International Journal of Home Science 2023; 9(2): 283-287

ISSN: 2395-7476
IJHS 2023; 9(2): 283-287

www.homesciencejournal.com
Received: 26-04-2023
Accepted: 29-05-2023

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Appraisal of antioxidant content and activity of developed composite flour from finger millet, black soybean and moringa leaf powder

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Abstract
Malnutrition has become an urgent global health issue, with under nutrition killing or disabling millions of children each year. In India about 2/3 portion of the under five children of our country is malnourished among them 5-8% is severely malnourished whole rest fall in the group of mild or moderate malnutrition so it can be said that malnutrition one of the widest spread conditions affecting child health. The aim of present work was to investigate the antioxidant profile of composite flours of Finger Millet, Black soybean and Moringa Leaf Powder for different composition. The composite flour was prepared using Finger Millet, Black soybean, Moringa Leaf Powder and Durum Wheat Flour; Control (100% Durum Wheat Flour) respectively. The antioxidant evaluation DPPH Radical Scavenging Activity, FRAP Radical Scavenging Activity; Total Phenols Content and Total Flavonoids Content of composite flours were analyzed with standard procedure. The result revealed that among all the composite flour CF-3 had significantly highest antioxidant content when compared with Control, CF-1 and CF-2 and registered significant difference at $p \leq 0.05$ level. The addition of Finger Millet, Black soybean and Moringa Leaf Powder in composite flours has been shown in this study to improve the nutrition, antioxidant content and health benefits of the body which could be further used for alleviating child malnutrition.

Keywords: Antioxidant, malnutrition, finger millet, black soybean, moringa leaf powder

1. Introduction
A sustained force for health, development, and maximizing the genetic potential of humans is nutritional well-being. As a result, it is now understood that a community's nutritional quality is a key determinant of the growth of a country. In other words, because malnutrition hinders national development, it is considered a national issue (Fanzo, 2016) [6]. Dietary quality needs to be taken into account in order to address the issues of pervasive food insecurity and malnutrition. Along with improving yields, it is important to promote food production diversification at both the national and household levels. One of the potentially effective methods for enhancing family food security is the cultivation of traditional food crops appropriate for the region (Singh, 2018) [19]. Reactive oxygen species (ROS) and antioxidants are in a disapproving equilibrium, and this leads to oxidative stress, a physiological state. Numerous chronic illnesses, including diabetes, hypertension, inflammation and cancer, have oxidative stress as their underlying cause (Liu *et al*., 2018) [12]. Antioxidants are chemicals designed to quench or trap ROS and prevent oxidative damage, which is how oxidative stress is managed (Nimse & Pal, 2015) [16]. By giving electrons to neutralize the detrimental effects of *in vivo* free radicals, which cause cancer, cardiovascular disease, and aging-related problems, antioxidants are helpful in lowering and avoiding them. Antioxidative peptides produced from dietary proteins have drawn increasing interest from modern consumers and academics due to their variety of advantages (Jacob E, 2022) [8].

Generally, the millet is grounded and the whole meal is consumed for preparation of food products. Even though, the seed coat of the millet is edible, it imparts chewy texture and dark color to the food products and hence its separation is desired to prepare the product of enhanced consumer appeal. Hence, preparation of millet flour almost free from the seed coat by incipient moist conditioning, pulverizing and sieving the native as well as the malted millet and also by decortications of the hydrothermally processed millet is being practiced (Krishnan *et al*., 2016) [9].

A healthy coarse cereal that is higher in protein, dietary fibre, calcium, and phenolics than wheat is finger millet (*Eleusine coracana* L.). High phenolic content is known to have cholesterol-lowering qualities and aids in the management of Type -2 diabetes by reducing the activities of intestine -glucosidase and pancreatic amylase (Patil, 2016) [18].
The family Moringaceae, also known as the drumstick or horseradish tree, includes *Moringa oleifera* Lamis an endemic species to Northwestern India. It has the potential to end malnutrition since it is a cost-effective and widely accessible source of important critical nutrients and nutraceuticals (Kunyanga *et al*., 2013) [11]. Due of its strong tolerance to drought and desert environments because of its tuberous roots, the Moringa is sometimes seen as a crucial famine food. It is commonly grown in Cameroon and has expanded across Africa (Agamou, Fombang, & Mbofung, 2015) [1]. A substantial source of -carotene, vitamin -C, minerals and phytochemicals with shown antioxidant action is thought to be the moringa plant, which is prized mostly for its leaves, sensitive pods, seeds and flowers (Nobosse, Fombang & Mbofung, 2017) [21]. Additionally, *Moringa oleifera* leaf extracts show notable pharmacological effects against inflammation, diabetes and hyperglycemia (Azad *et al*., 2017; Fombang & Willy Saa, 2016) [3, 7], cancer (Boonsirichai & Jetawattana, 2014) [4] and neurodegeneration (Hannan *et al*., 2014) [22].
The Black soybean (*Glycine max.* L) is an East Asian legume that contains a variety of phytonutrient such as saponins, anthocyanins, kaempferol, and quercetin, all of which have antioxidant properties (Kumar, *et al*., 2018) [10]. Anti-obesity, Anti-hyperglycemic, and Anti-hyperlipidemic actions have also been demonstrated (Ganesan, 2017) [8]. They are therefore a possible ingredient in nutraceutical formulations. In addition to being a fantastic source of protein (35-40%), vitamins, minerals, and calcium, soybeans are also the only food that contains all nine necessary amino acids. Proteins from soybeans are high in lysine but low in sulfur-containing amino acids, whereas proteins from cereals are low in lysine but high in sulphur amino acids. To improve the overall necessary amino acid balance and assist address the global protein calorie malnutrition problem, adding soybean flour to cereal-based goods might be a useful solution (Elisa, 2015) [23]. The aim of this study was to determine the antioxidant content and activity of finger millet, moringa leaf powder and black soybean based composite flour which not only possess high protein and fibre but is also abundant in antioxidants.

2. Materials and Methods

2.1 Materials

The Black soybean seed or bhat (*Glycine max* L.) variety VL Soya-65 and Finger millet seed (*Elusine coracana*) variety VLMandua-352 was procured from ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand. Moringa Leaf (*Moringa oleifera*) was obtained from the Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, PUSA, New Delhi. The seeds were cleaned and made free of dust, dirt and foreign materials prior to processing and product development. Raw materials were stored and packed in an air-tight plastic container for further analysis.

2.2 Processing of Techniques with Drying Procedures Cereal, Legume and Moringa leaf

2.2.1 Milling of Finger Millet Seed

Finger Millet Seeds were processed by sun drying. The dry milling process started with the cleaning of seeds. After sun drying, the seeds was prepared in powdered form by milling and sieving the flour than storage of flour and packed in air tight container.

2.2.2 Milling of Black soybean Seed

Black soybean Seeds were first cleaned and free from broken seeds and remove the husk, dust and other foreign materials and after sun drying the seeds was prepared in powdered form by milling and sieving the powder than storage of flour and flour are packed in air tight container.

2.2.3 Drying of Moringa Leaf

Moringa leaves were clean and properly and shaded dry in room temperature and milled using grinder and sieved. Moringa Leaf Powder can be stored for up to 6 months under the following conditions: clean, dried powder stored in air-tight container.

2.2.4 Preparation of Different Composite Flours

Composite flour of Finger Millet, Black soybean, Moringa Leaf Powder and Durum Wheat Flour, samples were prepared as reported below. The three (3) composite flours blends were formulated by replacement as follows. One hundred percent Durum Wheat Flour (100% Durum Wheat Flour) was the Control. Composite flours (CF) are developed from Finger millet, Black soybean, Moringa leaf Powder and Durum Wheat Flour in different proportion i.e., CF-1: 20:10:2:68, CF-2: 15:15:2:68 and CF-3: 10:20:2:68, respectively. The blends were thoroughly mixed using a Blender/Grinder to achieve uniform blending. The composite flour was packaged in air-tight containers for later use.

2.3 Analysis of Antioxidant Content

2.3.1 Total Phenolic Content (McDonald, *et al*., 2001) [24]

Total phenol content was determined by using Folin-Ciocalteu Reagent. Phenols react with phosphomolybdic acid in Folin- Ciocalteau reagent to produce a blue-coloured complex in alkaline medium, which can be estimated spectrophotometrically at 650 nm. The Methanolic extracts (0.5ml) were added to a 25 ml of volumetric flask filled with 10ml double distilled water and 2.5ml of 0.2N Folin-Ciocalteau phenol reagent. A reagent blank using double distilled H2O instead of sample was prepared. After 5min, 2ml of 2% Na2CO3 solution were added with mixing. The solution was diluted to the volume (25ml) with double distilled H2O and then allowed to stand for 90min and the absorbance was measured at 780nm versus the prepared blank. The concentration of phenolic content was expressed as mg of gallic acid equivalents (GAE) per 100 g dry weight.

2.3.2 Total Flavonoids Content (Aryal, *et al*., 2019) [25]

The content of total flavonoids was measured using aluminium chloride colorimetric assay. A volume of 125 µl of flour extract was mixed with a volume of 75 µl of a 5% $NaNO_2$ solution. The mixture was allowed to stand for 6 minutes. 150 µl of aluminium trichloride (10%) was added and incubated for 5minutes, before being filled with 750 µl of NaOH (1M). With distilled water, the final volume of the solution was adjusted to 2500 µl. The mixture become pink after 15minutes of incubation, and the absorbance was measured at 510nm using spectrophotometer. The total flavonoids content was expressed as mg of quercetin equivalent (QE/g dry mass).

2.4 Antioxidant activity

2.4.1 DPPH Scavenging Activity (Wang *et al.*, 2007) [26]

The DPPH radical scavenging activities were determined according to the method previously described by Wang *et al*., (2007) [26] with slight modifications. DPPH (0.2mM) in methanol**,** 2 ml of aqueous plant parts extracts at various concentrations (20 to 200 µg/ml) were respectively mixed with 2 ml of DPPH methanolic solution (0.2 mM). The mixtures were shaken vigorously and left to stand in the dark for 30 min. Consequently, the absorbance of these mixtures was measured using a spectrophotometer at 517 nm and the DPPH scavenging activity was calculated in (% Inhibition).

2.4.2 FRAP Radical Scavenging Activity (Jung, *et al.*, 2011) [27]

The FRAP radical scavenging activity was tested according to Jung *et al*., 2011) [27]. 10 m TPTZ (2, 4, 6- tripyridyl-s-triazine), 300 mM sodium acetate buffer, 40 mM HCl, 20 mM $FeCl_3$ and $FeSO_4$. FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 750 nm. This reaction is pH-dependent (optimum pH 3.6). 0.1 ml extract is added to 3.0 ml FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) in 40 mM HCl and 1 part 20 mM $FeCl_3$) and the reaction mixture is kept in a water bath at 50 °C for 20 min and then the absorbance was measured at 750 nm. $FeSO_4$ (100 to 1000 µM ml-^{1}) was used as a positive control**.**

2.5 Statistical Analysis of Data

The data analysis was carried out by using Statistical Package for the Social Sciences (SPSS) for Mean ± SD, Paired t- test, LSD and ANOVA.

3. Results

Table 1: Antioxidant Content of Composite Flours Developed from Finger Millet, Black soybean, Moringa Leaf Powder in Methanol Extract

Treatment	Antioxidant Content	
	Total Phenols Content (mg GAE/g)	Total Flavonoids Content (mg QE/mg)
Control	30.72±0.02	64.56±0.06
CF-1	49.99±0.01*^{c}	98.96±0.04*^{c}
CF-2	53.02±0.02*^{b}	102.75±0.06*^{b}
CF-3	62.91±0.08*^{a}	110.87±0.03*^{a}
F-value	12.18	21.76
LSD	6.831	4.603

Data are reported as Mean ± SD of triplicate determination. *-Significant at $p \leq 0.05$ when compared to control and ^{NS} Non-significant. LSD refers to least significant difference. Different alphabets in superscripts in each row show a significant $p \leq 0.05$ difference between values. All test composite flours such as CF-1, CF-2 and CF-3 were compared to Control. CF-1; 20:10 (FM: BS), CF-2; 15:15 (FM: BS) and CF-3; 10:20 (FM: BS) with 2% MLP and 68% DWF. FM; Finger Millet, BS; Black soybean, MLP; Moringa Leaf Powder DWF; Durum Wheat Flour.

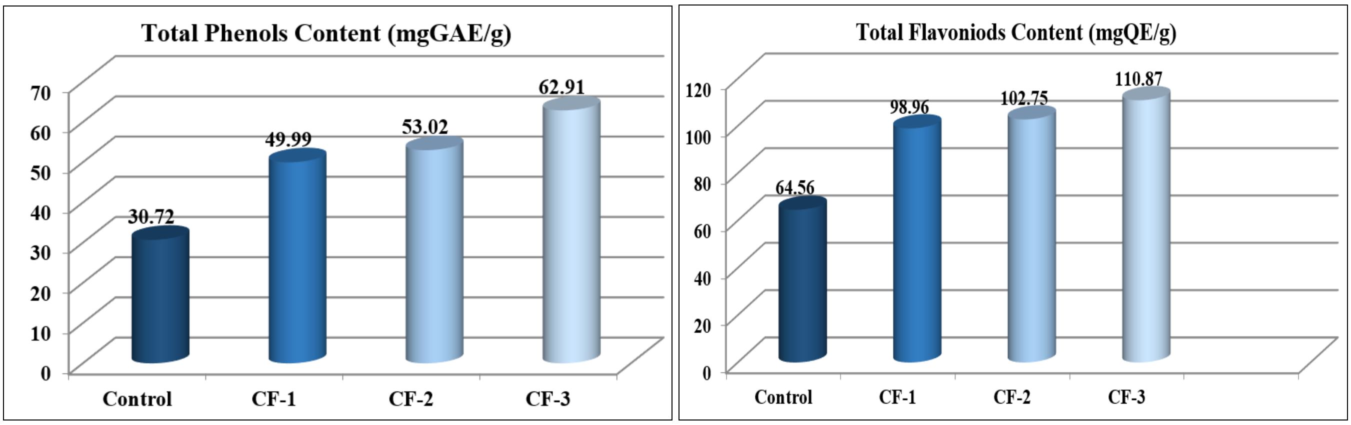

Fig 1: Antioxidant Content of Composite Flours Developed from Finger millet, Black soybean and Moringa Leaf Powder in methanol extract

Total phenols content was determined according to the Folin-Ciocalteu method in methanol extract and expressed as mg of gallic acid equivalent (GAE) per gram of extract (mgGAE/g) on composite flours. As showed in Table 1 and Figure 1 Total Phenols content were statistically different among three composite flours and Control. The values of total phenols content in the control and composite flours in different proportion of CF-1, CF-2 and CF-3 were 30.72±0.02, 49.99±0.01, 53.02±0.02 and 62.91±0.08 respectively. The values of phenols content were significantly different among all the composite flour and Control at ($p \leq 0.05$) level. CF-3 had the highest total phenols content and lowest amount was observed in Control and CF-2. The reason for the same may be the higher phenolic compound of the Black soybean flour attributing higher antioxidant activity. The data of total phenols content of composite flours indicates that phenolic activity in CF-3 was higher with value 62.91±0.08 mg GAE/g followed by CF-2 with mg GAE/g of 53.02±0.02 and CF-1 with mgGAE/g of 49.99±0.01. The phenolic content in composite flour of wheat, finger millet and jackfruit were found to be 57-77 mg GAE/100g in accordance with Palamthodi *et al*., (2021) [18].

Total flavonoids content in methanol extracts of different composite flours of Finger millet, Black soybean, Moringa leaf powder and durum wheat flourwere determined by using aluminum chloride method and the amount of flavonoids was expressed as mg quercetin equivalent (QE) per hundred gram of extract (QE/100g) dry weight basis (composite flours). The total flavonoids content in the Control and composite flours in different proportion of CF-1, CF-2 and CF-3 were 64.56±0.06, 98.96±0.04, 102.75±0.06 and 110.87±0.03 respectively. Data showed that there were significant differences among all the composite flour and Control at ($p<0.05$) level.CF-3 composite flours had the highest total flavonoids content and lowest amount was observed in Control and CF-1. The reason for the same may be the higher flavonoid compound of the Black soybean flour attributing higher antioxidant activity. The data of total flavonoids content of composite flours indicates that flavonols in CF-3 was higher with value 110.87±0.03 mg/QE/mg followed by CF-2 with mg/QE/mg of 102.75±0.06 and CF-1 with mg/QE/mg of 98.96±0.04. Gunashree *et al.*, (2014) [29] reported that finger millet contained 22.5 mgQE/g of Total Flavonoids content and 12.42-32.69 mgQE/g in moringa leaves.

Table 2: Antioxidant Activity of Composite Flours Developed from Finger Millet, Black soybean, Moringa Leaf Powder in Methanol Extract

Treatment	Antioxidant Activity	
	DPPH Radical Scavenging Activity (% inhibition)	**FRAP Radical Scavenging Activity (mgTrolox/g)**
Control	15.57±0.07	2.25±0.09
CF-1	26.66±0.05*c	11.56±0.07*c
CF-2	32.00±0.01*b	14.73±0.05*b
CF-3	37.62±0.05*a	17.55±0.07*a
F-value	18.77	21.47
LSD	4.464	2.282

Data are reported as Mean ± SD of triplicate determination. *- Significant at $p\leq$ 0.05 when compared to control and NS Non-significant. LSD refers to least significant difference. Different alphabets in superscripts in each row show a significant $p\leq$ 0.05 difference between values. All test composite flours such as CF-1, CF-2 and CF-3 were compared to Control. CF-1; 20:10 (FM: BS), CF-2; 15:15 (FM: BS) and CF-3; 10:20 (FM: BS) with 2% MLP and 68% DWF. FM; Finger Millet, BS; Black soybean, MLP; Moringa Leaf Powder DWF; Durum Wheat Flour.

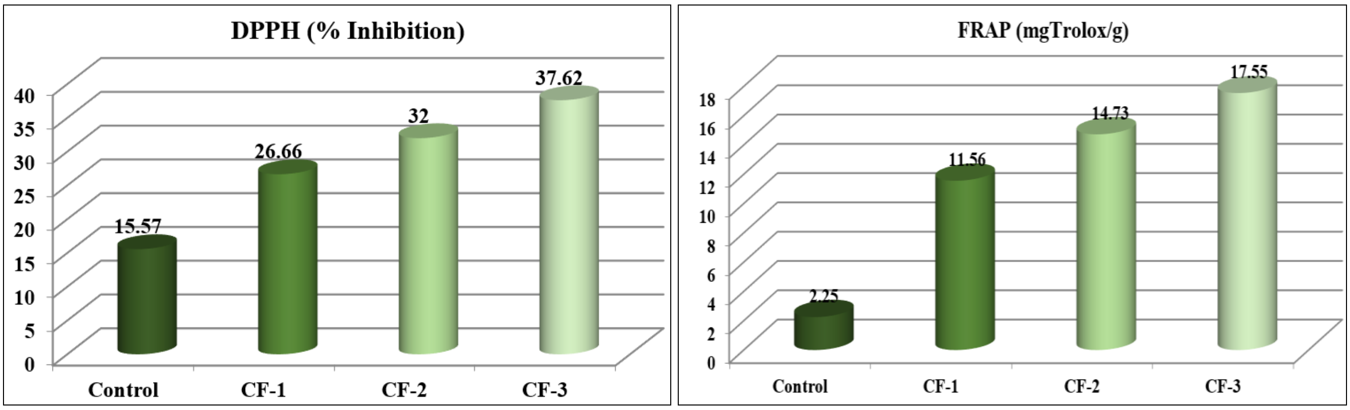

Fig 2: Antioxidant Activity of Composite Flours Developed from Finger Millet, Black soybean, Moringa Leaf Powder

The % inhibition of the radical DPPH by the composite flours of Finger millet, Black soybean, Moringa leaf powder and Durum wheat flour extract is showed in Table 2 and Figure 2. DPPH radical scavenging activity based antioxidant potential of the extracts was evaluated by using method described by Apak *et al.* (2008) [2] with some modification. Data are expressed as Mean ± SD of Control and composite flours in different proportion CF-1, CF-2 and CF-3 were antioxidant activity to a varying degree of 15.57±0.07%, 26.66±0.05%, 32.00±0.01% and 37.62±0.05% respectively. CF-3 composite flours showed highest antioxidant activity followed by Control and CF-2. CF-1 had the highest DPPH and lowest amount was observed in Control and CF-2. The reason for the same may be the higher inhibition percentage of the radical DPPH in Black soybean flour attributing higher antioxidant activity. The data of DPPH radical scavenging activity of composite flours indicates that percentage of inhibition in CF-3 was higher with value 37.62±0.05% followed by CF-2 with % inhibition of 32.00±0.01% and CF-1 with % inhibition of 26.66±0.05%. The DPPH % inhibition of CF-3 showed highest value because of high concentration of the of Black soybean flour. The higher DPPH % inhibition of a sample reflects that there is high amount of antioxidant compounds present in the sample extracts. Similar data was reported by Bourekoua *et al*., (2018) [28] i.e., 1.23 %. Also, in Black soybean 17.01% inhibition was recorded in consonance with Mitharwal & Chauhan, (2022) [15].

FRAP radical scavenging activity the Control and composite flours in different proportion CF-1, CF-2 and CF-3 were antioxidant activity to a varying degree of 2.25±0.09 mgTrolox/g, 11.56±0.07 mgTrolox/g, 14.73±0.05 mgTrolox/g and 17.55±0.07 mgTrolox/g respectively. The values of FRAP were significantly different with each other at ($p\leq$0.05) level. CF-3 had the highest FRAP and lowest amount was observed in Control and CF-2. The reason for the same may be the higher phenolic compound of the Black soybean flour attributing higher antioxidant activity. The data of FRAP radical scavenging activity of composite flours indicates that ferric reducing capacity in CF-3 was higher with value mgTrolox/g of 17.55±0.07 followed by CF-2 with mgTrolox/g of 14.73±0.05 and CF-1 with mgTrolox/g of 11.56±0.07. Patil *et al*., (2016) [18] reported that finger millet contained 29.34 of FRAP radical scavenging activity. The FRAP radical scavenging activity of CF-3 showed highest value because of high concentration of the of Black soybean flour. The higher FRAP mgTrolox/g of a sample reflects that there is high amount of phenolic antioxidant activity present in the sample extracts that converts ferric ion to ferrous ion which is a more stable compound.

4. Conclusion

The present study has provided with knowledge that Finger Millet Flour, Black soybean Flour, Moringa Leaf Powder and Durum Wheat Flour due to presence of high DPPH radical scavenging activity and FRAP radical scavenging activity, Total Phenols Content and Total Flavonoids Content indicated that they have all the possible preventive and curative properties. Amongst the composite flour (CF-3) 10:20:2:68 (Finger Millet Flour: Black soybean Flour: Moringa leaf Powder: Durum Wheat Flour) had the highest antioxidant content and antioxidant activity and have good potential to fight with malnutrition in children.

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