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# Health promoting properties of *Alternanthera brasiliana* leaves and *Hibiscus sabdariffa* calyces used in fortification of maize-bambara groundnut malt and maize-cowpea malt complementary foods

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**Abstract**

The study evaluated the chemical and antioxidant properties of *Alternanthera brasiliana* leaves and *Hibiscus sabdariffa* calyces used in iron and zinc fortification of maize-bambara groundnut malt and maize-cowpea malt complementary foods. *A. brasiliana* leaves and *H. sabdariffa* calyces were freshly harvested, dried at 50°C for 48 hours and analyzed for the relevant chemical components and antioxidant activities. The vitamin A content was 6996 and 745.6 µgRE/kg while the vitamin C was 238.26 and 294.78 mg/kg respectively. The aqueous extracts of *A. brasiliana* and *H. sabdariffa* calyces contained 509.5 mg/kg and 5234.72 mg/kg of alkaloids, 1545 mg/kg and 384 mg/kg of anthocyanins, 767.3 and 235.83 mg/kg of carotenoids, 14,702.8 and 26,428.3 mg/kg of phenols, 1043.5 and 897.63 mg/kg steroids and 462.0 mg/kg and 1006.5 mg/kg of flavonoids respectively. *A. brasiliana* and *H. sabdariffa* extracts had concentration-dependent DPPH activity with $IC_{50}$ of 1.76 mg/ml and 5.745 mg/ml, nitric oxide scavenging activity with $IC_{50}$ of 0.675 mg/ml and 3.976 mg/ml while the ferric reducing power had an absorbance range of 0.5 – 0.982 and 0.959 – 0.986 respectively. The study revealed that *A.brasiliana* leaves and *H. sabdariffa* calyces contain components that will impact positively on the health of the infants when used to formulate complementary foods.

## 1. Introduction

The role of diet and nutrition as determinants of chronic disease is well documented (Middleton *et al*., 2000; Yi-fang, 2002) and there is growing evidence that chemical components of plants and microbial foods may play an integral role in the link between food and long-term health (Ferrari, 2004). Complementary foods are any nutrient containing food given to young children along with breast-milk (Gibbs, 2010) when the breast milk nutrients become inadequate for their energy and growth needs (WHO, 2003) within the 6 - 23 months window. It is required to ensure adequate growth, to prevent malnutrition, stunting, and anaemia (Bhasin *et al.,* 2003). Poor complementary feeding is the immediate direct cause of malnutrition [which manifests as protein energy malnutrition (PEM) and micronutrient deficiencies] leading to growth faltering and high rates of infections during infancy.

In most traditional cultures of the developing world, cereal-based gruels are the first complementary foods to be introduced to infants, sometime between 4 and 6 months of age. They are followed by vegetables, fruits, fruit juices, and meat products. These plant-based traditional complementary foods do not meet these nutritional requirements because they are deficient in micronutrients such as calcium, iron, zinc and vitamin A (Dewey and Brown, 2003). This is because the level of these micronutrients falls below the Recommended Dietary Allowance (RDA) of 50 g/kg for calcium, 275 mg/kg for iron, 125 mg/kg for zinc and 5000 µgRE/kg for vitamin A. Hence, calcium, iron, zinc and vitamin A deficiencies continue to pose great danger to 6-12 months old infants leading to increased susceptibility to

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infections such as diarrhea, cholera and impaired immunity (Lutter and Dewey, 2003).

In infants, iron and zinc requirements are difficult to meet from non-fortified complementary foods (WHO/UNICEF, 2003). These can be prevented by fortification of infant foods. Fortification of plant-based complementary foods with vitamin and mineral pre-mix or animal supplements such as milk makes the foods expensive for low-income earners who earn less than 300 naira (1.5 USD) per day (Anon., 2012). The alternative is to adopt food-to-food fortification. This has fueled the interest in plant foods as sources of micronutrients for combating micronutrient deficiency of chemicals that may have useful roles in the health of infants. The suitability of a complementary food depends on a number of factors including the nature of raw materials and methods of processing and fortification practices adopted.

*Alternanthera brasiliana* (L) O. Kuntze belongs to the Family Amaranthaceae commonly known in Brazil as joy weed or Josephs coat is widely used as a medicinal agent to cure different disease, such as inflammation, wound healing, analgesic, antitumor activity, immune modulator and lymphocyte proliferation (Duarte and Debur, 2004; Saawan *et al.,* 2011). Roselle (*Hibiscus sabdariffa* L.) on the other hand belongs to the Family Malvaceae and is known in Nigeria as "Zobo". It is used effectively in folk medicines for the treatment of hypertension, inflammatory diseases and cancer (Kong *et al.,* 2003; Lin *et al*., 2007), decrease blood viscosity and reduce hypertension (Christian *et al.,* 2006).

Apart from these properties, there is evidence that the calyces of Hibiscus sabdariffa can be used in food–to-food- fortification to boost the iron and zinc contents in maize-bambara groundnut malt and maize–cowpea malt complementary foods (Attaugwu, 2015). It is against this background that the health promoting properties of *Hibiscus sabdariffa* and *Alternanthera brasiliana* which is known to have good iron and zinc contents were investigated.

## 2. Materials and methods

### *2.1 Materials*

Fresh leaves of Brazilian joy weed [*Alternanthera brasiliana* (L.) O. Kuntze] and calyces of Roselle [*Hibiscus sabdariffa* (L.) malvaceae] was obtained from a farm in the University of Nigeria Nsukka.

### *2.2 Methods*

One thousand grams (1000 g) of freshly harvested *A. brasiliana* leaves and *H. sabdariffa* calyces were chopped into 2 mm slices and dried at 50$^{o}$C in a convection Gallenkamp oven (Model IH-150, Gallenkamp, London, England). The extracts for the free radical scavenging activities were prepared according to the method described by Oyedemi *et al.* (2010) with a slight modification of using ethanol for extraction instead of methanol.

### *2.3 Analysis*

The steroid content, total carotenoid, ascorbic acid, thiamine, riboflavin, niacinamide**,** pyridoxine hydrochloride and cobalamin contents were determined using the method as described in AOAC (2010). The vitamin E content of the samples was determined by the α,α-dipyridyl method of Pearson (1976). The vitamin K content of the samples was determined by the 2, 4-dinitrophenyhydrazine method of Snell and Snell (1953). The flavonoid and alkaloid contents were determined using the method of Harborne (1973). The total phenolic content in the aqueous extracts (1:4 w/v) of dried *A. brasiliana* leaves and dried *H. sabdariffa* calyces were determined using the method of Wolfe *et al*. (2003). Total flavonoids were determined by the method of Ordoñez *et al*. (2006). Total flavonol content was determined by the spectrophotometric procedure described by Kumaran and Karunakaran (2007). Total proanthocyanidin determination was based on the spectrophotometric procedure of Sun *et al.* (1998). The DPPH and nitric oxide free radical scavenging activities were determined by the methods of Liyana-Pathiranan and Shahidi (2005) and Oyedemi *et al.* (2010) respectively. The ferric oxide reducing power of the ethanolic extract was evaluated according to the spectrophotometric method of Yen and Chen (1995).

## 3. Results and discussion

### *3.1 Vitamin composition of A. brasiliana leaves and H. sabdariffa calyces*

The vitamin composition of *A. brasiliana* leaves and *H. sabdariffa* calyces are presented in Table 1. The vitamin A content of *A. brasiliana* and *H. sabdariffa* calyces were 6996 µgRE/kg and 745.6 µgRE/kg respectively. The vitamin A content of *A. brasiliana* leaves and *H. sabdariffa* calyces are above the RDA

 

requirement of 500 µgRE/kg in complementary foods (Lutter and Dewey, 2003) implying that they could be a good source of vitamin A which is needed for good vision in infants. Hence the use of *A. brasiliana* leaves and *H. sabdariffa* calyces in complementary foods could improve the vision of infants taking the food. Saawan *et al.* (2011) reported 19260 µg/kg pro-vitamin A carotenoids in *A. brasiliana*.

Table 1. Vitamin composition of *A. brasiliana* leaves and *H. sabdariffa* calyces

| Micronutrient | *A. brasiliana* | *H. sabdariffa* |
|---|---|---|
| Vitamin A (µgRE/kg) | 6996.00 ± 0.0167[b] | 745.60±0.0042[a] |
| $B_1$ (mg/kg) | 11.12 ± 0.0551[a] | 12.37±0.0045[b] |
| $B_2$ (mg/kg) | 654.10 ± 0.0318[a] | 4094.50±0.0271[b] |
| $B_3$ (mg/kg) | 4184.40± 0.0242[b] | 2163.10±.0324[a] |
| $B_6$ (mg/kg) | 21.01± .0009[a] | 8.32±0.0042[b] |
| $B_{12}$ (mg/kg) | 1.44± .0015[a] | 43.80±0.0057[b] |
| C (mg/kg) | 238.36± 0.0159[a] | 294.78±0.0411[b] |

Results are the means of three replications. Values carrying different superscript in the same row are significantly different ($p<0.05$)

The vitamin B1 (thiamin) content of *A. brasiliana* and *H. sabdariffa* were 11.12 mg/kg and 12.37 mg/kg respectively. The vitamin $B_1$ content of *H. sabdariffa* of this report is higher than the 1.23 and 1.77 mg/kg reported by Luvonga *et al.* (2012) for fresh and dried *H. sabdariffa* respectively. The differences may be attributed to genetic variety and type of soil. These results implied that infants consuming these foods will have an improved appetite, a healthier nervous system and a higher release of energy from the complementary foods as these are the major functions of vitamin $B_1$ (thiamin) in the body.

The vitamin $B_2$ (riboflavin) content of *A. brasiliana* and *H. sabdariffa* were 654 mg/kg and 4094.50 mg/kg respectively. The vitamin $B_2$ content of *A. brasiliana* was higher than the 140 mg/kg content reported by Saawan e*t al.* (2011). The differences may be attributed to genetic variety and type of soil. Vitamin $B_2$ (riboflavin) is responsible for maintaining healthy blood cells, plays an important role in the conversion of food into energy and in fat and protein metabolism. Hence, infants consuming complementary foods containing *A. brasiliana* and *H. sabdariffa* will have healthy blood cells, healthier vision and skin as well as improved fat and protein metabolism.

The vitamin $B_3$ (niacin) content of *A. brasiliana* leaves and *H. sabdariffa* calyces were 4184.40 mg/kg and 1216.31 mg/kg respectively. The vitamin $B_3$ content of *A. brasiliana* in this report was lower than the 12,000 mg/kg reported by Saawan *et al.* (2011) for *A. brasiliana*. The differences may be attributed to genetic variety and type of soil. However, the vitamin $B_3$ content of *H. sabdariffa* (1216.31 mg/kg) of this report was higher than the 37.7 mg/kg reported by Wong *et al.* (2002) for *H. sabdariffa.* The difference in the vitamin $B_3$ content of the present report could be attributed to genetic variety and geographic differences. Vitamin $B_3$ helps in lowering the level of bad cholesterol and elevation of good (HDL) cholesterol level leading to significant decrease in heart disease. It also offers protection for certain skin cancers.

The vitamin $B_6$ content of *A. brasiliana* leaves and *H. sabdariffa* calyces were 21.01 mg/kg *and.* 8.32 mg/kg respectively. The high vitamin $B_6$ content of *A. brasiliana* and *H. sabdariffa* implies that the use of *A. brasiliana* leaves and *H. sabdariffa* calyces in complementary foods will help in fighting anemia in children if consumed since the health benefits of vitamin $B_6$ includes stimulating co-enzymatic activities, positive effect on hormone control, cardiac diseases, kidney disorder and anemia.

The vitamin C content of *A. brasiliana* leaves and *H. sabdariffa* calyces were 238.36 mg/kg and 294.78 mg/kg respectively. The vitamin C content of *A. brasiliana* (238.36mg/kg) was higher than the 170 mg/kg content reported by Saawan *et al*. (2011) while that of *H. sabdariffa* (294.78 mg/kg) was higher than 67.01 and 49.90 mg/kg reported by Luvonga *et al*. (2012) for fresh and dried *H. sabdariffa*. The difference could be attributed to genetic variety and type of soil. The high vitamin C content of the samples could enhance non-heme iron absorption from the fortified complementary foods hence reduce the prevalence of anemia in the infants consuming it. It will also help in fighting some cancers as vitamin C is a known antioxidant.

### *3.2 Phytochemical composition of A. brasiliana and H. sabdariffa calyces*

The phytochemical composition of aqueous extracts of *A. brasiliana* leaves and *H. sabdariffa* calyces are presented in Table 2. The alkaloid content of aqueous extracts of *A. brasiliana* leaves and *H. sabdariffa*

 

calyces were 509.5 mg/kg and 5234.72 mg/kg respectively. The anthocyanin content of *A. brasiliana* leaves and *H. sabdariffa* calyces aqueous extracts were 1545 mg/kg and 384 mg/kg respectively. The phenolic contents of aqueous extracts of *A. brasiliana* leaves and *H. sabdariffa* calyces were 14,702.80 mg/kg and 26,428.30 mg/kg respectively. The flavonoid content of extracts of *A. brasiliana* leaves and *H. sabdariffa* calyces were 462.00 mg/kg and 1006.50 mg/kg respectively. The carotenoid contents of the plant extracts were 767.30 mg/kg and 235.83 mg/kg for *A. brasiliana* leaves and *H. sabdariffa* calyces respectively. Saawan *et al.* (2011) reported 19.26 mg/kg carotenoids in *A. brasiliana* leaves while Wong *et al.* (2002) reported 0.3 mg/kg carotenoid content in *H. sabdariffa* calyces. The steroid content of *A. brasiliana* leaves and *H. sabdariffa* calyces were 1043.50 mg/kg and 897.63 mg/kg respectively. *H. sabdariffa* calyces had 4725.2 mg/kg higher alkaloid content than *A. brasiliana* leaves. The anthocyanin content of *A. brasiliana* was 1161 mg/kg higher than that of *H. sabdariffa*. *A. brasiliana* leaves would have more antioxidant/ health promoting benefit than *H. sabdariffa* calyces. The phenolic content of *H. sabdariffa* was 11,725.50 mg/kg higher than that of *A. brasiliana.* This suggests that the *H. sabdariffa* extract will possess higher antioxidant activity than *A. brasiliana.* The flavonoid content of *H. sabdariffa* calyces was 544.50 mg/kg higher than that of *A. brasiliana* leaves. This could be attributed to the fact that flowers generally have more flavonoids which are the most important pigments for flower and petal coloration and are used mainly for the attraction of pollinator animals. The result of this study is similar to that of Elija *et al*. (2010) who reported higher flavonoid content in flowers of *Ipomoea carnea* than in the stem and leaves antioxidant effects of which are attributed to their redox properties. They are therefore reducing agents, hydrogen donors, singlet oxygen quenchers and metal- chelators (Vladimir-Knezevic *et al*., 2011), properties which could contribute to their potential role in the prevention of cancer and heart disease.

### *3.3 Antioxidant properties*

The antioxidant activities of ethanolic extracts of dried *Alternanthera brasiliana* leaves and *Hibiscus sabdariffa* calyces are illustrated in Figures 1, 2 and 3.

Proanthocyanidins, vitamin C and phenols content have been reported to have high antioxidant activity (Oyademi *et al.,* 2010; Azza *et al.,* 2011). The DPPH activity of *A. brasiliana* increased with increasing concentration of extract while that of *H. sabdariffa* decreased with increasing concentration of extract. The latter could be due to exhaustion of DPPH reacting species at a lower concentration of extract or, due to the acidic pH of the extract (Luvonga *et al.,* 2012). A similar result of decrease has been reported by Luvonga *et al.* (2012).

Table 2. Phytochemical composition of *A. brasiliana* leaves and *H. sabdariffa* calyces

Results are the means of three replications. Values carrying different superscripts in the same row are significantly different ($p<0.05$)

| Phytochemical composition | *A. brasiliana* (mg/kg) | *H. sabdariffa* (mg/kg) |
|---|---|---|
| Alkaloids | 509.50 ± 0.0531[a] | 52,347.20 ± 0.1089[b] |
| Anthocyanin | 1545.00 ± 0.0473[b] | 384.00 ± 0.0300[a] |
| Carotenoids | 767.30 ± 0.0410[b] | 235.803 ± 0.0111[a] |
| Steroids | 1043.50 ± 0.0234[b] | 897.63 ± 0.0762[a] |
| Phenols | 14,702.40 ± 0.0212[a] | 26,428.33 ± 0.0122[b] |
| Flavonoids | 462.00 ± 0.0891[a] | 1006.50 ± 0.0140[b] |
| Total phenol (mg/kg gallic acid) | 67,320.00 ± 0.0222[a] | 67,440.00 ±0.0341[b] |
| Total flavonoids (mg/kg quercetin equivalent) | 69,030.00 ± 0.0541[b] | 57,810.00 ±0.0751[a] |
| Total flavonols (mg/kg quercetin equivalent) | 50,430.00 ± 0.0323[b] | 8710.00 ±0.0211[a] |
| Proanthocyanidins mg/kg catechin equivalent) | 461,340.00 ± 0.0412[a] | 729,290.00 ± 0.0122[b] |

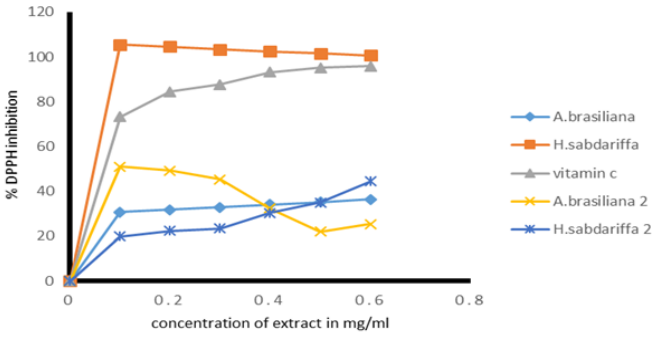


Figure 1. DPPH scavenging activity of *A. brasiliana* and *H. sabdariffa* extracts. *A. brasiliana = Alternanthera brasiliana extract, H. sabdariffa = Hibiscus sabdariffa extract,* Vitamin C = Vitamin C, A. *brasiliana* 2 = *Alternanthera brasiliana* extract adjusted with NaOH, *H. sabdariffa* 2 = *Hibiscus sabdariffa* extract adjusted with NaOH.

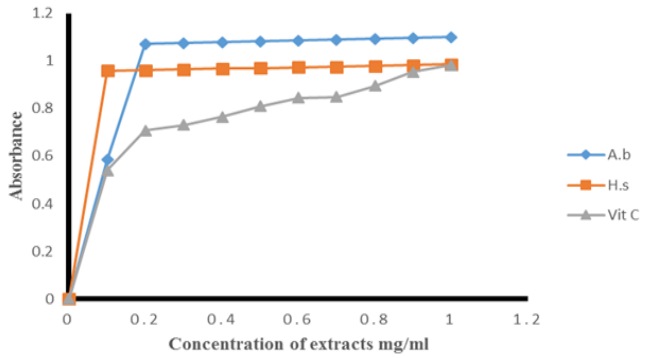


Figure 2. Ferric oxide reducing power of *A. brasiliana* and *H. sabdariffa* extracts. A.b = *Alternanthera brasiliana extract,* H.s = *Hibiscus sabdariff aextract,* Vit C = Vitamin C.

The abilities of the extracts to scavenge DPPH radicals (Figure 1) showed that *A. brasiliana* and *H. sabdariffa* extracts had DPPH radical scavenging activity with $IC_{50}$ of 1.76 mg/ml and 5.745 mg/ml respectively. The DPPH activity of *A. brasiliana* and *H. sabdariffa* extracts were lower than the $IC_{50}$ of 0.088 mg/ml for vitamin C, the reference antioxidant. Although the $IC_{50}$ of *A. brasiliana* was higher than the $IC_{50}$ of *H. sabdariffa,* which at a low concentration of 0.1 mg/ml inhibited 105.54% of DPPH radicals. This decreased gradually to 1.5 % at 10.05 mg/ml implying that *H. sabdariffa* possesses higher DPPH activity than *A. brasiliana* extract. The higher DPPH activity of *H. sabdariffa* could be attributed to its higher content of total phenol (67,440 mg/kg gallic acid equivalent), proanthocyanidins (729,290 mg/kg catechin equivalent) and vitamin C (294.78mg/kg) compared to the total phenol content of 67,340 mg/kg gallic acid, proanthocyanidins (461,340 mg/kg catechin equivalent) and vitamin C (238.26 mg/kg) in *A. brasiliana*.

The effect of pH of *H. sabdariffa* extract was evident when the different concentrations of extracts (0.1 -1.0 mg/ml) used for the assay were treated with 5M NaOH to neutralize the acidity of the extracts. The DPPH activity of the neutralized extract of *H. sabdariffa* increased with increasing concentration of the extract; the $IC_{50}$ was 16.75 mg/ml, implying that the DPPH scavenging activity of *H. sabdariffa* decreased as pH increased from 2 to 7. In *A. brasiliana,* however, the DPPH scavenging activity of the extract adjusted with 5 M NaOH decreased with increasing concentration and had an $IC_{50}$ of 0.229 mg/ml. *A. brasiliana* leaves extract showed a concentration-dependent DPPH scavenging activity while *H. sabdariffa* showed a pH dependent DPPH scavenging activity.

The ability of ethanolic extracts of *A. brasiliana* and *H. sabdariffa* to reduce $Fe^{3+}$ to $Fe^{2+}$ was high (Figure 2). *A. brasiliana* extract had a wider absorbance range of 0.5841-1.1012 at 0.1 – 1 mg/ml than *H. sabdariffa* extract (0.959 - 0.986) and vitamin C (0.5 – 0.982). *A. brasiliana* extract with an absorbance range of 0.5841-1.1012 had higher ferric ion reducing power than *H. sabdariffa* (0.959 – 0.986) and vitamin c. This could be attributed to the higher total flavonoids (69,034 mg/kg quercetin equivalent) and flavonols (50,430 mg/kg quercetin equivalent) in *A. brasiliana* compared to the 57,810 mg/kg and 8,710 mg/kg quercetin equivalent for flavonoids and flavonols in *H. sabdariffa.* This indicated a higher ability of the *A. brasiliana* extract to reduce ferric ion than vitamin C which at 0.1 – 1 mg/ml had an absorbance range of 0.5 – 0.982. The ferric reducing power of the extracts was concentration dependent implying that they increased with increasing concentration of extracts.

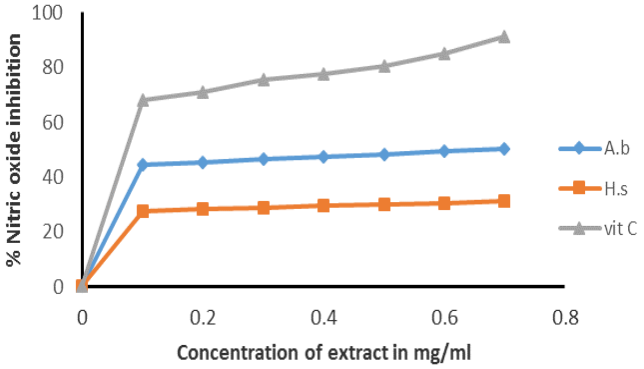


Figure 3. Nitric oxide scavenging activity of *A brasiliana* and *H. sabdariffa* extracts. A. b = *Alternanthera brasiliana extract,* H. s = *Hibiscus sabdariffa extract,* Vit C = Vitamin C

The nitric oxide radical scavenging activity of *A. brasiliana* and *H. sabdariffa* extracts (Figure 3) were high and dose-dependent, with $IC_{50}$ of 0.675 mg/ml and 3.976 mg/ml respectively. The nitric oxide scavenging activity of *A. brasiliana* and *H. sabdariffa* extract were lower than the $IC_{50}$ (0.322 mg/ml) of vitamin C. *A. brasiliana* extract with $IC_{50}$ of 0.675 mg/ml had a higher nitric oxide scavenging activity than *H. sabdariffa* calyces extract with $IC_{50}$ of 3.976 mg/ml. This could be attributed to its higher total flavonoid content (69,034 mg/kg quercetin equivalent); higher flavonols (50,430 mg/kg quercetin equivalent) content compared to the total flavonoid (57,810 mg/kg quercetin equivalent) and total flavonols (8,710 mg/kg quercetin equivalent) of *H. sabdariffa*. Brachado *et al.* (2003) identified six flavonoids in *A. brasiliana* leaf extract, out of which 3 inhibited lymphocyte proliferations. The high nitric oxide scavenging activity of the extracts suggests that the extracts be used in preventing inflammation, carcinomas

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and other diseases caused by nitric oxide radicals derived from cellular by-products as nitric oxide radical is known to play an important role in various inflammatory processes such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Hazra *et al.,* 2008).

## 4. Conclusion

The study has revealed that *A. brasiliana* leaves and *H. sabdariffa* calyces are good sources of vitamin $B_1$, $B_2$, $B_3$, and vitamin C as well as other health-promoting components such as flavonoids and phenols. The results implied that infants consuming these foods may have an improved appetite, a healthier nervous system and a higher release of energy from the complementary foods as well as be able to fight cancer better.

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