-hexane	DCM	EA	AQ	MeOH
TPC	26.40 ± 0.033	15.70 ± 0.039	15.12 ± 0.063	2.37 ± 0.095	11.41 ± 0.071
TFC	83.40 ± 0.099	28.30 ± 0.080	23.19 ± 0.103	17.51 ± 0.176	35.31± 0.099

*Units: TPC (mg GE/g of dry extracts); TFC (mg QE/g of dry extracts).

Presence of phenolics, flavonoids, tannins etc. may be responsible for the potent antioxidant, anti-inflammatory activities of the inflorescence sample.[29] Phenolics and flavonoids are said to exert antioxidant activity through either redox reactions or scavenging and chelating with the metal ions of the active species.[30]

Antimicrobial activity. The crude methanolic extract (MESF) of the sample and its hexane (HSF), dichloromethane (DCMSF), ethyl acetate (EASF) and aqueous (AQSF) fractions were tested against 16 different microbes. Sample extracts showed no activities against the gram-positive bacteria. DCMSF exhibited the highest zone of inhibition (15 mm) against the bacterium *Shigella dysenteriae* compared to the standard ciprofloxacin (30 mm). This bacterium is generally known to cause bacillary dysentery or shigellosis. DCM fraction also had moderate activity against *Salmonella paratyphii* (10 mm) and *Vibrio parahemolyticus* (8 mm). All the fractions were inactive against the tested fungi. The resultant activities of the plant extracts against the microbes lies in line with the traditional use of the inflorescence in treating diarrhoea and dysentery.[31]

Thrombolytic activity. The ability of the crude methanol extract of the sample and its different partitionates to lyse the human blood clot was tested by comparing their activity with standard streptokinase. Observed results are shown in Figure 4.

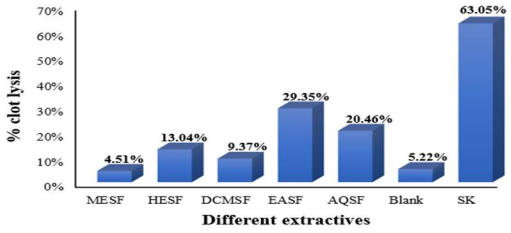

Figure 4. Thrombolytic activity of *M. paradisiaca* inflorescence extracts.

Thrombolytic activity indicates the ability to disrupt the blood clot inside the blood vessels. This activity is required in drugs related mostly to cardiovascular diseases. The ethyl acetate fraction showed thrombolytic activity of 29.35% compared to the standard (63.05%) demonstrating the potential of the sample as a source of anti-coagulant drugs.

CONCLUSION

M. paradisiaca inflorescence is still in use for the ailment of a number of pathological conditions in rural areas of developing countries. Present study on its bioactivities not only confirms the validity of these ethnopharmacological uses, but also reveals the prospects of this sample for further detailed studies regarding the *in-vivo* bioactivities and active chemical constituents. Also, wide availability of the sample as agricultural by-product with latent therapeutic activities could promise a low-cost alternative lead for the development of new drugs.

Conflict of Interest

The authors declare that there's no conflict of interest.

Funding

The authors are grateful to Ministry of Science and Technology, Government of People's Republic of

Bangladesh for providing financial aid to carry out the research work through National Science and Technology Fellowship program.

ACKNOWLEDGEMENTS

Authors' heartfelt gratitude goes out to Center for Advanced Research in Sciences (CARS), University of Dhaka and Department of Pharmacy, State University of Bangladesh for supporting the research work.

Data availability statement

The raw data used to support the findings of this study are available from the corresponding author upon request.

REFERENCES

1. Chowdhury, N.S. 2005. In vitro Studies on the toxicological property of the root and stem bark extracts of *Oroxylum indicum*. *Dhaka Univ. J. Sci.***4**, 1-5.
2. Nagaranjan, M., Rajasekaran, S. and Ganesh, K.S. 2013. Antibacterial activity of *Lawsonia inermis*L., *Int. J. Modern Bio. Med.* **4**, 169-175.
3. Nicholson, R.L. and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance, *Ann. Rev. Phytopatho.***30**, 369-389.
4. Nelson, S.C., Ploetz, R.C. and Kepler, A.K. 2006. Musa Species (Bananas and Plantains): An overview with emphasis on pacific island cultivars. In *Species Profiles for Pacific Island Agroforestry* (Elevitch, C.R., Ed.),Permanent Agricultural Resources, Holualoa, Hawai'i.
5. Reddy, C.K., Haripriya, S. and Vidya, P.V. 2015. Morphology, physico-chemical and functional characteristics of starches from different banana cultivars. *J. Food Sci. Technol.* **52**, 7289-7296.
6. The State of Food and Agriculture. 2006. In *World and Regional Review: Facts and Figures* (Food and Drug Administration (FAO) Statistics), Rome, Italy, part II, pp. 83-107
7. Ojewole, J.A. and Adewumni, C.O. 2003. Hypoglycemic effect of methanolic extract of *Musa paradisiaca* green fruits in normal and diabetic mice. *Methods Find. Exp. Clin. Pharmacol* **25**, 453-460.
8. Pari, L. and Umamaheswari, J. 2000. Antihyperglycaemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan induced rats. *Phytotherapy res*.**14**, 136-138.
9. Salau, B.A.*et. al.*2010. Methanolic extract of *Musa sapientum* sucker moderates fasting blood glucose and body weight of alloxan induced diabetic rats. *Asian J. Exp. Bio. Sci.***1**, 30-35.
10. Jo, S. and Megawati, R. 2010. Effect of pisang ambon (*musa paradisiaca* colla) on adult female blood pressure on cold stress test. *J. Med. Planta* **1**, 21-25.
11. Mokbel, M.S. and Hashinaga, F. 2005. Antibacterial and antioxidant activities of banana (Musa, AAAcv. Cavendish) fruit peels. *Am. J. Biochem. Biotech*. **1**, 125-131.
12. Poongzhali, P.K. and Cheju, H. 1994. The influence of banana stem extract on urinary risk factors for stones in normal and hyperoxaluric rats. *Br. J. Urol.* **741**, 23-25.
13. Wickramarachchi, K.S. and Ranamukharachchi, S.L. 2005. preservation of fibre-rich banana blossom as a dehydrated vegetable. *Scienceasia.* **31**, 265-271.
14. Divya, R.S., Venkatalakshmi, P., Vadivel, V. and Brindha, P. 2016. In vitro studies on the biological activities of flowers of banana (*Musa paradisiaca* L.), *Der Pharm. Lett.* **8**, 238-246
15. Nisha, P. and Mini, S. 2014. In vitro antioxidant and antiglycation properties of methanol extract and its different solvent fractions of *Musa paradisiaca* L. Inflorescence. *Int. J. Food Prop*. **17**, 399-409.
16. Bhaskar, J.J., Mahadevamma, S. and Vishwanath, S. 2010. Effect of banana (Musa sp. Cultivar elakki bale) flower and stem on enzyme activities of intestinal and renal disaccharidases in streptomycin-induced diabetic rats. *J. Food Biochem.* **34**, 564-580.
17. Ramu, R. *et al.* 2014. Inhibitory effect of banana (Musa sp. var Najangud rasa bale) flower extract and its constituents umbelliferone and lupeol on alpha-glucosidase, aldose reductase and glycation on multiple stages. *S. Afr. J. Bot.* **95**, 54-63.
18. Arun, K.B., Thomas, S., Reshmita, T.R., Akhil, G.C. and Nisha, P. 2017. Dietary fibers and phenolic rich extracts of *Musa paradisiaca* inflorescence ameliorates type 2 diabetes and associated cardiovascular risks. *J. Funct. Foods.***31**, 198-207.
19. Rahman, M.H., Rashid, M.A. and Chowdhury, T.A. 2019. studies of biological activities of the roots of *Bombax ceiba* L. *Bangladesh Pharm. J*.**22**, 219-223.
20. Mimi, S.S., Hasan, M.M., Rahman, M.H. and Chowdhury, T.A. 2024. Qualitative phytochemical screening, fatty acid profile and biological studies of the bark of *Mallotus nudiflorus* (Pitali) plant. *Toxicol. Int*. **31**, 63-72.
21. Chowdhury, V., Shristy, N.T., Rahman, M.H. and Chowdhury, T.A. 2022. Qualitative phytochemical screening, fatty acid composition analysis and biological Studies of *Trianthema portulacastrum* L. Leaves, *Dhaka Univ. J. Pharm. Sci*.**21**, 33-43.
22. Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T. 1995 Use of a free radical method to evaluate antioxidant Activity. *LWT-Food Sci. Tech.***28**, 25-30.
23. Singleton, V.L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents, *Am. J. Eno. and Vit.***16**, 144-158.
24. Chang, C.C., Yang, M.H., Wen, H.M. and Charn, J.C. 2002. Estimation of total flavonoid content in propolis by two complimentary colorimetric methods, *J. Food Drug Anal.* **10**.

25. Balouiri, M., Sadiki, M. and Ibnsouda, S.K. 2016. Methods for *in vitro* evaluation of antimicrobial activity: A review. *J. Pharm. Anal*. **6**
26. Prasad, S., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J., Taori, G.M. and Daginawala, H.F. 2006. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thromb. J*. **4**.
27. Mozaffarian, D., Cao, H. and King, I.B. 2010. Trans-palmitoleic acid, metabolic risk factors and new-onset diabetes in US adults, *Annals Int. Med*. **153**, 790-799.
28. Wang, L., Ding, L., Yu, Z., Zhang, T., Ma, S. and Liu, J. 2016. Food Science of Animal Resources. *Food Res Int.* **90**, 33-41
29. Shruthi, D. 2019. Medicinal Uses of Banana (*Musa paradisiaca*). *Drug Invention Today*. **12**, 104-107.
30. Kumar, S., Mishra, A. and Pandey, A.K. 2013. Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using *in vitro* models. *BMC Complement. Alt. Med*. **13**
31. Yakubu, M.T., Nurudeen, Q.O., Salimon, S.S., Yakubu, M.O., Jimoh, R.O. and Nafiu, M.O. 2015. Antidiarrhoeal activity of *Musa paradisiaca* Sap in wistar rats. *Evid. Based Complement. Alt. Med*. **2015**