Inhibitory potentials of Methanolic extract of flowers from *Musa acuminata* in human colorectal cancer

S.Jayakumar[1*], A.Vignesh[1], R.Aruna[1], G. Sriram Prasath[2], M.Hemalatha[1], Mukesh Kumar Dharmalingam Jothinathan[3] and R. Shenbhagaraman[3]

[1]*Department of Biochemistry, SRM Arts and Science College, Kattankulathur, Chengalpattu*

[2]*Department of Biochemistry, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai.*

[3]*Centre for Materials Engineering and Regenerative Medicine, Bharath Institute of Higher Education and Research, Selaiyur, Chennai.*

[*]*Corresponding Author: jaikumarsbio@gmail.com*

S.Jayakumar

Assistant Professor, Department of Biochemistry, SRM Arts and Science College, Kattankulathur, Chengalpattu.

ABSTRACT

Colorectal cancer (CRC) recorded to be the second most deadly cancer in world, treatment of these type of cancers have severe chemotherapeutic side effects. Alternative medicine comes from the folk knowledge of indigenous people and these plant-derived drugs were originally explored through the study of traditional cures with very minimal side effects. Flowers of *Musa acuminata* possess a wide range of pharmacological properties such as antihypertensive, immunomodulatory, anti HIV, anti-tumor activity, Anti-Helmintic, hence the present study was aimed to evaluate effect of *Musa acuminata* flower extract in Human colon cancer cell line Colo320.The primary phytochemical analysis of methanolic extract of *Musa acuminata* flower exposed the presence various phytochemical such as alkaloids, flavonoids, steroids, tannins, total phenols. The assays of enzymatic antioxidants in the methanolic extract of *Musa acuminata* flower shows a notable amount of Super oxide dismutase (14U/g) in it. methanolic extract of *Musa acuminata* flower contains good amount of vitamin C, vitamin A and E. The methanolic extract of *Musa acuminata* flower showed the

evidence of induction of apoptosis in the colo320 cell line, were confirmed by DNA fragmentation studies and AO/EB staining. Thus the methanolic extract of *Musa acuminata* flower has an inhibitory role on human colon cancer cell line Colo320.

Key Words: *Musa acuminata, Antioxidant property, MTT assay; DNA fragmentation; Anticancer property*

INTRODUCTION

Colorectal cancer (CRC), occurs in the colon and/or rectum region and represents third most commonly diagnosed cancer in the world and second most important cause for death due to cancer globally [1]. Global incidence and mortality of colorectal cancer may tend to increases in the fourth coming decades. High fatality rate associated with CRC are noted in well developed countries, moreover the incidence of CRC and fatality rate are growing in emerging countries too. In 2020 WHO reported that CRC accounts for 9.4% of cancer related deaths [2]. However, in light of the substantial rise in the number of identified cases in the elder population, it is assessed that the global occurrence of CRC will increased to double by 2050, with the most noteworthy increase occurring in under developed countries [3]. CRC is not only a common disease but categorised by a gradual progression of the adenoma–carcinoma sequence, hence early screening is appropriate way to cure the population having the colorectal cancer [4]. The time taken for CRC progression from early adenoma to an established cancer is yet unknown, most scientific evidence suggests that might be less than ten years [5], providing abundant chance for monitoring via routine screening techniques followed by treatment. Earlier detection of colorectal adenomas and their removal helps in the prevention of CRC [6], and the earlier the CRC is detection, better the survival rate of CRC patient [7]. Thus earlier treatment results are completely impacted by interferences in the progression of colorectal adenoma–carcinoma.

As of now herbal medicine remains backbone of about 75–80% of the world's population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. It isestimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances. Aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine are a few important examples of what medicinal plants have given us in the past. Most of these plant-derived drugs were originally

discovered through the study of traditional cures and folk knowledge of indigenous people and some of these could not be substituted despite the enormous advancement in synthetic chemistry. Thus to explore more on herbal medicine, my area of interest has been on banana plant flower [8].

Banana is a monocotyledonous, perennial herb within the order Zingiberales, and the family Musaceae. The Musaceae is divided into two genera: *Musa* and *Ensete*. Musa consist of about 40 species and is distributed through India, New Guinea, Australia and Southeast Asia [9]. The Musa genus is grouped into four sections: Eumusa, Callimusa, Rhodochlamys and Australimusa. Eumusa is the most widespread and contains the greatest number of species and forms, for it includes all the edible seedless bananas. Almost all cultivars of the edible banana are now classified under two species M. acuminata (AA) and M. balbisiana (BB), both belonging to Eumusa section. According to Simmonds (1990) most cultivated bananas were derived from natural hybridization between two diploid species M. acuminata and M. balbisiana. Musa acuminata surpasses Musa balbisiana in variability and in diversity of species, and at least nine sub species have been described (ssp.malaccensis, ssp. microcarpa, ssp.burmannica, ssp. burmannicoide, ssp. siamea, ssp. banksii, ssp. errans, ssp. zebrine and ssp. truncate [10], whereas Musa balbisiana is less diverse with no subspecies recognized. Most of edible types that are derived from these species are triploid, although diploid (AB) and Tetraploid (ABBB) cultivars are also known.

The banana flower is an excellent source of vital nutrient and mineral. It provides cheapest sources of nutrients when compared to other sources.They are part of many cuisines in the world. They also possess immense medicinal value. However, there is no scientific information to determine the side effects and drug interactions of banana flower.Health Benefits of Banana Flower for Children include rich in antioxidant, treats infection, helps manage diabetes and anaemia, heals wounds and burns, rich in vitamins and minerals, reduces anxiety, prevents cancer and heart disease, abundant in dietary fibres, soothes the digestive system, minimizes menstrual pain and bleeding. *Musa acuminate possess a wide range of pharmacological properties such as antihypertensive* [11], immunomodulatory [12], anti HIV [13] anti-tumor activity [14] and Anti-Helmintic [15]. Having known the importance of the Musa acuminate flowers, the present study was aimed to evaluate effect of Musa acuminata flower extract in Human colon cancer cell line Colo320.

MATERIALS AND METHODS

Materials and Methods

Collection and Extraction of Plant Material:

The flower of *Musa acuminata* was collected from a cultivator in Neyveli, Tamilnadu. The flower material was dried and powdered through a blender and stored in an air tight container. This powdered flower was material used for extraction process.

Methanolic extract of *Musa acuminata*: 10 g of powdered flower material was subjected extraction process using methanol as solvent.

Antioxidant studies

Qualitative Phytochemical Analysis of Methanolic Extract of *Musa Acuminata* Flower [16]. Protein in the flower extract was estimated according to the method of Lowry et al [17]. The antioxidants such as SOD, Catalase, Glutathione peroxidase, Glutathione-S-transferaseand Reduced glutathione were estimated [18, 19, 20, 21 & 22]. Vitamin A was estimated by the method of Bayfield and Cole [23].Vitamin E was estimated by the method of Kayden et al [24].Ascorbic acid was estimated by the method of Roe and Keuther [25].

In Vitro Studies

Cell line

Human colon carcinoma- Colo320 was procured from National centre for cell studies (NCCS), Pune, India. The cells were grown in T75 culture flask containing DMEM supplemented with 10% FBS. Upon reaching confluence, the cells were detached using trypsin-EDTA solution.

MTT Assay for Cell Viability

The MTT assay [26] is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37^{o}C in humidified atmosphere with 5% CO_2.

Procedure

- Colo320 cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X 10^4 cells/well and allowed to attach overnight at 37^{o}C. The medium was then discarded and cells were incubated with different concentrations of the extract (200, 40, 600, 800 & 1000 µg/mL) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added

to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as positive control.

Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) X 100

Cytotoxicity % = 100 – Viability%

Experimental Design

Based on MTT assay we selected the doses at 3 different concentrations like 400,600 and 800µg/ml for 24hrs time period.

- Group 1: Control Colo320 cells.
- Group 2: Colo320 cells treated with 400µg/ml of extract for 24hrs.
- Group 3: Colo320 cells treated with 600µg/ml of extract for 24hrs.
- Group 4: Colo320 cells treated with 800µg/ml of extract for 24hrs.

DNA Fragmentation Studies

DNA extraction and agarose gel electrophoresis were carried out by the method of Alexei G.Basnakian [27].

Acridine Orange/Ethidium Bromide Staining (AO/EB Staining or Dual Staining):

AO/EB staining was carried out by the method of Deborah Ribble [28].

RESULTS AND DISCUSSION:

Qualitative analysis of phytochemical in methanolic extract of *Musa acuminata* flower

Preliminary phytochemicals screening for major secondary metabolites of the flower was carried out using standard qualitative methods. The sample extract was screened for the presence of bioactive compounds such as alkaloids, steroids, terpenoids, saponins, flavonoids, tannin and glycosides.

Table 1: Qualitative Phytochemical Analysis of Methanolic Extract of *MusaAcuminata* Flower

Test	**Methanolic extract**
Carbohydrates	+
Reducing sugar	+
Amino acids	+
Alkaloids	+

Flavonoids	+
Tannins	+
Total phenol	+
Terpenoids	-
Steroids	+
Saponins	-

The preliminary phytochemical analysis of methanolic extract of *Musa acuminata* flower revealed the presence various phytochemical as shown in table 1 namely; alkaloids, flavonoids, steroids, tannins, total phenols. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Research suggests that phytochemicals may slow the ageing process and reduce the risk of many diseases, including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis and urinary tract infections [29].

Table 2: Quantitative analysis of protein in methanolic extract of *Musa acuminata* flower

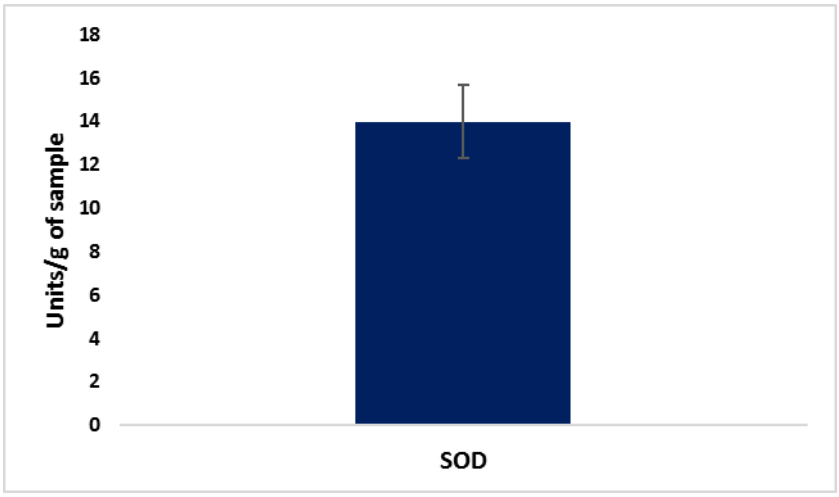
S.NO	Particular	Value in g/100g of dry sample
1	Protein	1.25±0.14

Results are expressed in mean ± S.D for the sample. Unit g/100g of dry sample.

Antioxidant Status of Methanolic Extract of *Musa Acuminata* Flower

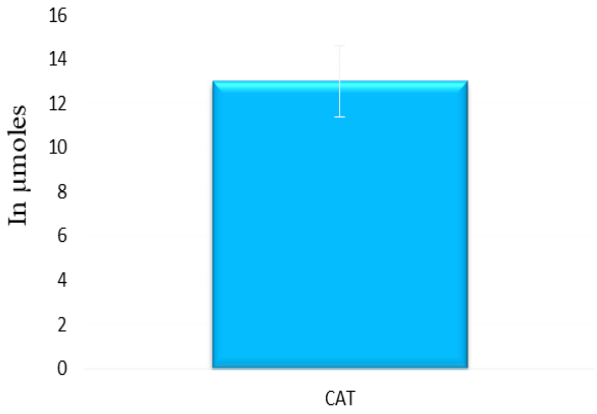
The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione s-transferase (GST). SOD, the first line of defence against free radicals, catalyses the dismutation of superoxide anion radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is transformed into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx). The selenoprotein GPx enzyme removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). The non-enzymatic antioxidants are also called as Nutrient antioxidants. These are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids etc.

Figure:1 Antioxidant Activity of SOD in Methanolic Extract of *Musa Acuminata* Flower



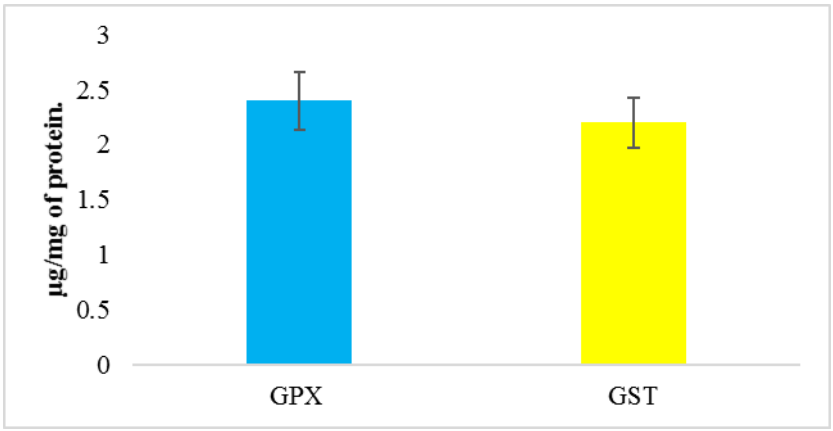
Results are expressed in mean ± S.D for the sample. Unit- SOD in units/g of sample

Figure 2: Antioxidant Activity of CAT in Methanolic Extract of *Musa Acuminata* Flower



Results are expressed in mean ± S.D for the sample. Unit- µmoles of H_2O_2consumed/min/mg of protein.

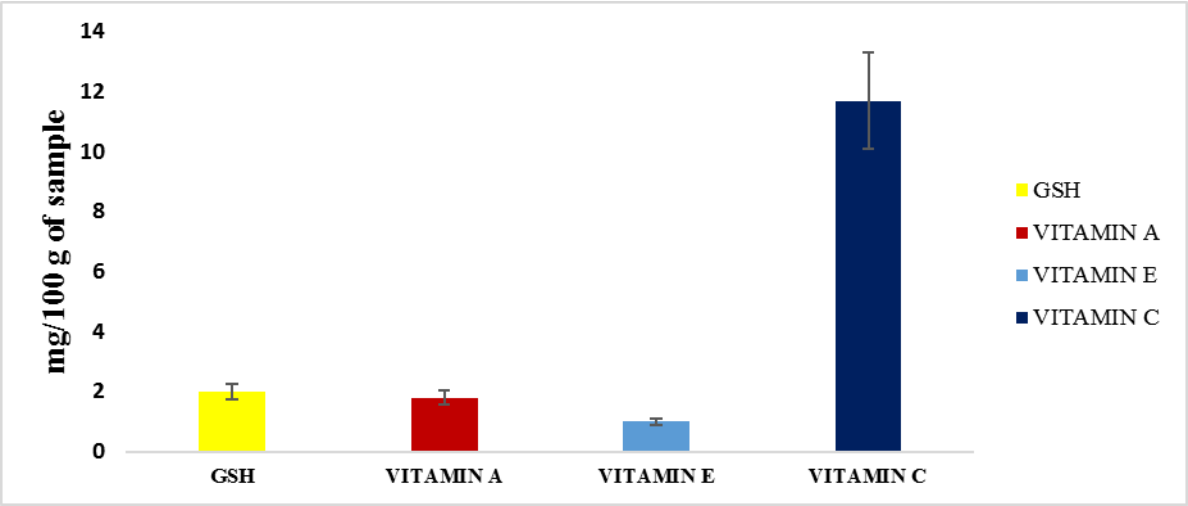
Figure: 3 Antioxidant Activity of GPX and GST in Methanolic Extract of *Musa Acuminata* Flower



Results are expressed in mean ± S.D for the sample. Unit- GPX AND GST in µg of GSH/mg of protein.

The assays of enzymic antioxidants in the methanolic extract of *Musa acuminata* flower shows a notable amount of enzymic antioxidant in it. Among them the level of Sod is significant shown in figure: 1. One gram of the sample contains 14 units of SOD. Antioxidants are broad spectrum of compounds which provides a primary defence to the cell from oxidative stress. These are protective agents which donate its electron to reactive oxygen species and prevents them from attacking the lipid bilayer of the cell membrane. Production of free radicals rapidly increases when toxic chemical accumulates in the body. Under these conditions supplement of antioxidants through diet promotes the scavenging activity of antioxidants [30].

Figure: 4 Antioxidant Activity of Non-Enzymic Antioxidants of Methanolic Extract of *Musa Acuminata* Flower



Results are expressed in mean ± S.D for the sample. Units – GSH, Vitamin A, E and C in mg/100g of sample.

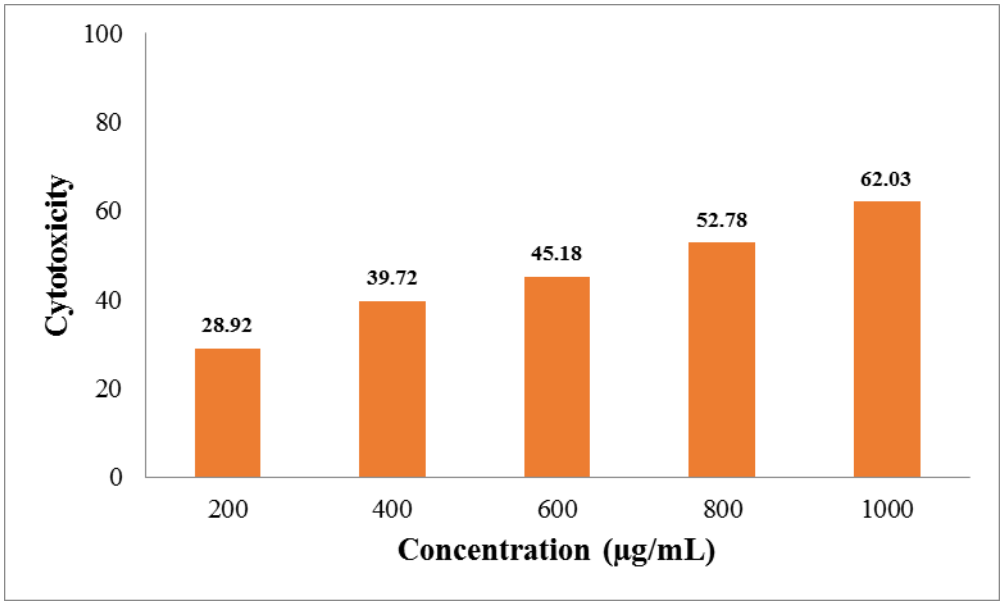
Nutrient antioxidants

Antioxidants from our diet play an important role in helping endogenous antioxidants for the neutralization of oxidative stress. The non enzymic antioxidants such as Reduced Glutathione, vitamin A and E are also were estimated in the methanolic extract of *Musa acuminata* flower. The values of these are also expressed as mg per 100g of sample. When compared to levels of vitamin C the levels of vitamin A and E were lesser in methanolic extract of Musa acuminata flower.

Vitamin E> Vitamin A > Vitamin C

MTT Assay

Figure 5 Effect of Methanolic Extract *Musa Acuminata* Extract at Various Doses on Colo320 Cells for 24hrs Assessed By MTT Assay



IC50 Value of Methanolic Extract of *Musa Acuminata* Flower is 719 µg/ml

Dosage Fixation Studies

The cytotoxic effects of *Musa acuminata* was evaluated using MTT assay. Figure 5 shows the effect of *Musa acuminata* at different doses (200-1000µg/ml) on Colo320 cells for 24 hrs by MTT assay. *Musa acuminata* inhibited the growth of Colo320 cells in a dose dependent

manner. Based on this study, we fixed the doses at 400,600 and 800µg/ml at time period of 24hrs for further studies.

Apoptotic Studies:

Methanolic Extract of *Musa Acuminata* Flower Induced DNA Fragmentation:

Figure: 6 Agarose Gel Electrophoretic Pattern of Nuclear DNA in Control and extract treated cells of Colo320 cell line

0 1 2 3 4

Lane 0 -1kb ladder

Lane 1 -Colo320 control cells

Lane 2 -400µg/ml of Methanolic extract treated at 24hrs.

Lane 3 - 600µg/ml of Methanolic extract treated at 24hrs.

Lane 4 - 800µg/ml of Methanolic extract treated at 24hrs.

Figure 6 shows the Agarose Gel Electrophoretic Pattern of Nuclear DNA in Control and extract treated cells of Colo320 cell line. *Musa acuminata* extract treated cells resulted in apoptosis of Colo320 cells as evidenced by the shearing of the DNA which is considered as the hallmark of apoptosis.

Acridine Orange/ Ethidium Bromide Staining

Figure: 7 Morphological changes of Colo320 cells analysed under Fluorescence Microscopy AO/EB staining (20x).

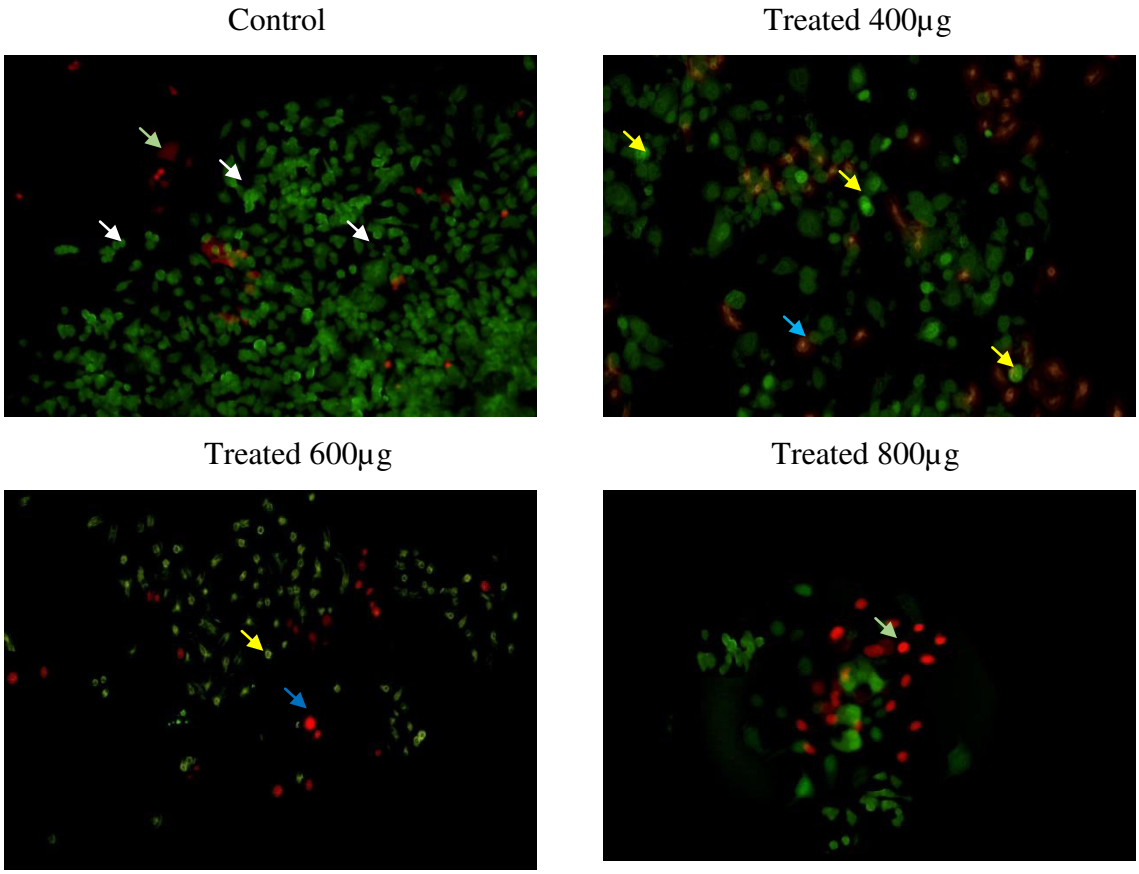


Figure: 7 Depicts the morphological changes of control and *Musa acuminata* extract treated Colo320 cells under Fluorescence microscopy after staining with Ethidium bromide/ Acridine orange stain. (Plate 1) Control cells: Less than 4% cells were apoptotic. Plate 2: Colo320 cells Treated with 400µg - 40% cells were in early apoptotic stage. Plate 3: Colo320 cells treated with 600µg - 15% cells were in early apoptotic stage and 30% cells were in late apoptotic. Plate 4: Colo320 cells treatedwith 800µg – Less than 10% cells were in late apoptotic stage and 64% of them were necrotic cells.

Role of *Musa Acuminata* Flower on Apoptosis Induction

The induction of tumor cell death by apoptosis is a major goal of cancer therapy. The cell line studies of methanolic extracts of *Musa Acuminata* flower on human colon cancer cell line Colo320 has shown the inhibitory role of *Musa Acuminata* flower against Colo320 cell line. The methanolic extracts of *Musa Acuminata* flower has induced apoptosis in the Colo320 cell line were confirmed by the assay of DNA fragnmentation studies and AO/EB staining. The Acridine orange dye binds to live and death cells in the cell culture whereas Ethidium bromide binds to dead once cells once which emits reddish orange color which can be seen

through fluorescence microscope. Thus, the methanolic extract of *Musa Acuminata* flower has an inhibitory role on human colon cancer cell line colo320

Conclusion

The result of present studies reveals that the methanolic extract of *Musa acuminata* flower has various phytonutrients in it. The phytonutrients such as alkaloids, flavonoids, tannins, phenolic compounds have eminent role in combating range of diseases in the human. The presence of both enzymic and non-enzymic antioxidant promotes the anticancer effect of *Musa acuminata* flower extract against cancer. The presence of Vitamin C in the *Musa acuminata* flower in enormous amount is quite significant. The presence of primary enzymic antioxidant in appreciable level also alleviates the anticancer properties of *Musa acuminata* flower. The presence of SOD, CAT, GPX,GST, and GSH were confirmed by the antioxidant assay of methanolic extract of *Musa acuminata.* The induction tumor cell death by apoptosis is a major goal of cancer therapy. The cell line studies of methanolic extracts of *Musa acuminata* flower on human cancer cell line Colo320 has shown the inhibitory role of Musa acuminata flower against colo320 cell line. The methanolic of *Musa acuminata* flower has induced apoptosis in the colo320 cell line were confirmed by the assay of DNA fragmentation studies and AO/EB staining. The Acridine orange dye binds to live and death cells in the cell culture whereas Ethidium bromide binds to dead once which emits reddish orange colour which can be seen through fluorescence microscope. Thus the methanolic extract of **Musa acuminata** flower has an inhibitory role on human colon cancer cell line Colo320.

References

1. WHO Cancer. [(accessed on 14 July 2021)]. Available online: https://www.who.int/news-room/fact-sheets/detail/cancer [Ref list]
2. Ferlay J., Ervik M., Lam F., Colombet M., Mery L., Piñeros M., Znaor A., Soerjomataram I., Bray F. Global Cancer Observatory: Cancer Today. [(accessed on 14 July 2021)].
3. Papamichael D., Audisio R.A., Glimelius B., de Gramont A., Glynne-Jones R., Haller D., Kohne C.H., Rostoft S., Lemmens V., Mitry E., et al. Treatment of colorectal cancer in older patients: International Society of Geriatric Oncology (SIOG) consensus recommendations 2013. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* 2015;26:463–476.
4. Morson B.C. The evolution of colorectal carcinoma. *Clin. Radiol.* 1984;35:425–431. doi: 10.1016/S0009-9260(84)80033-1.

5. Winawer S.J., Fletcher R.H., Miller L., Godlee F., Stolar M.H., Mulrow C.D., Woolf S.H., Glick S.N., Ganiats T.G., Bond J.H., et al. Colorectal cancer screening: Clinical guidelines and rationale. *Gastroenterology.* 1997;112:594–642.
6. Winawer S.J., Zauber A.G., Ho M.N., O'Brien M.J., Gottlieb L.S., Sternberg S.S., Waye J.D., Schapiro M., Bond J.H., Panish J.F., et al. Prevention of colorectal cancer by colonoscopicpolypectomy. The National Polyp Study Workgroup. *N. Engl. J. Med.* 1993;329:1977–1981
7. Ciccolallo L., Capocaccia R., Coleman M.P., Berrino F., Coebergh J.W.W., Damhuis R.A.M., Faivre J., Martinez-Garcia C., Møller H., De Leon M.P. Survival differences between European and US patients with colorectal cancer: Role of stage at diagnosis and surgery. *Gut.* 2005;54:268–273.
8. Akerele O (1988) Medicinal plants and primary health care: An agenda fo action. Fitoterapia.;59:355–63.
9. Simmonds NW and Weatherup STC (1990) "Numerical taxonomy of the cultivated bananas". Tropical Agriculture, Trinidad; 67, 90–92.
10. De Flora S, Ferguson LR (2005) Overview of mechanisms of cancer chemopreventive agents.Mutat Res.; 591(1-2):8-15
11. Kailash and Varalakshmi. (1992) - "Effect of banana stem juice on biochemical changes in liver of normal and hyperoxaluric rats" Indian J. Exp. Biol., 30,440-442
12. Singhal et al., 2013 Investigation of Immunomodulatory Potential of Methanolic and Hexane Extract of Musa acuminate Peel (Plantain) Extracts, Global Journal of Pharmacology 7 (1): 69-74.
13. Swanson *et al.,* 2010. Isolated from Bananas Is a Potent Inhibitor of HIV Replication, Journal of Biological Chemistry 285: 8646-8655
14. Roobha *et al.,* 2011 In vitro evaluation of anticancer property of anthocyanin extract from Musa acuminate bract.Research in Pharmacy 1(4):17-21
15. Prasanta et *al.,* 2013. Comparative Studies on Anthelmintic Activity of Leaf Extract of MusaAcuminateColla and CajanusCajan(Linn.) Leaf Extract. Mintage Journal and Pharmaceutical and Medical Sciences 2(1): 24-25.
16. Gunavathy N *et al., (2014)* "phytochemical evaluation of musa acuminata bract using Screening, ftir and uv-vis spectroscopic analysis" Journal of international

academic research for multidisciplinary impact factor 1.393, issn: 2320-5083, volume 2, issue 1

17. Lowry *et* al., (1951) J.Biol.Chem 193: 265 (The original method).
18. Misra HP and Fridovich (1972) - I J Biological Chem 247, 3170-3175.
19. Sinha, (1972) Colorimetric Assay of Catalase. Analytical Biochemistry, 47, 389-394.
20. Ellman GL and Fiches FT (1959) Quantitative determination of peptides by sulfhydryl groups Arch, BiochemBiophys.,; 82: 70-72.
21. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. J BiolChem 1974; 249: 7130-7139
22. Moron *et al.,* (1978) Levels of glutathione reductase and glutathione-Stransferase activities in rat lung and liver. BiochimBiophysActa; 582: 67–68
23. Bayfield RF, Cole ER (1980). Colorimetric estimation of vitamin A with trichloroacetic acid. Methods Enzymology., 67: 189 –195
24. Kayden *et al.(1973),* Spectrophotometric method for determination of tocopherol in red blood cells
25. Roe J.H. and Keuther.A (1953) - "The determination of ascorbic acid in whole blod and Wine through 2, 4–dinitrophenyl hydrazine derivative of dehydroascorbic acid", Journal of Biochemistry, 147: 39–404.
26. Mossman, T. (1983) Rapid colorimetric assay for cellular growth and survival – application to proliferation and cytotoxicity assays. *J.Immunol.Methods*65: 55-63.
27. Alexei G.Basnakian and S.Jill James (1994) A rapid and sensitive assay for the detection of DNA fragmentation during early phases of apoptosis- Nucleic Acids Research, Vol. 22, No. 13: 2714-2715.
28. Ribble D, et al., (2005) A simple technique for quantifying apoptosis in 96-well plates. BMC Biotechnology. doi:10.1186/1472-6750-5-12.
29. Pandey A, et al. (2012) Identification of a Nfs1p-bound persulfide intermediate in Fe-S cluster synthesis by intact mitochondria. *Mitochondrion* 12(5):539-49
30. Halliwell B. (2007) – "Biochemistry of oxidative stress". Biochem. Soc. Trans.; 35:1147–1150.