

Antioxidant Properties, Anti-oxidative Potentials and Phytochemical Properties of Wheat-Pearl Millet-*Andrographis paniculata* Leaf Flour Blends: Possible Implications of the Composite Functionality

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Abstract The study investigated and evaluated the influence of the inclusion of varying proportion of *Andrographis paniculata* leaf flour on the antioxidant properties, anti-oxidative activities and phytochemical properties of wheat-pearl millet- *Andrographis paniculata* leaf flour blends and the values of these quality parameters are used to predict the blends functionalities. The flour blends were prepared using whole wheat flour, whole pearl millet flour, and *A. paniculata* leaf flour. The recommended combination ratio (50:50) of wheat and pearl millet flour was adopted as the blending baseline while graded levels of *A. paniculata* leaf flour of 2, 4, 6, 8 and 10% were respectively included in the blending baseline to obtain wheat-pearl millet-*Andrographis panniculata* flour blends. In the formulation, the inclusion of *A. paniculata* leaf flour was carried out to replace the wheat and pearl millet flour respectively on equal basis. Each flour blends was evaluated for phytochemical properties, antioxidant properties and anti-oxidative activities. The oxalate content of the raw material flour ranged from 9.27mg/g in PMF to 15.85mg/g in APLF, while the value ranged from 7.74mg/g in WPMAPLF3 blend to 9.81mg/g in WPMAPLF4 blend. Tannin content of the raw material flours and flour blends ranged from 0.32 mg/g in WF to 1.91 mg/g in APLF, and from ranged from 0.43mg/g in WPMF blend to 0.84mg/g in WPMAPLF5 blend. Phytate content for the raw material flours ranged from 0.03mg/g in PMF to 0.08mg/g in APLF, while the phytate content of the flour blends ranged from 0.04mg/g in WPMAPLF1 to 0.06mg/g in WPMAPLF4, and 0.06mg/g in WPMAPLF5, respectively. The alkaloid value was observed to increase as the level of inclusion of the leaf increased from 2% to 4%, and gradually start decreasing from 6% to 10% inclusion. The free radical scavenging capacity of the flour blends against, 1,1-Diphenyl-2- picrylhydrazyl (DPPH), 2, 2-Azino-bis (3- ethylbenzthiazoline-6-sulphonic acid) (ABTS) and FRAP was observed to increase as the percentage of *A. paniculata* leaf inclusion increased. The antioxidant activities and free radical scavenging abilities of the flour blend samples were observed to increase as the percentage of inclusion of *Andrographis paniculata* leaf flour increased. Consequently, the formulated flour blends made from Wheat, pearl Millet and *Andrographis paniculata* exhibited good free radical scavenging ability against DPPH[•], OH[•], and FRAP. The antioxidant and phytochemical composition observed among the flour blends posit a viable staple possessing some predictive health benefiting potentials and healthy food functionality which could be exploited as preventive or curative food therapy against chronic diseases.

Keywords: phytochemicals, antioxidants, *Andrographis paniculata* leaf, Cereals flour

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1. Introduction

Cereals and legumes as well as their flours has served as major food staples in developing countries, especially Africa countries and they are equally serving as the flour base for the production of confectioneries products. Epidemiological studies have shown that regular consumption of whole grain foods is one of the vital ways of protecting the body against degenerative diseases [1,2]. The inclusion of the pericarp and germ components, have made whole grains to be rich sources of micronutrients and antioxidants [3]. Cereals such as Pearl millet and wheat formed the basis for the production of functional flour we intended to produce.

Wheat (*Triticum Aestivum*) is an important cereal and well utilized proteinous cereals in bread making and other aerated baked products [4]. Nigeria importation of wheat stands at 4.1million metric tonnes in 2011. Wheat importation has led to depletion in foreign earning and reserves, therefore the need to discourage further wheat importation in Nigeria by sourcing for protein containing and complementing food material alternatives.

Pearl millet (*Pennisetum glaucum* L.), species are the most cultivated in Nigeria. Pearl millet is rich in protein and some amino acid such as niacin [5,6]. Pearl Millets have been used in the production of ready-to-eat snacks [7]. But, no report yet on the inclusion of *Andrographis paniculata* leaf in the dual blend of wheat and pearl millet.

Andrographis paniculata, generally known as “King bitters” belong to the Acanthaceae family. Twenty eight (28) species of *Andrographis* genus is all there is [8]. *Andrographis paniculata* (A.P) is the most powerful and one of the few possessing healing properties [9]. This plant has been used centuries to treat respiratory infection, fever, herpes, and recently used to treat chronic and infectious disease [8,9]. Overall, the herb is known to be a powerful immune booster.

The increasing interest in therapeutic foods reflects the fact that a specific diet or component of a diet is associated with a lower risk of certain diseases. Plant-based functional foods are now getting more attention than ever before, reason being that they have the potential of myriad benefit to the society or indeed to the entire mankind especially in the line of nutrition, medicine and pharmacology. The medicinal values of these plant-based foods lies in being able to access their bioactive compound Such as phytochemicals and proteins that produce definite physiological action on the human body [10,11].

Therefore, the use of highly nutritious, under-utilized minor cereal like pearl millet to complement whole grains flours in the production of functional flour blends, Will nutritionally, complement and counterbalance each other to form a wholesome snack like biscuit, bread [7,12]. However, since there is no information yet on the combination of wheat, pearl millet and *Andrographis paniculata* flour blend for the production of functional confectioneries. The present study aims at producing functional flour blends from wheat, pearl millet and *Andrographis paniculata* leaf and evaluate the influence of the inclusion of the leaf on the antioxidant properties, anti-oxidative potentials, and phytochemical properties of the flour blends for possible health application.

2. Materials and Methods

2.1. Materials

2.1.1. Source of Food Materials

Wheat grain (*Triticum aestivum*), pearl millet grain (*Pennisetum glaucum*) were purchased at Apata market, Ibadan, Oyo state, Nigeria); and ‘King of bitter’ leaf (*Andrographis paniculata*) was cultivated and harvested from David’s Farm at Araromi Ogo Oluwa Area of Davog, Akure. Nigeria).

2.1.2. Food Materials Authentication

The ‘King of bitter’ leaf (*Andrographis paniculata*) was identified and authenticated at the Forest Research Institute (FRIN), Jericho, Ibadan, Nigeria with the herbarium number of 111951; while both wheat and pearl millet grains were also authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria.

2.2. Methods

2.2.1. Wheat Flour Processing

Wheat flour was processed into flour using the method of Amani, Ishola and bolade [7,13] with slight modifications. The wheat grains collected were cleaned to remove the stones, dust, woods and any other foreign materials from the grains. The clean and healthy wheat grain was finely grind in an electric grinder and passed through a 60 mesh size sieve. The powdered sample was stored in air tight container until further use for experiments.

2.2.2. Pearl Millet Flour Processing

Pearl Millet flour was processed using the method of Vaijapurkar, Ishola and Bolade with slight modifications [13,14]. The pearl millet grains were cleaned to removed dirt, sand and other extraneous material by winnowing. The cleaned grains were briefly washed and oven-dried until constant moisture content was obtained. It was later milled in an attrition mill, sieved and the pearl millet flour obtained stored in sealed polythene bags at room temperature for analysis.

2.2.3. *Andrographis paniculata* Flour Processing

A. paniculata leaf was processed into flour according to the method of Ishola and Bolade [13]. The freshly harvested ‘King of Bitter’ leaves were washed to remove dirt and soaked in 1% saline solution (NaCl) for 5 minutes to get rid of microbes. The leaves were drain of excess water and dried under shade to avoid loss of nutrients. The dried sample was milled into flour using Philips laboratory blender (HR2811 model) and stored in an air tight polythene bags for further analysis.

2.3. Chemical Methods

Antioxidants properties, antioxidative activities as well as the phytochemical properties of the flour blends were analyzed using standard methods.

2.3.1. Determination of Antioxidant Properties of Flour Blends

The total flavonoid content of the extract from flour blends were determined using a colorimeter assay developed by [15]. The total phenol content of the extract was determined according to the method of [16]. The Carotenoid content of the flour samples were determined using spectrophotometric method [17].

2.3.2. Determination of Anti-oxidative Potential of Flour Blends

The scavenging effect of the inclusion of *Andrographis paniculata* leaf flour in wheat-pearl millet based flour samples on 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was measured according to the method described by [18] with slight modifications. The ferric reducing antioxidant power (FRAP) property of the extract was determined by Pulido et al. method [19]. The OH scavenging ability of the extract of the flour blends was determined according to the method describe by Guttridge.

2.3.3. Determination of Phytochemical Properties of Flour Blends

Phytate was determined according to Wheeler and Ferrel method [20]. The alkaloid content was determined using Harborne method [21]. The spectrophotometric method of Brunner was used for Saponin determination [22]. Tannin Content was evaluated by the method of Makkar and Goodchild [23]. Oxalate determination was determined according to Day and Underwood, method [24]. The Carotenoid content of the flour samples were determined using spectrophotometric method [14]. The total phenol content of the blends were determined by the method of [16]. The total flavonoid content of the extract from flour blends were determined using a colourimeter assay developed by [15]

2.4. Determination of Functional Properties of Flour Blends

The functional properties of the flour blends determined were water and oil absorption capacities, bulk density, least gelation concentration, foaming and swelling capacities.

Water absorption capacity (WAC) of the flour blends according to the method described by Adebowale et al. [26] Oil absorption capacity (OAC) was estimated according to the method of Adebowale et al. [26] Bulk density was estimated by method described by Onwuka, [27]. Least gelation concentration (LGC) done according to Coffman and Garcia method [28]. Foaming capacity was studied as described by Coffman and Garcia [28]. Swelling capacity was carried out by the method described by Abbey and Ibeh [29].

2.5. Formulation of the Flour Blends

The wheat and Pearl millet was used as a base material at equal proportion for the preparation of flour mix with varying proportion of *Andrographis paniculata* leaf flour. The composite flour was formulated with reference to 50%Wheat: 50% Pearl millet standard blend ratio for biscuit production, a method described by Amani and Vaijapurkar with slight modifications (Table 1) [7,14].

2.6. Statistical Analysis

Data were subjected to analysis of variance using SPSS (IBM version. 20.0, SPSS Inc., Quarry Bay, Hong Kong) and presented as means (\pm SEM). Comparisons between different groups were done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $p < 0.05$ were considered as statistically significant as described by Yalta and Talha [30].

3. Results and Discussion

3.1. Some selected Phytochemical Properties of Flours from Wheat, Pearl Millet, and *Andrographis paniculata* Leaf and Their Blends

The result of the influence of the *Andrographis paniculata* leaf inclusion on the anti-nutritional composition of flours from Wheat, Pearl Millet, and *Andrographis paniculata* leaf and their blends are present in the Figure 1 (i-v). The oxalate content of the raw material flour ranged from 9.27mg/g in PMF to 15.85mg/g in APLF (Figure 1v), while the value ranged from 7.74mg/g in WPMAPLF3 blend to 9.81mg/g in WPMAPLF4 blend (Figure 1v). Tannin content of the raw material flours ranged from 0.32 mg/g in WF to 1.91 mg/g in APLF (Figure 1iii), while the value ranged from 0.43mg/g in WPMF blend, to 0.84mg/g in WPMAPLF5 blend. Tannins are responsible for antibacterial activity of plant seed [31]. One of the biggest concerns with tannins is their potential ability to hinder iron absorption. In the digestive tract, tannins can easily bind with iron present in plant-based foods, rendering it unavailable for absorption. But, considering the very low Tannin value of all the flour understudied, the result indicates that this result is not likely to cause significant harm in people with healthy iron levels, and may not be problematic for those with iron deficiency. Despite this, tannin-rich diets may have potential beneficial cardiovascular and cancer-fighting properties because of the antioxidant activity of tannins [32]. Phytate content for the raw material flours ranged from 0.03mg/g in PMF to 0.08mg/g in APLF (Figure 1i), while the phytate content of the flour blends ranged from 0.04mg/g in WPMAPLF1 to 0.06mg/g in WPMAPLF4, and 0.06mg/g in WPMAPLF5, respectively. It was observed that the inclusion of *A. paniculata* leaf into the biscuit increase the phytate content of the biscuit samples. Considering the low phytate level of all the flours understudied, it can be infer that the flours may not hinder the absorption of minerals such as iron, zinc and calcium. phytic acid can cause a significant problem when diets are largely composed of high-phytate foods while at the same time low in meat or other animal-derived products [33]. Phytate is also regarded as healthy plant compound, because not only is phytate acts as an antioxidant, it may also be protective against kidney stones and cancer. Scientists have even suggested that phytic acid may be part of the reason why whole grains have been linked with a reduced risk of colorectal cancer [34].

Table 1. Formulation of composite flour for physical and chemical tests and biscuit production (blends ratios by weight)

| SAMPLE | Formulations | Wheat Flour | Pearl Millet Flour | <i>Andrographis paniculata</i> leaf flour | Total (%) |
|----------|---|-------------|--------------------|---|-----------|
| WF | Wheat | 100 | ---- | ---- | 100 |
| PMF | Pearl millet | 100 | ---- | ---- | 100 |
| APLF | <i>Andrographis paniculata</i> leaf (APL) | 100 | ---- | ---- | 100 |
| WPMF | Wheat + Pearl millet | 50 | 50 | ---- | 100 |
| WPMAPLF1 | Wheat + Pearl millet + APL 1 | 49 | 49 | 2 | 100 |
| WPMAPLF2 | Wheat + Pearl millet + APL 2 | 48 | 48 | 4 | 100 |
| WPMAPLF3 | Wheat + Pearl millet + APL 3 | 47 | 47 | 6 | 100 |
| WPMAPLF4 | Wheat + Pearl millet + APL 4 | 46 | 46 | 8 | 100 |
| WPMAPLF5 | Wheat + Pearl millet + APL 5 | 45 | 45 | 10 | 100 |

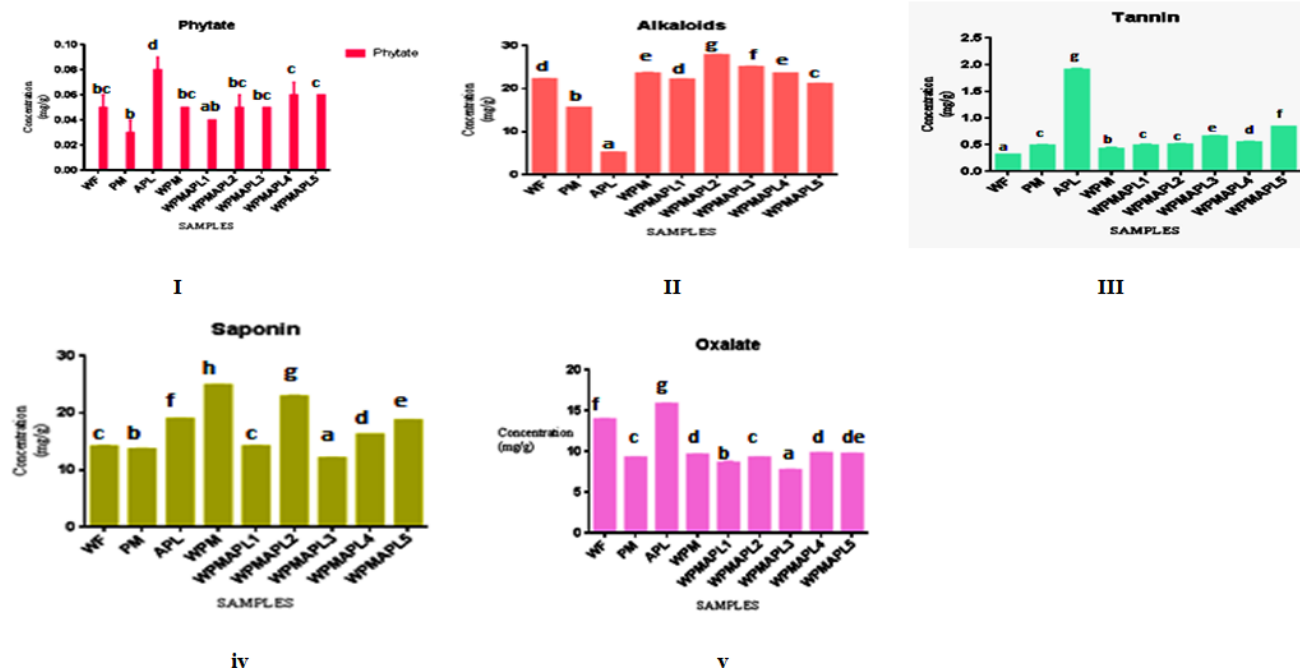


Figure 1 (i - v). Selected Phytochemical composition of composite flour from wheat, pearl millet and *Andrographis paniculata* flour [WF-Wheat flour (100%); PMF-Pearl millet flour (100%); APLF- *Andrographis paniculata* leaf flour (100%); WPMF- Wheat pearl millet flour (50:50) %; WPMAPLF1 – Wheat- pearl millet-*Andrographis paniculata* leaf flour (49:49:2) %; WPMAPLF2– Wheat- pearl millet- *Andrographis paniculata* leaf flour (48:48:4) %; WPMAPLF3– Wheat- pearl millet- *Andrographis paniculata* leaf flour (47:47:6) %; WPMAPLF4 – Wheat- pearl millet- *Andrographis paniculata* leaf flour biscuit (46:46:8) %; and WPMAPLF5 – Wheat- pearl millet- *Andrographis paniculata* leaf flour biscuit (45:45:10) %]

Saponin content for the raw material flours ranged from 13.67mg/g in PMF to 18.96 mg/g in APLF (Figure 1iv), while the phytate content of the flour blends ranged from 12.08mg/g in WPMAPLF3 to 24.85mg/g in WPMF (Figure 1iv). The saponin value was observed to increase among the raw materials flours but decreased in an undulating manner as the level of inclusion of the leaf increased among the flour blends. Clinical studies have suggested that these health-promoting components, saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels [35]. Plants produce saponins to fight infections by parasites. When ingested by humans, saponins also seem to help our immune system and to protect against viruses and bacteria. Saponins have hypolipidemic and anticancer activity, antioxidant and anti-mutagenic properties and are also necessary for activity of cardiac glycosides [35,36]. Considering the saponin content of all the flours investigated, the result suggest food staples with viable potentials for use as antiviral, antibacterial and immune boosting food therapy.

The Alkaloids content of the raw material flours ranged from 5.20% in APLF to 22.23% in WF (Figure 1ii), while the value for the flour blends ranged from 21.16% in WPMAPLF5 to 27.85 in WPMAPLF2 (Figure 1ii). The alkaloid value was observed to increase as the level of inclusion of the leaf increased from 2% to 4%, and gradually start decreasing from 6% to 10% inclusion. Alkaloids are multi-purpose compounds active in various environmental interactions, such as defense against herbivores, bacteria, fungi, viruses or competing plants [37]. At a molecular level, especially in humans, alkaloids can interfere with membrane permeability, membrane proteins (ion channels and receptors), enzymes and other proteins, DNA, RNA and corresponding proteins, electron chain, and the cytoskeleton [37]. Because of these pharmacological properties, some alkaloids can be used in medicine to treat infections, health disorders, and even cancer [37,38]. The results of the flours understudied explicated the observed increase in the alkaloid contents as the percentage of the leaf inclusion increased to be implicative in the treatment of infectious disease and

health disorder, but, that as it were, is recommended for further studies.

3.2. Some Anti-oxidant Properties and antioxidative Activities of Flours from Wheat, Pearl Millet, and *Andrographis paniculata* Leaf and their Blends

The results of antioxidant properties of the raw material flours and the flour blends made from wheat, pearl millet and *Andrographis paniculata* leaf flours are presented Figure 2a (I-III). The ability of flour from Wheat, Pearl Millet, and *Andrographis paniculata* leaf and their blends to scavenge free radical against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay ranged from 28.68% in WF to 40.49% in APLF, and the values were significantly ($p < 0.05$) higher than PMF (31.37%) among the raw materials flour sample, while the value ranged from 9.32 in WPMF blend, to 37.40 in WPMAPLF3 blend, and the value were significantly different ($p < 0.05$) higher than WPMAPLF1(15.59), WPMAPLF2 (28.24), WPMAPLF4 (25.10) and WPMAPLF5 (33.82).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [39]. The results from the assay revealed that as the percentage of *A. paniculata* leaf inclusion increases, the free radical scavenging capacity of the flour blends increased.

The ability of the flour blends samples and the raw flour samples to scavenge free radical against 2, 2-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) ranged between 0.01 in WPMAPLF blend, and 0.03 mg TEAC/g

in WPMAPLF2 (0.03), WPMAPLF3 (0.03), WPMAPLF4 (0.03), and in WPMAPLF5 (0.03), respectively. The values were not significantly difference among the flour blends.

The ferric reducing antioxidant power (FRAP) of the raw materials ranged from 6.27 in WF to 30.35 mg AAE/g in APLF, the value which is significantly ($p < 0.05$) higher than the value of PMF of 7.40 mg/g among the raw material flour samples. The value for the flour blends samples ranged from 1.89 mg/g in WPMAPLF1 to 8.08 mg/g in WPMAPLF3, while that of WPMF, WPMAPLF2, WPMAPLF4 and WPMAPLF5 were 2.19 mg/g, 5.06 mg/g, 4.68 mg/g, and 7.93 mg/g, respectively. The flavonoid antioxidant power of the raw material flour samples ranged from 0.23mg/g in WF to 0.89 mg/g in APLF. The value for the flour blends samples ranged between 0.08 mg/g in WPMAPLF1 to 0.38mg/g in WPMAPLF3, while WPMF sample was 0.14 mg/g, in WPMAPLF2 was 0.19 mg/g, in WPMAPLF4 was 0.18mg/g and WPMAPLF5 was 0.30 mg/g. The phenol antioxidant power of the raw material flour samples ranged from 8.67 in WF to 19.20 mg/g in APLF, which is significantly ($p < 0.05$) higher than 9.24 value of PMF of the raw material flour samples. The value for the flour blends samples ranged between 7.52 mg/g in WPMAPLF1 to 11.68mg/g in WPMAPLF3, while WPMF sample was 7.97 mg/g, WPMAPLF2 was 8.57 mg/g, WPMAPLF4 was 8.15 mg/g and WPMAPLF5 was 9.90 mg/g. The ability of flour samples to prevent free hydroxyl radicals ranged from 0.70% in APLF and 12.70% in WF, and 12.00% IN PMF in the raw materials flour samples. The values for the flour blends samples ranged from 0.55% in WPMAPLF2, to 12.50% in WPMAPLF5, and the value was 11.30% in WPMAPLF4, 0.75% in WPMAPLF3, 1.20 % in WPMAPLF1, and 7.95% in WPMF.

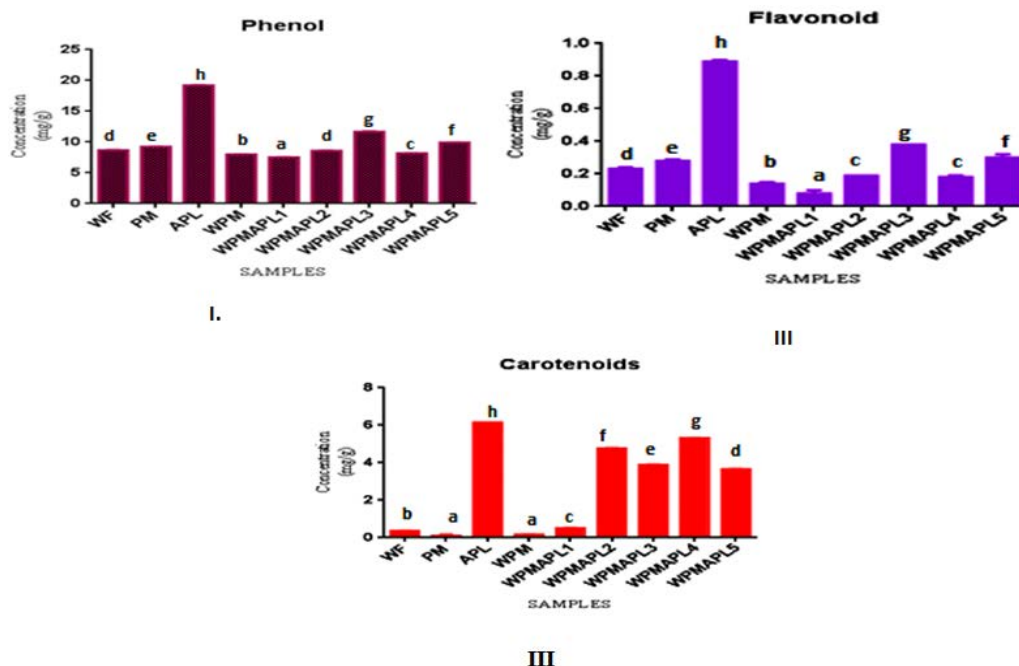


Figure 2a (I- III). Some selected Antioxidant of composite flour from wheat, pearl millet and *Andrographis paniculata* flour [WF-Wheat flour (100%); PMF-Pearl millet flour (100%); APLF- *Andrographis paniculata* leaf flour (100%); WPMF- Wheat pearl millet flour (50:50) %; WPMAPLF1– Wheat-pearl millet-*Andrographis paniculata* leaf flour (49:49:2) %; WPMAPLF2– Wheat- pearl millet- *Andrographis paniculata* leaf flour (48:48:4) %; WPMAPLF3– Wheat- pearl millet- *Andrographis paniculata* leaf flour (47:47:6) %; WPMAPLF4 – Wheat- pearl millet- *Andrographis paniculata* leaf flour biscuit (46:46:8) %; and WPMAPLF5 – Wheat- pearl millet- *Andrographis paniculata* leaf flour biscuit (45:45:10) %]

The carotenoid content of the raw material flours ranged from 0.12mg/g in PMF to 6.15mg/g in APLF (Figure 2iii), while carotenoid content of the flour blends ranged from 5.32mg/g in WPMAPLF4. The carotenoid contents of the flour blends were observed to increase and decrease like copious, moderate and slightly as the percentage of inclusion of *A. paniculata* leaf increased. There is evidence that carotenoids, in addition to beneficial effects on eye health, also produce improvements in cognitive function and cardiovascular health, and may help to prevent some types of cancer. Despite the evidence for the health benefits of carotenoids, large population-based supplementation studies have produced mixed results for some of the carotenoids [37].

The antioxidant activities and free radical scavenging abilities of the flour blend samples were observed to increase as the percentage of inclusion of *Andrographis paniculata* leaf flour increased. This finding could be attributed to variations in food composition and bioactive components like phytochemicals, fibres and bioactive proteins, which were significantly present in these experimental food samples, especially in *Andrographis paniculata* leaf. In the last decades, several studies have demonstrated the significant of antioxidant in diseases prevention and managements [39,40]. For instance, studies have reported on the contributions of antioxidants in diabetes and its complications [40].

4. Conclusion

The study established that the formulated flour blends made from Wheat, pearl Millet and *Andrographis paniculata* exhibited better nutritional qualities in terms of good free radical scavenging ability against DPPH[•], OH[•], FRAP. Considering the antioxidant and phytochemical composition observed among the flour blends, this shows that the blends are viable staples possessing some predictive health benefiting potentials and healthy food functionality which could be exploited as preventive or curative food therapy against chronic diseases. This prediction could be validated through further animal or clinical studies. The flour blends also posit a potential to promote good health status on regular ingestion and may reduce the risk of chronic diseases and may provide health and physiological benefit beyond the traditional nutrients it contains. Development of this functional flour through the inclusion of *A.paniculata* leaf in way could increase further usage of this under-utilized medicinal plant part in food industries.

5. Recommendation

Further studies is envisage into confectionaries production from the blends and animal studies is envisaged with the blend to validate the prospective nutritional potential claims as observed in the blends understudied.

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Competing Interest Statement

The authors declare no conflict of interest.

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