PlantArchives Volume 20 No. 2, 2020 pp. 3627-3633 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EVALUATION OF THE PHYTOCHEMICALS CONSTITUENT OF ANTIOXIDANT ACTIVITIES IN FREE RADICAL SCAVENGING OF *MUSA PARADISIACA* FLOWERS

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Abstract

Banana blossom is an excellent source of crude fiber in the human diet. Dietary fiber has demonstrated its benefits in health and disease prevention in medical nutrition therapy. The objective of this study is to find out qualitatively and quantitatively phytochemical constituents and antioxidant activities, in the flower of *Musa paradisiaca* extracts. The extracts from, ethanol was investigated for the presence of alkaloid, glycoside, tannin, flavanoid, terpenoids. Particular reagents were used to screen phytochemicals in the samples and their presence was indicated by the changes of colour, precipitation. Then quantitative analysis by colorimetric method showed that the flower of *Musa paradisiaca* contains 100gm quantity crude Extract flower extraction % of yield in 3.5gm. Than quantity in 1g crude Extract 300µg of alkaloid; 200µg of flavonoids, in colorimetric method, the flower also contains 200µg of total phenolic, and 150µg of tannin. The extracts from, ethanol were investigated for the presence DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay of ethanol extract demonstrated stronger antioxidant activity than aqueous extract in which the IC50 value were 49.32 µg/ml and 57.433 µg/ml, respectively.

Key words : Phytochemicals, antioxidant activity, banana flower, *Musa paradisiaca.*

Introduction

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provides health benefits for human further than those attributed to macronutrients and micronutrients. Phytochemicals have an important role in preventing chronic diseases like cancer diabetes and coronary heart diseases. Phytochemicals accumulate in different part of plant, such as leaves, flower, fruits, or seeds (Ahuja *et al.,* 2017). It is estimated in five thousand individual phytochemicals have been identified. photochemical are classified as primary or secondary constituents, depending on their role in plant metabolism, primary constituents include the common sugar, amino acids, chlorophyll. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Sheng *et al.*, 2010). Therefore, they tend to be considered beneficial for general health and well being.

Antioxidants are the substances that may pressure cells from the damage caused by free radicals. Antioxidants inter change with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. The antioxidants may be exogenous or endogenous in nature. The endogenous antioxidants can be classified as enzymatic and non-enzymatic. The antioxidant enzymes incorporate Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) (Young 1990). The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic includes, glutathione, L-arginine, uric acid, bilirubin etc. While nutrient antioxidant belonging to exogenous antioxidants are compounds which cannot be produced in the body and must be provided through foods such as vitamin E,

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vitamin C, carotenoids, trace elements (Se, Cu, Zn, Mn) (Willcox *et al.,* 2004). Different types of biological antioxidants include, for instance, Glutathione, Vitamin C and vitamin E etc. It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes. ROS level elevation in diabetes may be due to decrease in destruction and increase in the production by CAT–enzymatic/non-enzymatic, SOD and GPx (Pham, *et al.,* 2008).

Medicinal plants are frequently used in traditional medicine to treat different diseases in different areas of the world (Palombo *et al.,* 2005). This indigenous knowledge, passed down from generation to generation various parts of the world, has significantly contributed to the development of different traditional systems of medicine (Jachak and Saklani, 2007). As well as helped in exploration of different medicinal plants to find the scientific basis of their traditional uses. This exploration of biologically active natural products have played an important role in finding new phytochemical (Newman *et al.,* 2003).

M. paradisiaca is often consumed as a vegetable (Ruvini liyanage, *et al.,* 2016). It is consumed as a curry as well as a boiled or deep-fried salad with rice and wheat bread. *M. paradisiaca* is generally valued as a fiber-rich source. Dietary fiber has demonstrated its benefits in health and disease prevention in medical nutrition therapy (Chandalia *et al.,* 2000). Along with dietary fibers, proteins and unsaturated fatty acids. The present study was undertaken to investigation this dietary plant flower Phytochemical analyze, free radical and antioxidant potential of *M. paradisiaca*. The work highly effect of *M.paradisiaca* compound produce from Phytochemical analyze, free radical and antioxidant potential

Materials and Methods

Materials

Chemicals and all Glassware's were obtained from the Department of Biochemistry, Government Arts College for women, Krishnagiri. All chemicals used were of analytical grade with 99 % purity. All the chemicals and reagents used for screening test, quantitative analysis and antioxidants activity were of analytical grade obtained from various companies.

Collection of plant samples

The freshly and healthy of Musa paradisiaca flowers were collected from local market of panjaliyur village, Krishnagiri (DT), Tamilnadu, in India. The flower was cut into small pieces and dried under shade for three to four weeks. The dried materials were grinding in to fine powder. The entire mixture was homogenized in blander than the homogenized mixture was left at room temperature for about 48 hr.

Preparation of flower extract

9g of *Musa paradisiaca* powder was extracted with 150 ml ethanol in a soxhlet apparatus for 48 hours. After extraction the solution was left out for 24 hrs was filtered and the clear filtrate was evaporated to dryness using water bath at 40^0C. After completion of the reaction, the entire slurry was filtered to get blossom powder extract (Singh, *et al.,* 2017). All extracts obtained were stored in a refrigerator until required for use. Further Phytochemical screening of flower extract were analysed by qualitatively and quantitatively.

Qualitative analysis of phytochemical screening assay (Harborne JB., 1998)

Detection of alkaloids

The presence of Alkaloids was determined by four methods. A small quantity of the flower extract was treated with few drops of dilute hydrochloric acid. The filtrate was tested with alkaloid reagent such as

1. Mayer's test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2. Wagner's test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

3. Dragendroff's test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

4. Hager's test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of flavonoids

The presence of flavonoids was determined by four methods.

1. Alkaline reagent test: Extracts was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2. Lead acetate test: Extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

3. Shinoda test: To 2-3 ml extract, few fragments of magnesium metal were added in a test tube, followed by drop wise addition of concentrate HCl. Formation of magenta colour indicated the presence of flavonoids.

Detection of Carbohydrates

A minimum amount of the extract was suspended in 5 ml of distilled water. The suspension was subjected to the following chemical tests.

1. Molisch's test: The extract was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2 ml of concentrated sulphuric acid was added along the side of the test tube. The formation of purple ring between two layers shows the presence of carbohydrates.

2. Fehling's test: The extract was treated with Fehling's A and B solution and heated for few minutes. Formation of brick red precipitate shows the presence of reducing sugar.

Detection of amino acids

1. Ninhydrin test: About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Detection of tannins

1. Lead acetate test: The extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Detection of Phenol

1. Ferric chloride test: The five mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins.

Quantitative analysis of bioactive phytochemical components

Determination of total alkaloids analysis of bioactive phytochemical components by the method of Fazel Shamsa *et al.*, 2008 and Mallikarjuna Rao *et al.*, 2012. The flower extract (1mg) were dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2N HCl and filtered. This solution was transferred to separating funnel, 5ml of bromocresol green solution and 5ml of phosphate buffer was added. The mixture was shaken with 4ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract. By using standard atropine calibration curve, measured the concentration of alkaloid content in atropine equivalents using unit's mg/g of gallic acid (FazelShamsa *et al.,* 2008).

Determination of total flavonoids (Jia *et al.*, 1999)

Total flavonoids content was determined using the method of Jia *et al.*, 1999. Add 0.5ml of various polysaccharide flower extracts into a test tube containing 1.25ml of distilled water. Then added 0.075ml of 5% sodium nitrite solution and allowed to stand for 5min. Added 0.15ml of 10% aluminium chloride, after 6min, 0.5ml of 1.0 M sodium hydroxide were added and the mixture at 510nm was measured immediately. The flavonoid content was expressed as milligram of catechi, quercetin and rutin equivalents of per g flower extract.

Determination of total phenolics (Singleton and Rossi, 1965)

Total phenolic content was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965) with some modifications. Stock solution of ethanol extract was prepared to get a final concentration of 1.0ml of various polysaccharide extracts was mixed with 1.0ml of folin ciocalteau's phenol reagent. After 3 min, 1.0ml of saturated Na_2CO_3 (~ 35%) was added to the mixture and made up to 10ml by adding distilled water. The reaction was kept in the dark for 90min, after which its absorbance were read at 725nm. A calibration curve was constructed with different concentrations of gallic acid, caffeic acid, ferulic acid and catechol. The results were expressed as mg of gallic acid caffeic acid, ferulic acid and catechol equivalents per g of extract.

Determination of total condensed tannins (Sun *et al.*, 1998)

Tannin content in banana flower was determined in accordance with Sun *et al.*, 1998 with some modifications method in 0.2 ml of extract, 1.0 ml of freshly prepared vanillin reagent was added. After incubation for 20min at 30 C, the absorbance was measured at 500nm against a reagent blank. Catechin ranging from 50 to 250µg/ml was used for the preparation of the calibration curve. The results are expressed as mg catechin equivalents (CAE) per g of the extract.

Free Radical Scavenging Activity

The antioxidant activity of flower extracts of *M. paradisiaca* was determined using DPPH free radical scavenging assay (Ripa & Haque, 2009) with

modifications. The stock solution of 500 mg/100mL was prepared by mixing the crude extracts with 95% ethanol. A series of test samples were prepared from stock solution by diluting with ethanol to attain a concentration in the range of 2.5 -12.5µg /ml ethanolic extract of *M. paradisiaca* flower showed inhibition percentage at a range of 15-105% respectively. It was used as a positive control and DPPH solution was used as negative control. A solution of 95% ethanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation: - % DPPH radical scavenging = [1-(As/Ac)] ×100 Where, Ac=absorbance of control, As=absorbance of sample solution. Percent of inhibitions (IC50) were plotted against respective concentrations used. Linear regression analysis was used to calculate IC50 values of the extracts and the standard.

Results and Discussion

a) Qualitative analysis

The phytochemicals screening use in *M. paradisiaca flower in extraction*

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, tannins, antioxidants and phenolic compounds. (Table 1).

Table 1 show that the presents the qualitative analysis of Phytochemicals in ethanolic solvent of *M. paradisiaca* crude extract. Crude extracts were found to contain alkaloids, flavonoids, Phytosterol, carbohydrates, tannins, spaonin, proteins, and phenols. Alkaloids where found to be enriched with 4+, while flavonoids and carbohydrates are present at 2+ grades, where found to be enriched with 1+, while Phytosterol, tannins and phenols where protein and saponin are present at 1- grades, where rest of the phytochemicals are present at 1+ grade. From the above study and supportive reports suggest *M. paradisiaca* crude extract is enriched with alkaloids and other phytochemicals.

b) Quantitative Analysis of phytochemicals: The phytochemicals screening in quantitative analysis of *M. paradisiaca* flower extraction.

Units: Total alkaloids mg of BSA/g; Total flavonoids mg of CE/g, Total phenols mg of GAE/g. Total tannins mg of GAE/g. Table 2 Shows that the quantitative analysis presents of yield in crude extraction for 3.5 gm percentages in *M. paradisiaca* flower extract at the rate of 100gm. (Table 3) shows the quantity of phytochemicals: Alkaloids 300µg, Flavonoids 200µg, Phenols 200µg and Tannins 150µg.

Table 1: Presents the qualitative analysis of Phytochemicals in ethanolic solvent of *M. paradisiaca* crude extract.

S.No.	Experiments	Ethanol Crude Extract of *M. paradisiaca*
1.	Test for Alkaloids	
	a. Mayer's test	+
	b. Wagner's test	+
	c. Dragendroff's test	+
	d. Hager's test	+
2.	Test for Flavonoids	
	a. Alkaline reagent test	+
	b. Lead acetate test	+
3.	Test for Phytosterol	
	a. Salkowski test	+
4.	Test for carbohydrates	
	a. Bendict's test	+
	b. Fehling's test	+
5.	Test for Tannins	
	a. Lead acetate test	+
6.	Test for Saponin	
	a. Foam test	-
7.	Test for protein	
	Million's test	-
8.	Test for phenols	
	a. Ferric chloride test	+

'+' Present '- 'Absent

Table 2: The quantitative analysis of yield in crude and *M. paradisiaca* extraction.

Plant used	Quantity for Extraction	% Of yield (crude Extraction)
Musa paradisiaca	100gm	3.5gm

Table 3: Shows the quantity of phytochemicals present in ethanolic crude extract of *M. paradisiaca* flower.

S.No.	Name of Phytochemicals	Quantity in 1g crude Extract
1	Alkaloids	300µg
2	Flavonoids	200µg
3	Phenols	200µg
4	Tannins	150µg

c) Determination of alkaloids

Fig. 1 & Table 4 show the Alkaloids reveals about 300µg of BSA/g of extract alkaloid content in ethanolic extract of *Musa paradisiaca flower. The* alkaloid contents expressed in terms of bovine serum albumin equivalent using mg of extract (the standard curve equation Y=0.001 R^2=1)

Fig. 1 &Table 4: Present the determination of content alkaloid from standard BSA/G.

Concentration	OD at 470nm	Quantity in crude extract of *M. paradisiaca*
20	0.03	300µg
40	0.06	
60	0.09	
80	0.12	
100	0.15	

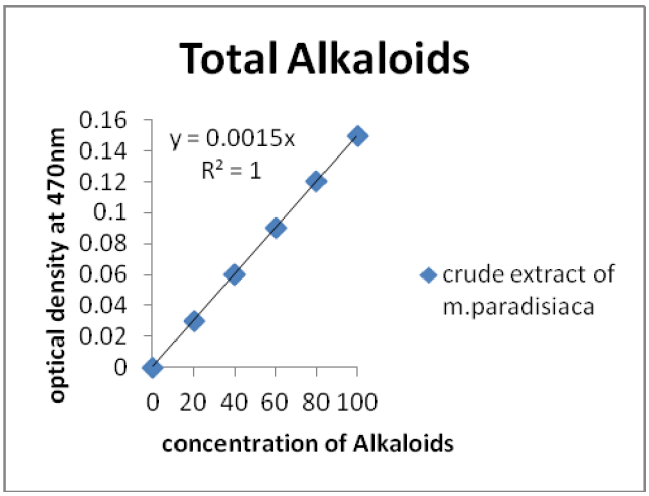
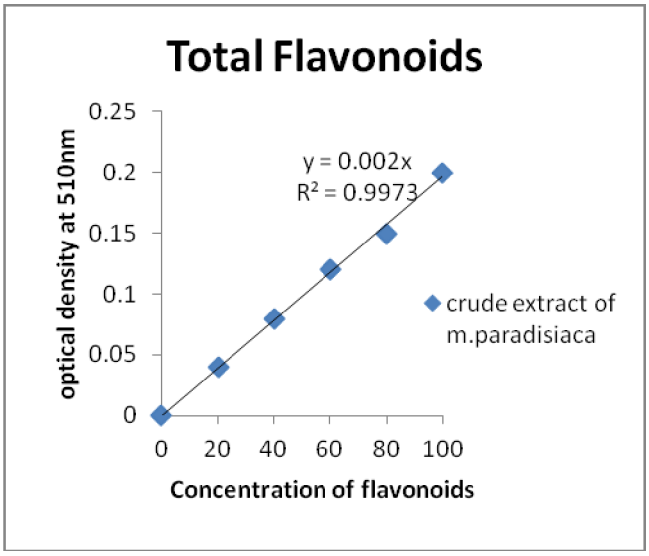


Fig. 2 & Table 5: Present the determination of content flavonoids from standard CE/G.

Concentration	OD at 510nm	Quantity in crude extract of *M. paradisiaca*
20	0.04	200µg
40	0.08	
60	0.12	
80	0.16	
100	0.20	



d) Determination of flavonoids

Fig. 2 & Table 5 Flavonoids reveals about 200µg of CE/g of extract flavonoids content in ethanolic extract of *Musa paradisiaca*. The flavonoid contents expressed in terms of catechin equivalent using mg of CE/g of extract the standard curve equation Y=0.002x R^2=0.997

e) Determination of phenols

Fig. 3 & Table 6 phenol reveals about 200 µg of GAE/ g of extract phenolic content in ethanolic extract of *M. paradisiaca* flower. The phenolic contents expressed in terms of catechin equivalent using mg of GAE/g of extract the standard curve equation Y=0.002 R=1.

f) Determination of Tannins

Fig. 4 & Table 7 Tannins reveals about 150 µg of GAE/g of extract tannins content in ethanolic extract of *M. paradisiaca* flower. The tannins contents expressed in terms of Gallic acid using unit's mg of GAE/g of extract (the standard curve equation Y=0.000x R^2=1)

g) Antioxidant Activities of *In vitro* antioxidant assay

Free radical Scavenging potential of crude extract of *M. paradisiaca* flower. DPPH radical scavenging assay.

The essence of DPPH method is that the antioxidants react with the stable free radical *i.e.* α, α-Diphenyl-β-

Fig. 3 & Table 6: Present the determination of content phenols Gallic acid standard GAE/G.

Concentration	OD at 725nm	Quantity in crude extract of *M. paradisiaca*
20	0.05	200µg
40	0.10	
60	0.15	
80	0.20	
100	0.25	

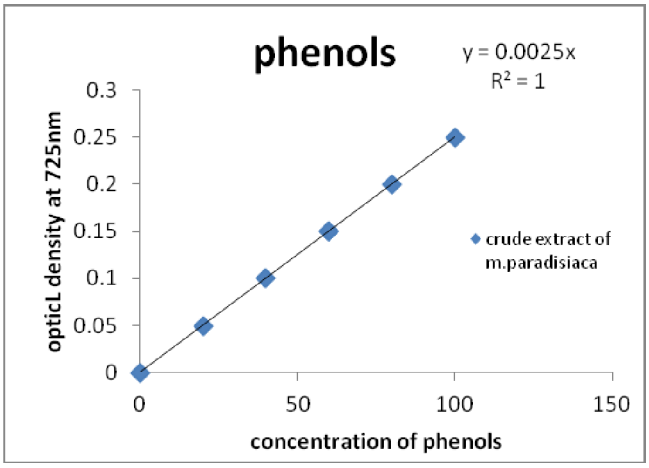


Fig. 4 & Table 7: Represent the determination of content tannins Gallic acid standard GAE/G.

Concentration	OD at 500nm	Quantity in crude extract of *M. paradisiaca*
50	0.2	150µg
100	0.4	
150	0.6	
200	0.8	
250	0.10	

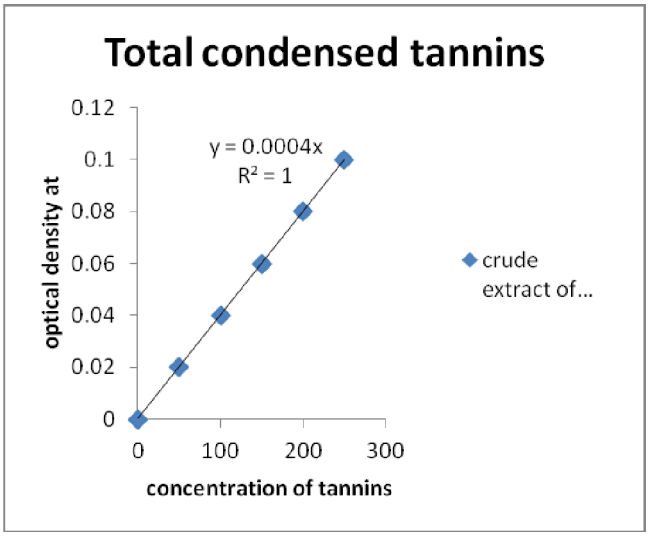


Table 8: Present DPPH scavenging potential of *M. paradisiaca* flower crude extract.

Contents	Concentration of *M. paradisiaca* µg/ml	%Inhibition	IC_{50} µg/ml
DPPH	2.5	15	49.32µg/ml
	5	45	
	7.5	66.25	
	10	92.5	
	12.5	105	
Ascorbic Acid	10	28	57.433 µg/ml
	20	39.3	
	30	58.32	
	40	67.4	
	50	79.5	

picrylhydrazyl (deep violet colour) and convert it to α, α-Diphenyl-β-picrylhydrazine with discolouration. The degree of discolouration indicates scavenging potentials of the sample antioxidant. Table 8 & Fig. 5 Presents the free radical DPPH scavenging potential of the crude extract of *M. paradisiaca* flower and ascorbic acid (ASB) For the concentration range of 10 -50µg /ml ethanolic extract of *M. paradisiaca* flower showed inhibition percentage at a range of 2.5-12.5 %

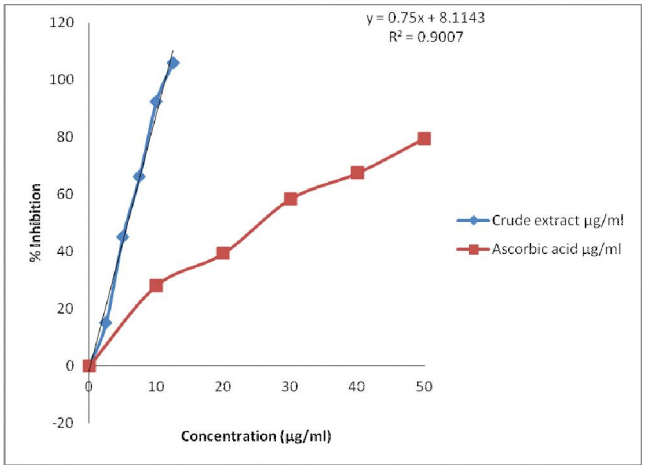


Fig. 5: Present DPPH scavenging potential of *M. paradisiaca* flower crude extract.

respectively. In concentration dependent manner the ethanolic extract of *M. paradisiaca* flower was found to be effective in neutralizing DPPH radical @ Mean IC_{50} found to be 49.32 µg /ml, Ascorbic acid inhibited at IC_{50} rate 57.433µg/ml. Potential of ethanolic extract was found to be equivalent to that of standard.

Conclusion

Phytochemicals in the flower of *M. paradisiaca*, antioxidant activity in the plant extracts can be attributed to at least one of the compounds. These findings confirmed that the flower may have potential use in pharmaceutical, cosmetic, and food products. Therefore, the findings are of the great impact in going further in research relevance. Antioxidant in *M. paradisiaca* flower demonstrated that ethanol extract required lower concentration of substrate than the aqueous extract to inhibit a 50% DPPH colour. The results conclude that phytochemicals defend free radicals and protects from diabetic disease, is expressed in the crude ethanolic extract of

M. paradisiaca flower concentration of phytohemicals markedly drops from the outer to the inner leaves. Therefore, it is possible for instance that sample taken from the inner tissues might contain low phenolic content compared to the outer skin layers.

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