The Pharma Innovation Journal 2019; 8(5): 36-40

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(5): 36-40

www.thepharmajournal.com
Received: 16-03-2019
Accepted: 17-04-2019

Godage S
MIT College of Food Technology, MIT Art, Design and Technology University, Pune, Maharashtra, India

Shere PD
MIT College of Food Technology, MIT Art, Design and Technology University, Pune, Maharashtra, India

Gaikwad ST
MIT College of Food Technology, MIT Art, Design and Technology University, Pune, Maharashtra, India

Agrawal RS
MIT College of Food Technology, MIT Art, Design and Technology University, Pune, Maharashtra, India

Correspondence
Shere PD
MIT College of Food Technology, MIT Art, Design and Technology University, Pune, Maharashtra, India

Antioxidant synergism: An approach for development of antioxidant rich blend for snack bar

Godage S, Shere PD, Gaikwad ST and Agrawal RS

Abstract
The intake of antioxidants helps to decrease oxidative stress which may lead to cancer, cardiovascular disease and pre-mature ageing. Phytochemicals from plant are more effective in reducing the free radical formation and act as a natural antioxidant. Vitamin A, C, E and phenolic compounds are partners in defence and have a synergistic relationship working together so that their combined effect is greater than the sum of their individual actions. The attempts were made in present investigation to formulate the natural antioxidant rich snack bar blends and assess the *in vitro* antioxidant activity of blends. Three blend formulations rich in natural antioxidants were developed with plant food ingredients rich in vitamin A, C, E and polyphenols. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method was standardized for sample extraction and estimation of *in vitro* antioxidant activity. The red kidney bean-based blend formulation found to contain highest % inhibition activity compared to other two formulations. Ascorbic acid was used as a positive control. The antioxidant rich snack bar blend formulation was developed with natural plant food ingredients.

Keywords: Snack bar, natural, antioxidant, blends

1. Introduction
The food bars are snacks of good sensory and nutritional characteristics due to their high carbohydrates, proteins, lipids, and minerals contents. Increasing demand from consumers for nutritious snacks, has provoked the food manufacturers to develop food bars that provide nutrition and convenience. Snack bar is a compact product having high energy and micronutrients which is able to fulfil the need of consumers at all ages. Snack bars are nutritional product which is made by processed cereals and other ingredients. The selection of ingredients depends upon the targeted consumers. The ingredients should be combined in well manner to ensure that they complement each other in the characteristics of flavor, texture and physical properties (Izzo and Niness, 2001) [8].

Carotenoids are natural pigments which are synthesized by plants have antioxidant activity. Studies have reported that a diet high in carotenoids may reduce the risk of heart attack and candidates for cancer prevention (Steinmetz and Potter, 1996; Federmann and Federmann, 2000) [20]. This fortification is one of the best ways used to deliver the antioxidant health benefits to human that may be useful for the prevention of these diseases.

Vitamin E (α-tocopherol) is an efficient lipid soluble antioxidant that functions as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including low-density lipoprotein (LDL). It functions to intercept lipid peroxyl radicals and to terminate the lipid peroxidation chain reactions. Vitamin C or ascorbic acid is a water-soluble free radical scavenger. Moreover, it regenerates vitamin E in cell membranes in combination with compounds capable of donating reducing equivalents.

Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries contains up to 200–300 mg polyphenols per 100 grams fresh weight. Epidemiological studies have repeatedly shown an inverse association between the risk of chronic human diseases and the consumption of polyphenolic rich diet (Scalbert *et al*., 2005) [19]. It is well established that polyphenol-rich foods and beverages may increase plasma antioxidant capacity.

Combining antioxidants may increase cumulative effectiveness. Synergism can be defined as the coordinated or correlated action of two or more structures, agents, or physiologic processes so that the combined action is greater than the sum of each acting separately. The interactions among different antioxidant components can be synergistic (the combined effect of two components is much greater than the sum of the effects of each agent given alone),

or antagonistic (opposite of synergism). It may be successful to use antioxidant combinations in food matrix producing antioxidant synergistic effect (Sharma and Gluleria, 2017) [21]. Therefore, efforts have been made in present investigation to develop antioxidant rich snack bar with synergism approach of antioxidants and to *in vitro* assess the antioxidant activity of snack bar.

2. Materials and methods

2.1 Materials

Red kidney beans, soybeans, finger millet and other ingredients were purchased from local market.

2.2 Methods

2.2.1 Preparation of vegetable powder: Carrot and spinach leaves were washed, thin sliced and steam blanched for 15 minutes and 2 minutes respectively. The blanched vegetables were dried in cabinet drier at 60°C for 5-6 hours.

2.2.2 Process for amaranth puffing: Puffing of amaranth was carried as per the method described by John *et al.,* (2014) [14] with some modifications. Grains were puffed by heating in pan at 220°C with continuous stirring for 20-25 seconds.

2.2.3 Preparation of flour: Red kidney bean, soybean and finger millet were cleaned, roasted in an open pan for 8-10 minutes at 200°C, cooled at room temperature, milled to flour and sealed in polyethylene packaging for further use.

2.2.4 Preparation of blend

Three blends were prepared by mixing required proportions of ingredients. All the three blends were formulated with ingredients rich in polyphenols, beta carotene, ascorbic acid and tocopherol.

Red kidney beans- 332.60±6.41 mg/100gm.
Soybeans- 100.54±1.08 mg/100gm.
Finger millet- 373.98±8.06 mg/100gm.

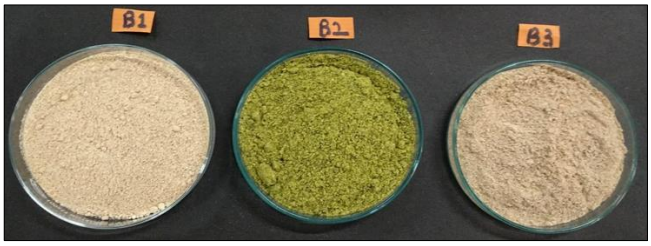

Plate 1: Snack bar blends

2.3 Proximate composition

All the chemicals used in the present research work were of analytical grade. The proximate composition (moisture, crude fat, crude protein, total ash and crude fibre) were determined by AOAC. (2000) [2] methods. The total carbohydrates were determined by difference method. Crude fibre was determined by following the method No 32-10 as described in.

2.4 Estimation of phenolic compounds

2.4.1 Preparation of sample extract: Extraction of sample was carried out as described in Ansari *et al*., (2013). Blend sample extracts were prepared with different solvents such as diethyl ether, ethanol and methanol for optimising the extractions by solvent extraction method using Soxhlet apparatus. The extracts were concentrated by evaporating the solvents on water bath.

2.4.2 Total phenolic content

Total phenol content was determined with folin-Ciocalteu reagent (FCR). Phenols react with an oxidizing agent Phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions and result in the formation of a blue coloured complex, the molybdenum blue which is measured at 650nm. The total phenol content of Blend B1, B2, B3 was calculated from the regression equation of standard gallic acid curve ($y = 1.8728x + 0.1629 R^2 = 0.9982$) and is expressed as mg gallic acid equivalent.

2.5 Antioxidant and radical scavenging assay

2.5.1 Determination of total antioxidant capacity

The determination of total antioxidant activity was done as per the phosphomolybdenum method with some modifications (Sahu *et al.,* 2011) [6]. The total antioxidant activity was expressed as mg of equivalents of ascorbic acid.

2.5.2 DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of non-radical form DPPH-H (Chanda *et al.,* 2009) [5]. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1- diphenyl-2-picryl-hydrazyl by the method of (McCune and Johns, 2002) [14] with some modification. The reaction mixture (3.0 ml) consist of 2.0 ml of DPPH in methanol (0.004%) and 1.0 ml of various concentrations of extract and. It was incubated for 10 min in dark and absorbance was measured at 517 nm against methanol as a blank and control was prepared by DPPH and methanol in place of sample extract. The percentage of inhibition can be calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1) \times 100}{A_0}$$

Where;
A_0 is the absorbance of control
A_1 is the absorbance of test.

IC50 (µg/mL) is the concentration of an antioxidant extract which was required to quench 50% of the initial DPPH· under the experimental conditions given. It was obtained by interpolation from linear regression analysis.

2.6 Statistical analysis

All the experiments were carried out in triplicate, and the results were expressed as mean ± SD (Standard deviation). Statistical analysis was performed using Excel 2007.

3. Results and Discussion

3.1 Total phenol content in blends

The standard curve was plotted using various concentrations of gallic acid against absorbance measured which is depicted in fig. 1. The total phenolic compounds were determined by using standard curve of gallic acid and expressed as gallic acid equivalent (GAE).

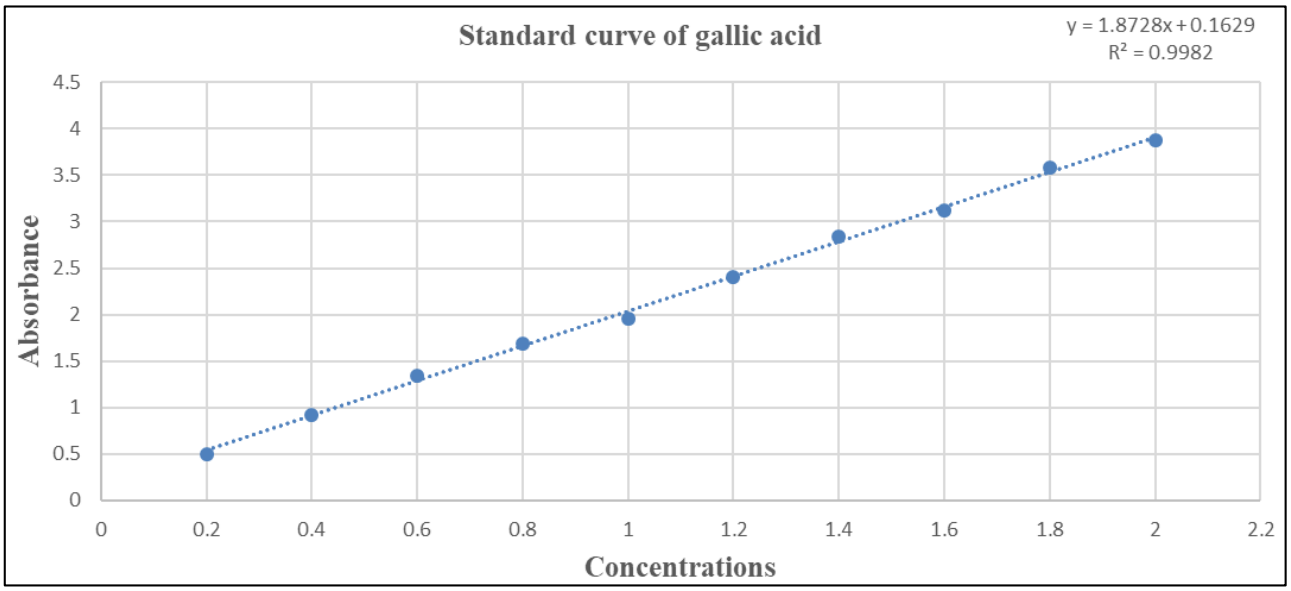

Fig 1: Standard curve of gallic acid

Table 1: Total phenol content of blends

Blends	Total phenol content (mg GAE/100g)
B_1	437.5
B_2	265
B_3	72.5

GAE- Gallic acid equivalent
B_1 – Red kidney bean-based blend
B_2 – Soybean based blend
B_3 – Ragi based blend

The total phenolic compounds present in blend B_1, B_2 and B_3 was found to be 437.5, 265 and 72.5 mg GAE/100g respectively as shown in table 3. There are numerous studies indicating relationship between enhanced antioxidant activity due to presence of high phenolic compounds in samples (Holasova *et al.*, 2002) [14]. The highest content of total phenolics was found in blend B_1 compared to other blends (B_2 and B_3). The total phenolic content showed strong correlations with the antioxidant capacities of the red kidney beans. Thus, total phenolic content could be used as an indicator in evaluating the antioxidant capacity of beans which may preliminarily applied as natural sources of antioxidant functional foods (Golam *et al*., 2011) [7].

3.2 *In vitro* antioxidant activity of blends

3.2.1 DPPH radical scavenging assay

Standard curve of DPPH scavenging activity was plotted using various concentrations of ascorbic acid against the %inhibition as shown in Figure 2.

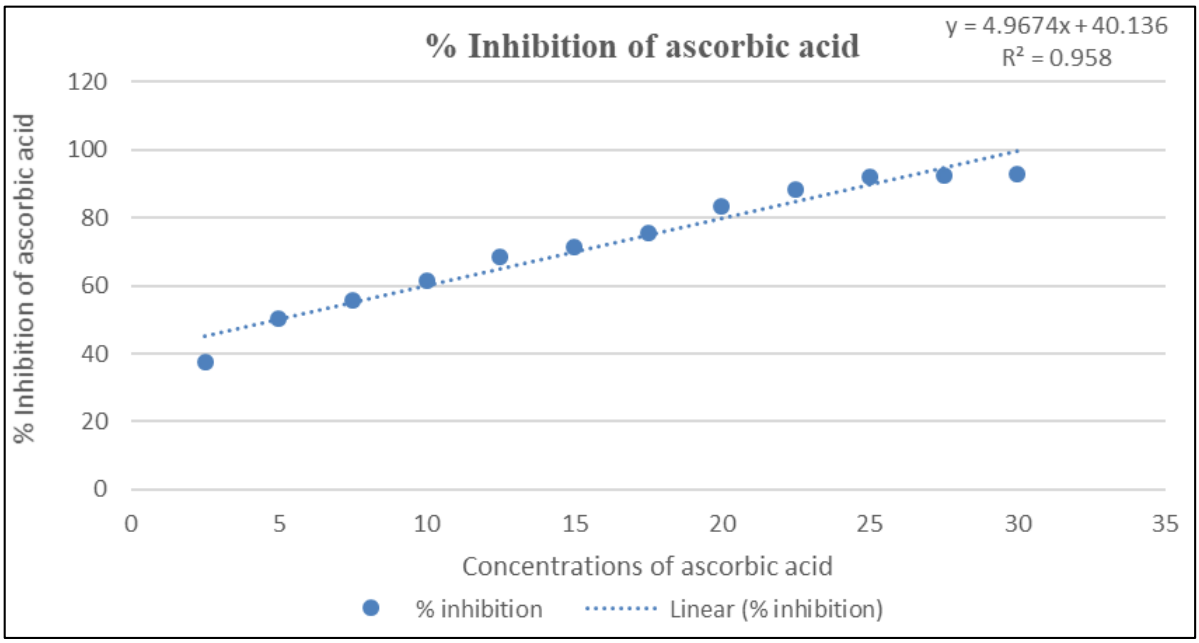

Fig 2: Standard % inhibition curve for ascorbic acid

The IC50 value of standard ascorbic acid was determined from the regression line of concentration versus % of inhibition (y = 4.9674x + 40.136, R^2 = 0.958). The ascorbic acid was found to have 30.21 µg IC50 value under the given experimental set-up. Kumawat *et al.*, (2012) [4] reported IC50 value of standard ascorbic acid 21.22 µg, 58.92 µg/ml. (Saha *et al*., 2008) [18] 13.7 µg/ml raghavendra *et al*., (2013) [13]

Blend B_1, B_2 and B_3 were analysed for DPPH radical

scavenging activity and expressed in terms of IC50 value. Table 4 shows comparative evaluation of IC50 values for the three blends and standard ascorbic acid. The IC50 values of blends were found to be B_1= 33.5 µg, B_2= 200 µg and B_3=350 µg in comparison to IC50 value of standard ascorbic acid (30.21 µg). The results indicated that blend B_1 had the highest DPPH radical scavenging activity compared to other two blends (B_2 and B_3). The combination of ingredients in blend B_1 such as red kidney bean flour which contained high phenolic compounds whereas other ingredients such as fruit and vegetable powder were added as a source of vitamin A, vitamin C and Vitamin E respectively might have synergistically improved the % DPPH radical scavenging activity.

Table 2: Comparative evaluation of % inhibition of DPPH free radical scavenging activity and IC50

Concentration (µg/ml)	Ascorbic acid (%I)	IC50 (µg/ml)	B_1 (% I)	IC50 (µg/ml)	B_2 (%I)	IC50 (µg/ml)	B_3 (% I)	IC50(µg/ml)
50	65.71	30.21	50.86	3.5	28.21	200	20.13	350
100	74.32		63.12		35.41		25.31	
150	88.8		74.54		43.58		30.47	
200	90.02		83.71		51.02		33.94	

% I= % Inhibition
B_1 – Red kidney bean-based blend
B_2 – Soybean based blend
B_3 – Ragi based blend

3.2.2 Total antioxidant activity of blends

Table 3: Total antioxidant activity of blends

Blend samples	Total antioxidant activity (mg AAE/100g)
B_1	475.86
B_2	341.78
B_3	288.11

AAE- Ascorbic acid equivalent
B_1 – Red kidney bean-based blend
B_2 – Soybean based blend
B_3 – Ragi based blend

Table 5 indicates that the total antioxidant activity of blend B_1 was highest (475.86 mg AAE/100g) compared to other blends. The synergistic action of phenolic compounds with vitamin A, C and E could have resulted in higher antioxidant activity in blend B_1. Phenolic compounds are the most abundant structures in plants. Antioxidant compounds are usually in the phenolic form. The antioxidant properties of phenolic compounds originate from their properties of proton loss, chelate formation, and dismutation of radicals. B_1 contains polyphenolic rich components that have the ability to destroy free radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxy radicals; hence may be responsible to play an important role in antioxidant activity. Vitamin A, C, E also helps to induce antioxidant activity. Hence, blend B_1 can be selected for development of antioxidant rich snack bar blend.

4. Conclusion

In the present study, the antioxidant capacities of snack bar blends were analyzed using DPPH free radical scavenging activity. The DPPH test is an indirect method for the determining the antioxidant activity based on the ability of free radical 2, 2–diphenyl-1- picryl hydrazyl to react with hydrogen donors with phenol. On the basis of results obtained in the present study it is concluded that the methanolic extract of snack bar blend B_1 possessed highest free radical scavenging activity with IC50 value of (33.5 µg/ml) and antioxidant activity (475.86 mg AAE/100g). The natural ingredients rich in phenols, vitamin A, C and E synergistically enriched the natural antioxidant potential of snack bar blend.

5. References

1. AACC. American association of cereal chemists, 1976. Approved methods of the AACC (8th ed). Method 32-10, approved. The association St. Paul, Mn, 1981.
2. AOAC. official Methods of Analysis of AOAC International, 2000.
3. Ansari QA, Ahmed AS, MA Waheed, Sayyed JA. Extraction and determination of antioxidant activity of Withania somnifera Dunal. European Journal of Experimental Biology. 2013; 3(5):502-507.
4. Kumawat KB, Gupta M, Tarachand, Singh Y. Free radical scavenging effect of various extracts of leaves of *Balanites Aegyptiaca* (L.) by DPPH method, Asian Journal of Plant Science and Research. 2012; 2(3):323-329.
5. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. African Journal of Microbiology and Research. 2009; 3(13):981-996.
6. Laloo D, Sahu NA. Antioxidant activities of three Indian commercially available Nagakesar: An *in vitro* study. Jornal Of Chemical and Pharmaceutical Research. 2011; 3(1):277-283.
7. Golam MA, Khandaker L, Berthold J, Gates L, Peters K. Delong H et al. Anthocyanin, total polyphenols and antioxidant activity of common beans. American Journal of Food Technology. 2011; 6:385-394.
8. Izzo M, Niness K. Formulating nutrition bars with inulin and oligofructose. Cereal Foods World. 2001; 46:102-105.
9. Indian Food Composition Tables, 2017.
10. John HM, Brain A, Geoffrey S. Effect of heat processing on selected grain amaranth physicochemical properties. Food Sci. and Nutrition. 2014; 2(1):9-6.
11. Martini FCC. Canniatti-Brazaca SG. Comparison between the availability of iron in the presence of vitamin a and b-carotene in foods and medications. Ciênc. Tecnol. Aliment., Campinas. 2011; 31(2):334-340.
12. McCune ML, Johns T. Antioxidant activity in medical plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. Journal of Ethnopharmacology. 2002; 82(2-3):197-205.
13. Raghavendra MA, Reddy M, Yadav RP, Sudharshan R. A, Kumar LS. Comparativey Studies on The *In Vitro* Antioxidant Properties of Methanolic Leafy Extracts from Six Edible Leafy Vegetables of India. Asian J

Pharm Clinical Research. 2013; 6(3):96-99.
14. Holasova M, Fiedlerova V, Smrcinova H, Orsak M, Lachman J, Vavreinova S. Food Res. Int. 2002; 35:207-211.
15. Butnariua M, Butub A. Chemical Composition of Vegetables and their Products. Handbook of Food Chemistry. 10.1007/978-3-642-41609-517.
16. Panase VG, Sukhatme VG. Stastical methods for agriculture workers. A handbook of statistics for use in plant breeding and agricultural problems, 1985.
17. Renaud SC, Gueguen R, Schenker J, d‟Houtaud A. Alcohol and mortality in middle- aged men from eastern France. Epidemiology. 1998; (9)184-188. Bangl. J Vet. Med. 2008; 6(2):197-202.
18. Saha MR, Hasan MS, Akter R, Hossain MM, Alam SM, Alam AM et al. *In vitro* free radical activity of methanol extract of the leaves of mimusops elengi linn. Bangl. J Vet. Med. 2008; 6(2):197-202.
19. Scalbert A, Manach C, Mornand C, remesy C, Jimenez L. Dietary Polyphenols and the Prevention of Disease. Critical Reviews in Food Science and Nutrition. 2007; 45(4).
20. Steinmetz Potter, Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. J Am Diet Assoc. 1996; 96:1027-1039.
21. Sharma KS, Gluleria S. Synergistic Antioxidant Activity of Natural Products. An Pharmacol Pharm. 2017; 2(16):1086.
22. Sreeramulu D, Vijaya Kumar Reddy C, Raghunath M. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. Indian Journal of Biochemistry & Biophysics. 2009; 46:112-115.
23. Temple. Temple NJ. Antioxidants and disease: more questions than answers. Nutrition Research. 2000; 20:449-459.