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Antioxidant Activity, Total Flavonoid and Total Phenolic Content of *Musa acuminate* Peel Extracts

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Abstract: *Musa acuminate* peel (Plantain peel) extract was evaluated for their antioxidant potential using the DPPH scavenging method, Total phenolic content determination and total flavonoid determination. Effect of extract on haematological parameters like total leukocyte count and total lymphocyte count of Wistar albino rats was also investigated. The result showed that methanolic extract is a more potent antioxidant than the hexane extract. Both extracts showed DPPH scavenging effect in dose dependent manner. Total flavonoid and total phenolic are higher in methanolic extract as compared to hexane extract of banana. Both the extracts significantly increased the total leukocyte and the percentage of lymphocyte in dose dependent manner.

Key words: Banana · Flavonoids · Phenolic Content · DPPH Scavenging · Antioxidant

INTRODUCTION

An increasing amount of evidence shows that the consumption of fruits and vegetables is, in general, beneficial to health due to the protection provided by the antioxidant compounds contained in them [1]. In fact, the presence of phytochemicals, in addition to vitamins and provitamins, has been considered of great nutritional interest in the prevention of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, ischemic and cardiovascular diseases and also in the aging process, in which oxidants or free radicals are involved [2-4]. Reactive oxygen species cause DNA damage which leads to mutation and chromosomal aberrations, oxidize cellular thiols and abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids. So there is need of development and utilization of more effective natural antioxidants which have minimal side effects [5].

Bananas are one of the most consumed fruits in tropical and subtropical regions. Bananas are tree like perennial herbs 2-9 meters in height. They are vegetatively propagated from the rhizome. The peel of banana represents 40% of the total weight of the fruit. Peel contains potassium (K), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn), Copper (Cu), bromine, rubidium, strontium, zirconium and niobium. Banana peel also demonstrated the presence of various phenolic compounds such as gallocatechin and anthocyanins like peonidin and malvidine and arabinoxylans [6-8].

MATERIALS AND METHODS

Plant Materials: *Musa acuminate* peels were collected from Jaipur, Rajasthan, India, in the month of August, 2012. The plant was authenticated and a voucher specimen (RUBL21136) was deposited in the herbarium department, Rajasthan University, Jaipur, Rajasthan, India.

Preparation of Methanolic Extract of Musa Acuminate Peel: Freshly collected *Musa acuminate* peels were dried in shade and coarse powder was prepared by maceration in methanol for one week. The macerated mixture was filtered through muslin cloth and evaporated at 40°C using rotary vaccum evaporator. The residue was designated as methanolic extract and used for further studies.

Preparation of Hexane Extract of Musa Acuminate Peel: Powdered peels were packed in Soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was extracted with Hexane in Soxhlet apparatus.

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The extract was concentrated under vaccum to get solid crude mass. The dried crude extract was stored in a dessicator and used for further experiment after suspending in 2% sodium carboxy methyl cellulose (CMC).

Antioxidant Activity Using DPPH Scavenging Assay: The free radical scavenging activity of Banana peel was measured by 1,1-Diphenyl-2-picryl-hydrazil (DPPH) [9]. 0.1mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of MAPE solution in water at different concentrations (5-50µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using UV-Vis spectrophotometer. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity.

$$\text{DPPH scavenging effect (\%)} = [(A_0-A_1/A_0) \times 100];$$

Where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of Standard and MAPE.

Estimation of Total Phenolic Content [10, 11]: From the stock solution (1mg/ml) of MAPE, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of folin-ciocalteu's reagent. After 5 min, 4ml of 20% (w/v) sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The absorbance was recorded at 765 nm, after 30 min. Percentage of total phenolics was calculated from calibration curve of gallic acid (5-50µg) plotted by using same procedure and total phenolics were expressed as % gallic acid.

Total Flavonoid Content [12]: The total flavonoid content was determined with aluminium chloride ($AlCl_3$) using rutin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml of $NaNO_2$ (5%) and incubated for 5 min at 25°C. Later 0.03 ml $AlCl_3$ (10%) was added and further after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. All tests were performed six times. The flavonoid content was calculated from a rutin standard curve.

Standard Drug: Septilin (Dabur India Ltd. Baddi, H.P.) was used as a standard drug at a dose of 500 mg/kg. *p.o.* Septilin, a proprietary herbal preparation has been reported to produce wound healing and immunomodulatory activities which contanins *Balsamodendron mukul, Tinospora cordifolia, Emblica officinalis, Rubia cordifolia, Moringa plerygosperma, Glycyrrhiza glabra,* Shankh bhasma and maharasnadi quath [13].

Animal: Wistar albino rats (120-150 g) of either sex were used. The animals were housed under standard laboratory conditions maintained at 25 ± 1°C and under 12/12 h light/dark cycle and fed with standard pellet diet (Amrut feed, chakan) and water *ad libitum*. Animal experiments were approved by the Institutional Animal Ethical Committee.

RESULTS AND DISCUSSION

Free radical scavenging activity measured by 1, 1-Diphenyl-2-picryl-hydrazil (DPPH): Percentage inhibition on free radical scavenging generation by MMAPE was found increasing in a concentration dependent manner, showing IC_{50} value 321.29 µg/ml and for HMAPE is 323.41 µg/ml, while IC_{50} value of reference standard ascorbic acid was found to be 29.50 µg/ml as shown in Figure 1.

Determination of Total Flavonoid Content: Total flavonoid content of MMAPE was found to be 75.97 mg rutin equivalent per gram of extract and total flavonoid content of HMAPE was found to be 71.95 mg rutin equivalent per gram of extract (Figure 2).

Estimation of Total Phenolic Content: Total phenolic content of MMAPE was found to be 114.4 mg gallic acid equivalent per gram of extract and total phenolic content of HMAPE was found to be 74.08 mg gallic acid equivalent per gram of extract (Figure 3).

Determination of Hematological Parameters: The two major groups of WBC's are granular leukocytes and agranular leukocytes. Neutrophils and macrophages are active in phagocytosis, they can ingest bacteria and destruct bacteria with lysosozyme defence and strong oxidants such as superoxide anion, hydrogen peroxide and hypochlorite anion dispose the matter. The two kinds of agranulocytes are lymphocytes and monocytes.

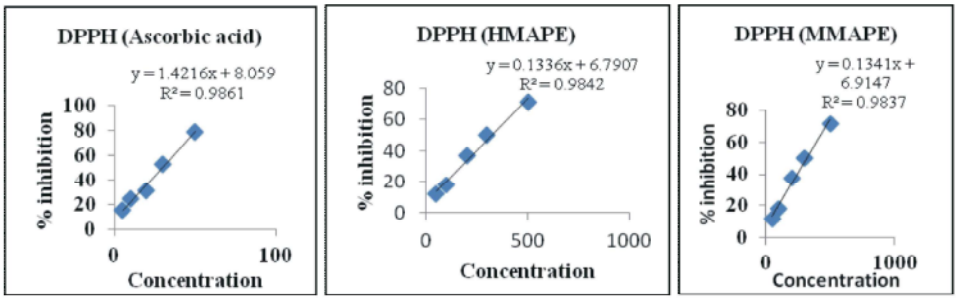

Fig. 1: DPPH scavenging activity of *Musa acuminate* peel extracts

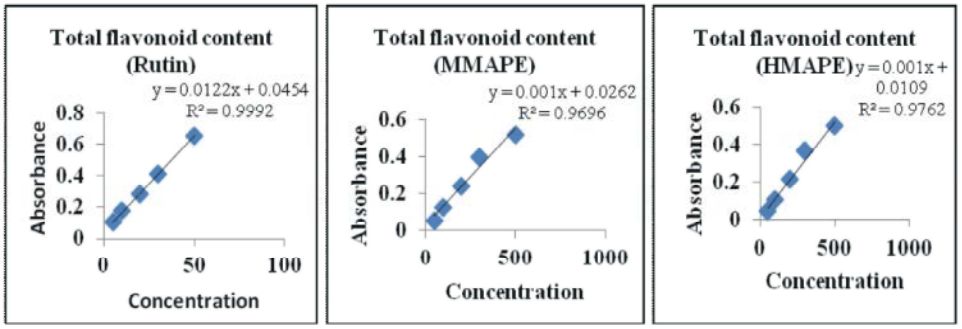

Fig. 2: Total flavonoid content determination of *Musa acuminate* peel extract

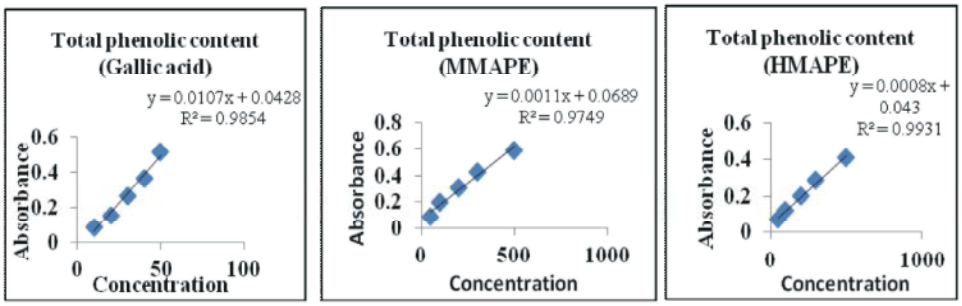

Fig. 3: Graphical representation of total phenolic content of MAPE and Gallic acid

Table 1: Effect of *Musa acuminate* peel extract (MAPE) on Total Leukocyte count in rat

		Days		
S.No.	Groups	0	7	14
1	Control	8.26±0.31	8.33±0.17	8.35±0.42
2	Standard drug	8.29±0.24	8.98±0.25***	9.22±0.42***
3	HMAPE-1	8.31±0.14	8.53±0.22[a]	8.75±0.23
4	HMAPE-2	8.21±0.17	8.68±0.27	8.89±0.21
5	HMAPE-3	8.28±0.32	8.87±0.21**	9.08±0.17**
6	MMAPE-1	8.34±0.28	8.78±0.17*	8.93±0.33*
7	MMAPE-2	8.25±0.09	8.90±0.19**	9.14±0.21**
8	MMAPE-3	8.21±0.19	8.98±0.26***	9.27±0.32***

All data were expressed as Mean ± SD for each group where n=6. Where *$p<0.05$, **$p<0.01$, ***$p<0.001$ *as compared to control*; [a]$p<0.05$ *as compared to standard.* Data were analysed using One Way ANOVA followed by tukey test.

Table 2: Effect of *Musa acuminate* peel extract (MAPE) on Total Lymphocyte count in rats

		Days		
S.No.	Groups	0	7	14
1	Control	74.5±0.31	74.57±0.19	74.64±0.34
2	Standard drug	74.56±0.56	74.84±0.4	75.5±0.38
3	HMAPE-1	74.19±0.78	74.34±0.47	74.67±0.28
4	HMAPE-2	74.12±0.31	74.45±0.10	74.79±0.48
5	HMAPE-3	74.5±0.42	74.95±0.45	75.11±0.32
6	MMAPE-1	74.38±0.19	74.76±0.21	74.87±0.65
7	MMAPE-2	74.54±0.87	74.97±1.23	75.28±1.47
8	MMAPE-3	74.29±0.98	74.99±0.38	75.59±0.49

All data were expressed as Mean ± SD of each group where n=6. Data were analysed using One Way ANOVA followed by tukey test.

Lymphocytes develop from lymphoblasts and monocytes develop from monoblasts. Lymphocytes mainly mediate the immune responses including antigen-antibody reactions. B-Lymphocytes develop into plasma cells which secrete antibodies. T-Lymphocytes attack invading viruses, cancer cells and transplanted tissues. Natural killer cells attack a wide variety of infection and certain spontaneously arising tumour cells. Monocytes are mainly involved in phagocytosis after transforming into fixed/wandering macrophages [14]. Before the scheduled treatment of animals by all the plant extracts, the total leukocyte count and total lymphocyte count were observed, thereafter, 7^{th} and 14^{th} day. The methanolic *Musa acuminate* Peel extract (MMAPE) increased the total leukocyte count significantly as compared to control and the hexane extract of the *Musa acuminate* Peel (HMAPE), (Table 1). (MMAPE) extract treatment also raised the total lymphocytes count significantly when compared to (HMAPE) which was further confirmed by increased phagocytosis and survivalency against inert particles and bacterial infection respectively as shown in Table 2. The result showed that the methanolic MMAPE and HMAPE increased the total leukocyte count and the percentage of lymphocytes in dose dependent manner, MMAPE exhibited more effect as compared to HMAPE.

Preliminary screening of the extracts was performed to determine the presence of various phytoconstituents. Both the extracts had glycosides, tannins, saponins and flavonoids in common. The investigation also revealed that the hexane extract of the plantain peel was found to contain carbohydrates while alkaloids were shown positive in the methanolic extract. From the results, it was observed that methanol extracts contain more phenolic and flavonoid compounds than hexane extract, because methanol can release the cell wall bound polyphenols from the cells and also it can neutralize the activity of poluphenol oxidase which degrades the polyphenols in plants [15, 16]. Free radicals and reactive oxygen such as super oxide anion, hydrogen peroxide and hydroxyl radical results in DNA damage, tissue injury and disease [5]. There was a close correlation between the antioxidant capacity and amount of polyphenol and flavonoid present in the plant. They are essential for the antioxidation process and for bioactivities in plants [17-21].

CONCLUSION

The present study indicated that Banana peel is the potent source of novel bioactive compounds like flavonoids and polyphenols with wide range of medicinal properties in particular the high free radical scavenging activity. Both extracts also increased the total leukocyte and the percentage of lymphocyte. Total amount of flavonoid and phenolic compounds were maximum in methanol than hexane. Because of antioxidant activity, Banana peel may show good biological activities and may be effective in various diseases.

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