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**PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND NUTRITIONAL
COMPOSITION OF FOOD BASED MIXES**

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ABSTRACT

Antioxidants is a molecule that has a capacity to slow down or prevent the oxidation of other molecules and thereby decrease the risk of various non communicable diseases. They help in quenching free radicals and maintain optimum health and well being. It is very quintessential to incorporate antioxidants in the diet. Therefore the present investigation was planned with focus on the development of food based antioxidant mixes. Three health mixes namely Mix C gms, Mix A, Mix B were formulated and there Phytochemical screening, Antioxidant and Nutritional composition was performed. The results of Nutritional components of the developed mixes clearly indicate that there is a significant increment in the crude fiber content of Antioxidant mixes A and B when compared to Standard Mix C. However there is no significant difference in the moisture, ash, protein, fat and carbohydrate content of the mixes. The results showed that there was a significant increase in the antioxidant components of mixes when compared with standard mix at (p>0.05) level. There is also a significant increase in the total Antioxidant activity of both mix A and B when compared to Mix C. The screening of phytochemicals confirms the presence of Alkaloids, Saponin and Flavanoids in all solvents namely ethanol ,methanol , ethyl acetate and water From this it can be concluded that it is essential to enrich our diets with natural fruits and vegetable for maintaining regular supply of antioxidants.

Keywords: Antioxidants, Total Antioxidant Activity, Phytochemical, DPPH, Non Communicable Diseases

INTRODUCTION

Antioxidants have the capacity to reduce free radicals in the body and they take part in protecting our body's immune system against reactive oxygen system (ROS). Number of recent researches have highlighted beneficial role of Antioxidant in our body. They act as oxidizable substance which delay the process of oxidation.¹ Antioxidants are classified into two major classes ie. Endogenous and Exogenous as well as enzymatic and non enzymatic natural plant based antioxidant. Vitamin C is water soluble antioxidant, which is considered to be the best for neutralizing the free radicals in the body before the initialization of lipid peroxidation. On the other hand Vitamin E known to be a fat soluble antioxidant, plays an important function in protecting membranes from oxidative damage. Vitamin E traps peroxy radical present in cellular membrane thereby preventing peroxidation of such membranes. The main function of β carotene and other carotenoids is to ensure antioxidant protection to tissues of the body which are rich in lipids.² Human body is not able to manufacture these nutrients therefore in order to reduce the oxidative stress these antioxidants should be provided daily in our diet.

All plant based material contains antioxidants and act as a radical scavengers transforming the free radicals to the species which are less reactive. Dietary sources like fruits and vegetables are fair sources of antioxidant which possess free radical quenching properties. Hence they play a remarkable role in maintaining optimum health including well being therefore act as first step of defense. We can ensure defense against chronic diseases by regular consumption of antioxidant rich fruits and vegetables³. Several researches have also proved that antioxidant rich diet ensure better positive health in long run⁴⁻⁵ Recent researches indicate that antioxidant vitamin and minerals can help in the prevention and possible reversal of numerous chronic diseases and promotes positive well being of populations. Food based approach is coming to the forefront as a major modifiable determinant of chronic diseases and facilitating humans in leading a healthy life, the evidence based scientific researches always support the alteration in diet and also verified the strong affection on health throughout life. A relative evaluation of foods revealed that natural plant food contribute much higher in combating chronic diseases and promote health. Phytochemicals are the

bioactive compounds that are present in fruits and vegetables, they also contain several other compounds that has capacity to improve quality of life. Conclusion drawn from various clinical, chemical and population based researches indicated the inclusion of antioxidant rich food in the diet to get rid of several major chronic degenerative diseases.⁶The diet consumed by human works as a tool to fight against damage caused by stress which increase the production of free radicals. Therefore the diet which has amalgamation of fruits , vegetables and generous mixture of natural plant based antioxidants like Vitamin C, Polyphenols, carotenoids and complex carbohydrates proves to be beneficial in maintaining antioxidant profile⁷. Various researches emphasizes on the development, evaluation and supplementation of health mixes utilizing whole and sprouted cereals , millets and oil seeds, studies related to incorporation of green leafy vegetables and amla are limited⁸. Therefore the present study was planned to develop Food based Antioxidant mix and estimate its Nutrient Antioxidant components, Total Antioxidant Activity and Screening.

MATERIAL AND METHODS

2.1 Procurement and Preparation of Sample

The ingredients used for the development of mix were selected on their nutrient content, cost as well as their antioxidant and phytochemical properties. Commercially released varieties of the ingredients were procured from Agriculture Research Station, Durgapura, Jaipur Some identified varieties of the ingredients include – Wheat –Raj 1482, Bengal gram-RSG-973, Soybean-Pratap soya, Amla-Banarsi and Curry leaves The grains were cleaned, washed dried and then stored in a polythene bag in a cool and a dry place prior to use. The curry leaves was firstly sorted and then washed. After washing blanching was done at 85 °C boiling water for 5 min, oven dried for 85°C for 1 hour and then grinded to convert in fine powder and stored in a air tight container in dry and cool place. The same procedure was followed for the amla instead of blanching it was grated.

2.2 Development of Food Based Mixes

Three mixes were developed in the present investigation, their antioxidant and phytochemical screening was performed. 100gms of mix contain Wheat –Raj 1482 (75 gms), Bengal gram -RSG-973 (15 gms), soybean Pratap soya (10 gms). Antioxidant rich mix A was prepared by using the above mentioned ingredients in which (5 gms) of wheat flour was supplemented by incorporating (5 gms) of Antioxidant rich

curry leaves. In Antioxidant rich mix B along with above mentioned ingredients 5 gms of wheat flour was replaced by 2.5 gms Amla-Banarsi and 2.5 gms Curry leaf powder. All the estimations were performed in Food Analysis lab of IIS (deemed to be) University. Readings of each test were taken in triplicates and finally reported as mean and Standard Deviation.

2.3 Determination of Nutritional components of Food Based Mixes

Nutritional analysis was carried out by using the standardized method of AOAC⁹. The moisture content of all the three prepared mixes were determined by drying at 105°C until a constant weight is attained. The nitrogen content thus determined was multiplied by factor 6.25 to get crude protein content. Fat was determined by extraction with petroleum ether (80°C) with the help of Soxhlet apparatus. Ash content was estimated by using Muffle furnace at a temperature of 550°C. The estimation of crude fiber content was carried out by acid alkali method using the apparatus fibra plus. Carbohydrate was calculated by difference by subtracting the values of moisture, ash, protein, fat and fiber.

2.4 Determination of Antioxidant Components

2.4.1 Extraction

The three samples of mixes were extracted with 80% aqueous methanol at 200 rpm for 2 hour at ambient temperature with continuous stirring in a dark bottle. Filtration was done by using filter paper (whatman No. 4). The filtrate were then used for estimating β -carotene, total polyphenols and total antioxidant activity.

The non enzymatic antioxidants like vitamin C were estimated by using dye method of AOAC.⁹ Total polyphenols were determined by using the FolinCiocalteu(FC) reagent and total phenolic content was expressed as mg gallic acid equivalent (GAE) /100 gm sample.¹⁰ B-carotene were determined by using standard method of HPLC⁹. Vitamin E was determined by using spectrophotometer.¹¹

2.4.2 Determination of Total Antioxidant Activity

Free radical scavenging activity of the mixes was measured using 1, 1-diphenyl-2-picrylhydrazil (DPPH) by the method of Gupta and Prakash¹² with slight modification. DPPH is a commercial oxidizing radical is reduced by antioxidants. The disappearance of the DPPH radical absorption at a characteristic wavelength is monitored by decrease in optical density.¹³

2.5 Determination of Phytochemicals

2.5.1 Preparation of Extracts

In the present study 20 gms of mix of all the three samples was macerated and extracted with 200 ml of solvent in sequential individually n hexane, ethyl acetate, methanol and water by solvent extraction method for each solvents but not exceeding the boiling point temperature. After that the preliminary test for various phytochemical were performed.

2.6 Preliminary Phytochemical Analysis

2.6.1 Test for Alkaloids

- a) **Dragandroff Reagent Test**¹⁴ Eight to Ten drops of freshly prepared Dragandroff reagent was added in 1 ml of extract and found formation of orange to brownish precipitate which shows the presence of alkaloids in extract.
- b) **Mayer's Test**¹⁵: 1 ml of extract was used with 1 ml of Mayes reagent (Potassium mercuric solution) and formation of white to yellowish or cream colour precipitate indicates the presence of alkaloids.

2.6.2 Test for Flavanoids:

- a) **Shinoda Test**¹⁶: In the solution of extract add three pieces of magnesium chips and then add some drops of hydrochloric acid . Apperance of “an orange, pink

or red to purple colour indicates the presence of flavanoids

- b) **Sulphuric Acid Test**: Dissolve the extract in concentrated sulphuric acid and observe the change in colour.
- c) **Ferric Chloride Test**¹⁶: A few drops of prepared chloride solution were mixed with the extract, intense green, blue or violet colour appears that shows the presence of flavanoids.
- d) **Sodium Hydroxide Test**: The extract was dissolve in 10% aqueous hydroxide solution and filtered to give yellow colour which decreases their intensity when treated with dilute HCL: indicated “the presence of alkaloids.

2.6.3 Test for Saponin

- a) **Foam Test**¹⁷: The extract was shaken with water in a test tube and a persistent foam for 10 minutes indicates the presence of saponin.

2.6.4 Test for Tannins

- a) **Ferric Chloride Test**¹⁷ The extract was boiled with water and filtered then add a little drops of ferric chloride solution were added into filtrate, development of a blue- black,

or green precipitate show the “presence of tannins.

- b) **Acetic acid test**¹⁶: In 2-3 ml of extract add a few drops of acetic acid solution, formation of red colour indicates the presence of tannins.

2.7 Statistical Evaluation

The results of developed antioxidant mix were reported “as mean \pm standard deviation of three parallel replicates. The data were statistical analyzed by one way analysis of variance (ANOVA) using statistical software (SPSS, version 21.0) and the means were separated at 95% confidence level ($p \leq 0.05$) applying Tukey’s multiple range tests.

3. RESULTS AND DISCUSSIONS

3.1. Nutritional Components of Developed Mixes

The results of the proximate composition viz moisture, ash, protein, crude fiber, carbohydrate and fat of the developed mixes was shown in **Table 1**. The moisture content of the developed mixes ranged between 10.3 to 11.33%. Similar trends were reported by^{18, 19} who developed millet based mixes using Bajra, Wheat, Bengal Gram, Moringa Leaves and the moisture content of the mixes ranged between 11 to 13%. The ash content was found to be highest in standard Mix C which was found to be 4.40 g /100 g and lowest was of Antioxidant mix B which was

reported to be 3.2 g /100 g may be due to the seed coats as majority of the grains were used with the seed coats. On contrary the results of ash content developed functional food mix by using different ingredients was found to be 8 g /100 g of standard mix and 9.44 g /100 g of the variation 1.²⁰ There is a slight difference in the protein content of the three samples of the mixes and ranged between 13.20 to 14.57 g /100 gm because the amount of the soybean remain the same in all the developed mixes. Crude fiber includes mostly lignin, cellulose and hemicelluloses²¹. Further there is a significant rise in the crude fiber content of the mixes, which is due to the incorporation of curry leaves and amla powder. According to well documented studies, it is obvious that dietary fiber plays a significant role in prevention of several chronic diseases like cancer, diabetes and cardiovascular diseases. Carbohydrate determined by composite method varied between 59 to 63 k cal among which Antioxidant Mix A has highest amount of carbohydrate. The Fat content of the mixes ranged between 5 to 6 gm /100 g respectively

3.2 Antioxidant Components of Developed Mixes

Antioxidants are the compounds that are capable in combating free radicals interfacing at various steps by oxidative process. The

antioxidant profile of the developed mixes were estimated viz Vitamin C, Vitamin E, β -carotene and polyphenol. There is a significant increase in the Vitamin C content of the Mix B as compared to the both Standard and Antioxidant Mix A which is due to the incorporation of amla. It is very well documented that Amla is the richest source of Vitamin C, which contains 600 mg of vitamin C. By adding just 2.5 gm of dried powder of amla in the Standard mix there is an increase in the Vitamin C content of the mix. Ascorbic acid is a water soluble antioxidant which readily scavenges free radicals²². Vitamin E (Tocopherol) is found in number of animal and plant product. Tocopherol are important antioxidant in foods specially vegetable oils. The mainly helps in inhibition of unsaturated fatty acids in the tissues, also helps to maintain healthy cells, protects unsaturated fatty acids and Vitamin A against oxidation.²³ Antioxidant premix B contain 64.5 mg/100gm of vitamin E followed by Anti-oxidant premix A which contain 63.66 mg/100gm and standard premix which only contain 52.46mg/100gm. However the vitamin A content of the antioxidant rich instant biscuit mix developed by²⁴ was analysed and it was estimated to be 20 I.U. which was quite high and act as a powerful antioxidant. Further nutritionally

enriched instant complimentary feeds enriched with vitamins were developed and Vitamin A was analyzed, the results revealed that mix has 289 ± 3.60 $\mu\text{g}/100\text{g}$ of Vitamin A which was quite high when compared with other complimentary feeds.²⁵ The β carotene level of antioxidant rich premix A was found to be $277.66\mu\text{g}/100$ gm when compared with the other two premixes. Phenolics are the secondary aromatic plant metabolites which is widely present throughout plant kingdom and are responsible for the colour, sensory attributes, nutritional and antioxidant properties. The green leafy vegetables and fruits like amla have varying level of polyphenols. The total polyphenol content of content of Anti-oxidant rich premix B was highest when compared to both the premixes. The total polyphenol content ranged between 126.3 to 142.3mg /100 gm, the variation in polyphenol content is widely depending on the variety of vegetables and fruits therefore the comparison is very difficult.¹². Researchers concluded in their study that the phenol content of the composite flour was found to be 549.70 ± 4.32 GAE/100g when compared to the wheat flour which was only found to be 379.0 ± 2.29 GAE/100g, significant difference was found between the phenol content of both the flours at $p < 0.01$ level of significance²⁶ (**Table 2**).

3.3 Total Antioxidant Activity

Mean with different superscript in a row denote significant difference at $p \leq 0.05$

The total Antioxidant Capacity of the developed food based mixes was concluded by DPPH Techniques generally used in plant and food research for screening of antioxidant capacity²⁰. The results obtained on the antioxidant activity of three mixes, determined by DPPH (IC₅₀) assay has been presented in the (Table 3). The IC 50 value of the developed mix C was found to be 66.50 μ l/ml, mix A has 72.3550 μ l/ml and mix B has 76.3150 μ l/ml. There was a significant increase in the Total Antioxidant Potential of the developed antioxidant mixes when compared with Control Mix C. The statistical analysis of the free radical scavenging activity of wheat flour and wheat legume flour indicated that WF-SF 30 and WF LF 30 samples showed higher antioxidant activity than other samples.²⁷ Free radical scavenging activities of for three green leafy vegetables highlighted that they possess >70% free radical scavenging activities²⁸ Therefore these dietary antioxidants from fruits and vegetables conferring the protection against free radicals damage to cellular, DNA, lipids and proteins.”

3.4 Phytochemical screening of the developed mixes

The present study carried out on Food Based Antioxidant mixes, the results of preliminary phytochemical screening revealed the presence of medically active constituents like Alkaloids, Flavanoids and Saponins and in all the developed mixes. However Tannins were not present in all the samples. Phytochemicals are secondary metabolites which are produced and used by plants for protection and process of repair within the natural environment. Different bioactive components have varied role in protection against oxidative damage. Flavanoids are known to possess anticancer activity and are potent water soluble antioxidants, it is considered to be as free radical scavenger which prevent oxidative cell damage. Alkaloids and saponin which are also isolated from plant are commonly found to have antimicrobial properties. These also help in protecting against chronic diseases specially hypercholesterolemia²⁹. Tannins are good antioxidant substances which have antidiarrhoeal activity and prevent disorders related to oxidative stress. Further the phytochemical screening of six selected green leafy namely *Amaranthus tritris*, *Centella asiatica*, *Hibiscus sabdariffa*, *Moringa oleifera*, *Sesbania Grandiflora* and *Solanum trilobatum* vegetables and the results tested revealed the presence of

alkaloids, tannins and flavanoid.³⁰ Similarly various bioactive components³¹⁻³³ (Table 4).
curry leaves powder and amla possess

Table 1: Proximate Composition of Food Based Antioxidant Mixes

S. No.	Nutrients	Mix C	Mix A	Mix B
1.	Moisture %	10.3 ± 0.04	10.9 ± 0.04	11.33 ± 0.30
2.	Ash (g/100gm)	4.40 ± 0.15	3.39 ± 0.12	3.2 ± 0.29
3.	Protein (g/100gm)	14.57 ± 0.33	13.20 ± 0.05	14.32 ± 0.37
4.	Crude fiber (g/100gm)	2.32 ± 0.01	6.03 ± 0.02	5.61 ± 0.29
5.	Carbohydrate (gms)	61.94 ± 0.19	63.39 ± 0.12	59.97 ± 0.77
6.	Fat (g/100gm)	6.47 ± 0.04	5.77 ± 0.04	5.57 ± 0.32

Mean ± SD

Mean with different superscript in a row denote significant difference at p ≤ 0.05

Table 2: Antioxidant content of the standard and Antioxidant Rich Mixes

Antioxidants	Mix C	Mix A	Mix B
Vitamin C (mg/100gm)	0.21 ± 0.0 ^a	0.25 ± 1.52 ^b	86.23 ± 0.28 ^c
Vitamin E (mg/100 gm)	52.46 ± 0.33 ^a	63.66 ± 1.52 ^b	64.5 ± 0.5 ^c
B-carotene (µg/100gm)	183.26 ± 0.65 ^a	277.66 ± 1.52 ^b	257.33 ± 1.52 ^c
Total polyphenols (mgGAE/100g)	126.43 ± 2.3 ^a	130.93 ± 0.83 ^b	142.33 ± 0.04 ^c

Mean ± SD

Mean with different superscript in a row denote significant difference at p ≤ 0.05

Table 3: Total Antioxidant Activity of the developed Antioxidant Mixes

Total Antioxidant Activity	Mix C	Mix A	Mix B
Total Antioxidant Activity 50µl/ml	66.5 ± 0.08 ^a	72.35 ± 0.25 ^b	76.31 ± 0.12 ^c

Mean ± SD

Table 4: Phytochemicals screening of the developed Food Based Antioxidant Mixes

Phytochemical	Mix	Solvent			
		Ethanol	Methanol	Ethyl Acetate	Aqueous
Flavanoid	C	+	+	+	+
	A	+	+	+	+
	B	+	+	+	+
Alkaloid	C	-	-	-	-
	A	+	+	+	+
	B	+	+	+	+
Saponin	C	-	-	-	-
	A	+	+	+	+
	B	+	+	+	+
Tannins	C	-	-	-	-
	A	-	-	-	-
	B	-	-	-	-

4. CONCLUSION

Exposure occurs due to radiation, chemicals or any other means generate free radicals in the body. This phenomenon only occurs when oxygen reacts to develop reactive

oxygen species, it can only stop or reduce in the body when we consume foods that are rich in antioxidant. The food which are potent sources of antioxidant are fresh fruits and vegetables. These are not only rich

sources of antioxidant but also possess phenolic compound which are responsible for antioxidant activity due to their hydroxyl groups. In the present study presence of Saponin, Alkaloids, Tannins and Flavanoids were confirmed through preliminary phytochemical screening. Analysis of Antioxidant revealed that both Antioxidant rich mix A and B possess good amount of Antioxidant. Therefore it is well documented that consumption of plant based diet or incorporation of cereal pulses based mixes enriched with antioxidant properties with fruits and vegetables are good for health. It also has an impact on positive health and well being of the individuals

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