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Research article

Phytochemical contents, antioxidant activity and functional properties of *Raphanus sativus* L, *Eruca sativa* L. and *Hibiscus sabdariffa* L. growing in Ethiopia

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ABSTRACT

Information on phytochemical contents, antioxidant activity and functional properties of underutilized plants Figl (*Raphanus sativus* L.), Girgir (*Eruca sativa* L.) and Karkade (*Hibiscus sabdariffa* L.) grown in Benishangul Gumuz, Ethiopia are limited. In view of this, leaves and roots of Figl, leaves of Girgir, calyces and seeds of Karkade were evaluated following standard analytical methods. The total flavonoids, total anthocyanins, β-carotene and L-ascorbic acid contents were ranged: 5.28–35.97, 0.01–2.53, 0.15–0.42 and 0.28–1.49 (db mg/g), respectively. The total flavonoids content, total anthocyanins content and antioxidant capacity were high in the brown calyces of Karkade, but are low in the roots of Figl. The antioxidant activity of roots of Figl and seeds of Karkade were low. The effective inhibitory concentration (IC_{50}) toward 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity demanded from brown calyces of Karkade was low and the IC_{50} was strong negatively correlated with β-carotene and L-ascorbic acid contents (i.e., achieve IC_{50} with low amounts of these bioactive compounds). The ferric reducing antioxidant power was positively strong correlated with total flavonoids and anthocyanins content. The finding showed that calyces of Karkade can be used as a candidate to substitute synthetic antioxidants and food colorant in food, beverage and pharmaceutical industries because of their high antioxidant capacity, desired color and as a good source of phytochemicals. The study also showed that the leaves of Figl and Girgir were found to exhibit good sources of vitamin C, β-carotene with low bulk density. Because of these properties, they can be regarded as good candidate to supplement micronutrients particularly for vulnerable groups like infants and young children.

1. Introduction

Natural bioactive compounds from plants perform specific biological activities and modify different physiological functions to improve health of human being (Niaz et al., 2020). Utilization of these compounds has become widespread to minimize occurrence of common non-communicable diseases in adults. Plant-based foods contain many phytochemical compounds along with nutrients such as proteins, fats, carbohydrates, vitamins, and minerals (Narzary et al., 2016). Plant phytochemicals are potent antioxidants against reactive oxygen species and have several health benefits (Narzary et al., 2016). Numerous phytochemicals can be identified in plants food and a single plant can have more than thousand different phytochemicals (Chipurura et al., 2013). The level of phytochemicals in different commercial, indigenous and underutilized plants is different. Particularly underutilized plants reported from different countries are believed that they are potential sources of different types of health promoting bioactive compounds.

There are several indigenous underutilized plants in Ethiopia like okra (*Abelmoschus esculents*) (Gemede et al., 2018), Figl (*Raphanus sativus* L.), Girgir (*Eruca sativa* L.) and Karkade (*Hibiscus sabdariffa* L.) in the western (Keyata et al., 2020), moringa (*Moringa oliferain*) in southern part (Mikore and Mulugeta, 2017) and anchote (*Coccinia abyssinica)* in south western (Parmar et al., 2017) of the country. In western part of Ethiopia Figl, Girgir and Karkade have been known in their exceptional properties in terms of drought resistant, better yield and fast commercial maturity in for local consumption. In Benshangul Gumuz regional state close to border to Sudan, dried calyx of Karkade is commonly used to make hot and cold beverage. The leaves of Girgir are used as a vegetable

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whereas the leaves and roots of Figl are used in salad or cooked vegetable preparations.

Based on scientific report from other countries, calyces of Karkade is documented as rich in total phenolic, total flavonoid and total anthocyanins content with strong antioxidant potential (Shruthi et al., 2017). Mazzucotelli et al. (2018) and Sarikurkcu et al. (2017) indicated that leaves of Figl and Girgir were good sources of phenolic, flavonoids and antioxidants, respectively. However, so far, no scientific evidences are available to support the contents of these phytochemicals in these indigenous and underutilized plants in Ethiopia. Increased production and consumption of Figl, Girgir and Karkade could provide cheap sources of phytochemicals and antioxidants which are very important for health of the consumer and can also be used for diet diversification. It is also necessary to evaluate the functional properties of the plants parts to use as an ingredients in food formulation to different target groups. Therefore, this work aimed was at characterizing the three underutilized plants in terms of their phytochemical contents, antioxidant activity and functional properties for further production and commercialization opportunities to contribute for food and health security efforts of the country.

2. Materials and methods

2.1. Geographical location of sample purchased area

Sample collections of the seeds of Figl, Girgir, calyces and seeds of Karkade were purchased from Homesha, Kurmuk, and Sherkole districts of Assosa Zone, Benishangul-Gumuz Regional State, Ethiopia (Figure 1). The zones is located at 10.07°N and 34.53° E, at 1417 m above sea level and receives an average annual rainfall of 1316 mm and have an annual minimum and maximum temperature of 19 °C and 35 °C. The Figl and Girgir were cultivated in Jimma University, College of Agriculture and Veterinary Medicines's (JUCAVM) horticultural research farm, Oromia Regional State, Southwestern Ethiopia. Jimma is located at 352 km southwest of Addis Ababa, the Ethiopian capital city (Figure 1). Geographically, Jimma is located at 7°13′ and 8°56′ N latitude and 35°52′ and 37°37′E longitude. The area has an altitude ranging between 1720 and 2110 m above sea level with an annual rainfall ranging between 1200 and 2000 mm. The annual mean temperature ranges from 12 to 28 °C.

2.2. Sample collection and preparation

The seeds of Figl and Girgir were purchased from Assosa zone, Benishangul gumuz region, Ethiopia and then cultivated at Jimma University College of Agriculture and Veterinary Medicine research farm as per described by Keyata et al. (2020). The leaves and roots of Figl and leaves of Girgir were harvested when reach for commercial maturity. Uniform size and color of fresh mature leaves of Girgir, leaves and roots of Figl were carefully harvested, washed with distilled water, chopped/sliced using a stainless steel knife, and dried in oven (DHG-9203A, Shanghai, China) at 45 °C. Becaues of unfavorable climatic conditions in Jimma University for Karkade, the seeds and calyces were randomly collected from three districts of Assosa zone. The collected seeds were bulked and mixed thoroughly to make representative working samples. The dried roots, leaves, calyces and seeds were milled separately into flour using laboratory mill (RRH-200, Zhejiang, China), sieved (0.5 mm sieve size), packed in moisture and air proof heavy duty polyethylene

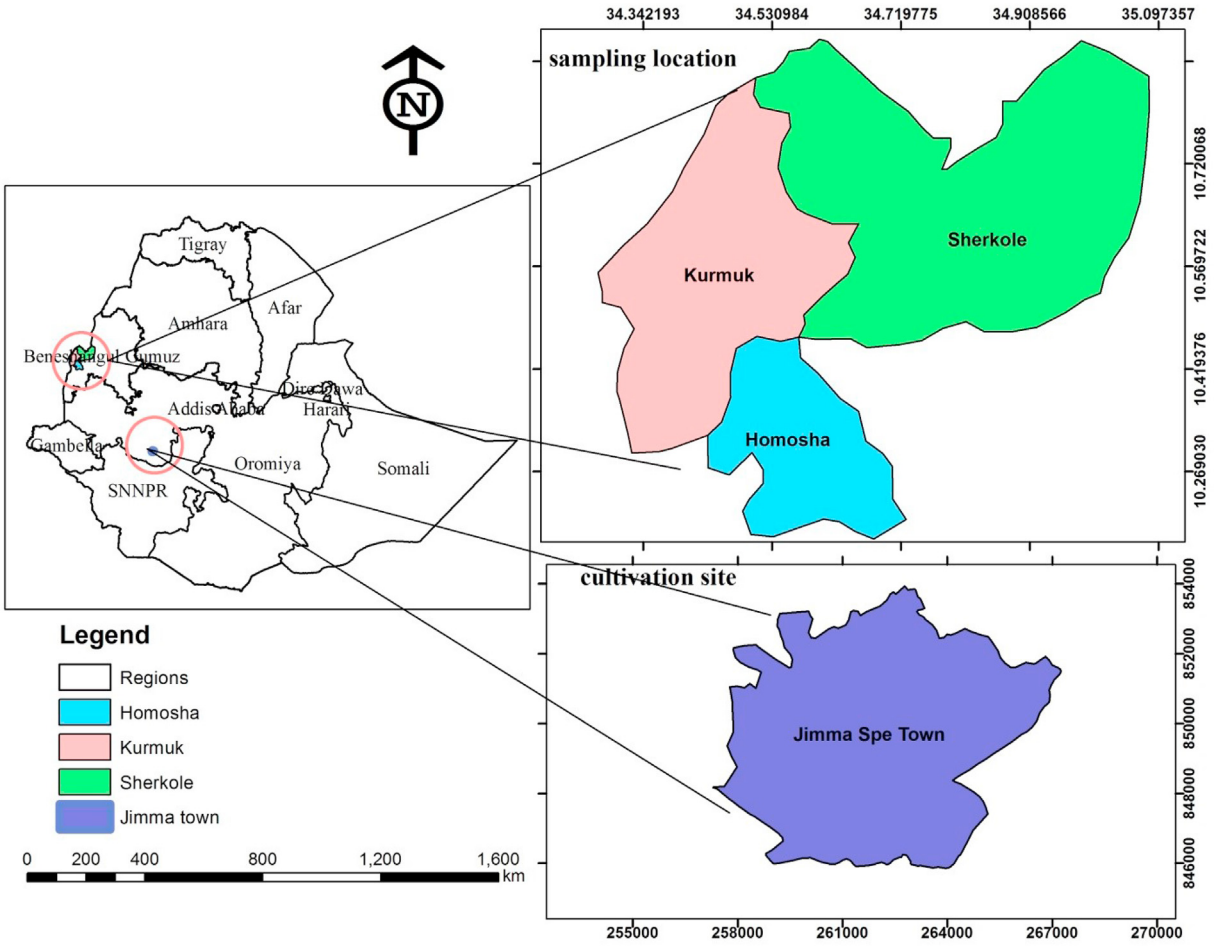

Figure 1. Map showing the sample locations and cultivation site of studied plants.

bag, wrapped with aluminum foil to exclude impact of light, and stored at -18 °C till analysed.

2.3. Preparation of the methanolic extract

Leaves and roots of Figl, leaves of Girgir, calyces and seeds of Karkade were extracted according to Handa et al. (2008). Ground sample (100 mg) were soaked in 100 mL of methanol (99.8%) to produce about 1 mg/mL of concentration using the maceration technique, soaking in the solvents for 24 h and shaking at ambient temperature and finally the extract sample was filtered using Whatman No. 1 filter paper. The filtered extract was used to determine the total flavonoids, total anthocyanins content and antioxidant capacity (DPPH and FRAP).

2.4. Phytochemical contents

2.4.1. Determination of flavonoid content

The total flavonoids content (TFC) was determined according to methods of Chang et al. (2002). One milliliter of the extract (1 mg/mL) mixed with 0.3 mL of 5% sodium nitrite followed by addition of 0.3 mL of 10% aluminum chloride after 5 min. Subsequently, after 6 min, 2 mL of 1-M sodium hydroxide then added, followed by the immediate addition of 2.4 mL of DW (distilled water) to produce a total volume of 10 mL. The color intensity of the flavonoids-aluminum complex was then measured at 510 nm using UV- VIS spectrophotometer. The total flavonoids content was determined as catechin equivalent (CE) (0.00, 6.25, 12.50, 25.00, 50.00 and 100.00 μg/mL, $R^2 = 0.992$) and was expressed as mg of CE/g.

2.4.2. Determination of total anthocyanins content

The total anthocyanins content was determined by pH dependence of color change of anthocyanins using spectrophotometric method (Lee et al., 2005). One mL of each sample extract was diluted to 10 mL of distilled water. One mL of the solution then diluted to 5 mL with acidic buffer pH 1.0 into test tubes (wrapped with aluminum foil). Another one mL of the sample solution was diluted to 5 mL with buffer pH 4.5. The mixture was allowed stand for 30 min at ambient temperature and then absorbance was measured, at 520 and 700 nm, using a UV-VIS spectrophotometer in 4 mL spectrophotometer glass cells. Results were expressed as equivalents of cyanidin-3-glucoside per g of sample (equation 1).

$$\text{CA (mg of cyanidin-3-glucoside per gram)} = \left(\frac{\text{A*MW*DF*V}}{\varepsilon\text{*L*W}}\right) \quad (1)$$

where, CA is the concentration of anthocyanin's, A is the absorbance difference of pH = 1 and pH = 4.5, A = [A520 nm - A700 nm]pH = 1 - [A520 nm - A700 nm] pH = 4.5), MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, V is the total volume of extract (mL), w is the weight of the sample used in the extraction (g), L is the quartz cuvette cell width (1 cm), ϵ is the coefficient of molar extinction for cyanidin-3-glucoside (26,900 L/mole-cm).

2.4.3. Beta (β) carotene content determination

Extraction of ß-carotene content was done as described in Sadler et al. (1990), with minor modifications. One gram of a sample flour was mixed with one gram $CaCl_2.2H_2O$ and 50 mL extraction solvent (50% hexane, 25% acetone, and 25% ethanol, containing 0.1% BHT) by shaking for 30 min at room temperature. After adding 15 mL of distilled water, the solution was frequently mixed by shaking for a further 15 min. The organic phase, containing the β-carotene was separated from the water phase, using a separation funnel, and filtered using Whatman filter paper No.1. The extraction procedure was conducted under subdued light to avoid degradation ofcarotenoids. The stock β-carotene (Sigma Aldrich from USA) standard solution was made by dissolving accurately weighed 0.01 g β-carotene in the solvent (50% hexane, 25% acetone, and 25% ethanol) used to extract samples and made the volume to one hundred milliliter using the same solvent. From stock solution, series of standard solutions (0.1, 0.2, 0.4, 0.6, 0.8 and 1 μg/mL, $R^2 = 0.994$) were used to construct calibration line from which β -carotene was estimated and expressed in mg/g. The absorbance of the sample extract and β-carotene standard solutions was measured at 450 nm wavelength using UV-Vis spectrophotometer.

2.4.4. Determination of L-ascorbic acid content

The L-ascorbic acid content was determined by 2,6-dichloroindophenol titration methods according to AOAC (2005) method 967.21. About 0.1 g flour sample was extracted with 40 mL of 15 g of metaphosphoric acid (HPO_3), mixed with 40 mL of acetic acid (Ac) in 500 mL of deionized H_2O. The extracted sample was filtered using Whatman filter paper No.1. The filtrated sample was titrated by using indophenol standard solution made by dissolving 50 mg of 2,6-dichloroindophenol sodium salt and 42 mg of $NaHCO_3$ to 200 mL with deionized water to a light but distinctive rose-pink end point lasting ≥ 5 s. The standard solution of L-ascorbic acid was prepared by taking about 50 mg of L-ascorbic acid into 50 mL of HPO_3- Ac extracting solution. The L-ascorbic acid content was calculated according to Eq. (2).

$$\text{L-Ascorbic } (mg/g) = \frac{(A-B)*C*40}{10S} \quad (2)$$

where: A = volume in mL of the 2,6-dichloroindophenol sodium salt solution used for the sample.

B = volume in mL of the 2,6-dichloroindophenol sodium salt solution used for the blank.

C = mass in mg of L-ascorbic acid equivalent to 1.0 mL of standard indophenol solution.

S = weight of sample taken (g).

40/10: 40 = volume of extract & 10 = volume of extract used for the determination.

2.5. Total antioxidant activity

2.5.1. DPPH free radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity of the methanolic extract of the sample was estimated as described in Kirby and Schmidt (1997) with minor modification. A 0.004% solution of DPPH (Sigma Aldrich from Germany) radical solution in methanol was prepared and then 4 mL of this solution was mixed with 1 mL of various concentrations (0.20–0.56 mg/mL) of the sample extracts in methanol (99.8%). The samples were incubated for 30 min in the dark at ambient temperature. Radical scavenging capacity was measured using UV-Vis spectrophotometer by monitoring the decrease in absorbance at 517 nm (AS). The absorbance of freshly prepared DPPH was used as control (blank) solution (AC). Butyl hydroxytoluene (BHT) and L-ascorbic acid were used as the positive control. Percent inhibition of free radical DPPH was estimated according to Eq. (3). The extract concentration that provides 50% of radical scavenging activity (IC_{50}) was calculated from the graph continuation of DPPH (percentage of inhibition versus extract concentration) (Buritsand Bucar, 2000).

$$\text{Inhibition}(\%) = \left(\frac{\text{AC} - \text{AS}}{\text{AC}}\right)*100 \quad (3)$$

AC = Absorbance of control (blank) solution; AS = Absorbance of sample extract solution.

2.5.2. Ferric reducing antioxidant power (FRAP)

The FRAP of sample extracts were determined as described by Dudonne et al. (2009) and with slight modification. The assay was based on the reducing power of antioxidant compound present in the sample extract having a potential of reducing colorless of the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), latter forms a blue complex (Fe^{2+}/TPTZ), which

increases the absorption at 593 nm. The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at a ratio of 10:1:1 (v/v/v). One hundred μL of samples extract (2 mg/mL) was added to 3 mL of prepared FRAP reagent and mixed thoroughly on a vortex mixer. The tube with its content was kept in the dark at ambient temperature and absorbance was read at 593 nm after 30 min by using UV- VIS spectrophotometer. FRAP values were expressed in terms of mM Fe^{2+}/g of sample using $FeSO_4.7H_2O$ standard curve (0.00, 0.28, 0.56, 0.84, 1.12 and 1.40 μg/mL, $R^{2=}$ 0.991).

2.6. Functional properties of the flours

2.6.1. Bulk density

Bulk density (BD) was determined according to the method stated by Gupta et al. (2015). About one gram of the powder sample was placed in 10 mL test tube by constant tapping until there was no further change in volume. The final bulk volume was recorded. Bulk density was then calculated as the weight of sample powder (g) divided by its final volume (mL) using Eq. (4).

$$\text{Bulk } (g/mL) = \frac{\text{weight}}{\text{volume}} \quad (4)$$

2.6.2. Water and oil absorption capacity

Water and oil absorption capacities of the samples were determined according to procedure described by Beuchat (1977) with slight modifications. About 1 g of flour sample was mixed with 10 mL of distilled water or oil in a pre-weighed 50 mL tube. The suspension was agitated for 1 h on a mechanical shaker (Hy-2(C), Shanghai, China) after which it was centrifuged (Sigma 2-16KC, UK) at 3500 rpm for 30 min. The separated water or oil was then removed with a pipette and the residues with the amount of oil or water retained were re-weighed. The water or oil absorption capacity was expressed as grams of water or oil absorbed per gram of the sample.

2.6.3. Solubility and swelling power

Swelling power and solubility were determined as described in Oladele and Aina (2007) with slight modification. About 0.35 g flour was mixed with 12.5 mL of DW (distilled water) in screw cap tube and heated at 60^{o}C for 30 min in a thermostatically controlled water bath. The tube was removed from the bath, wiped dry, cooled to room temperature and centrifuged for 20 min at 2500 rpm to separate gel and supernatant. The aqueous supernatant was removed and transferred into a tarred evaporating dish. The transferred supernatant was dried in air oven at 100 °C for 4 h and the dried residue was weighed to determine the solubility (equation 5).

$$\text{Solubility}(\%) = \left(\frac{\text{weight of dried sample in supernatant}}{\text{weight of original sample}}\right) * 100 \quad (5)$$

The swollen sample (paste) obtained from decanting the supernatant was weighed to determine the swelling power (equation 6).

$$\text{Swelling power}(g/g) = \left(\frac{\text{weight of wet mass of sediment}}{\text{weight of dry matter in gel}}\right) \quad (6)$$

2.7. Statistical analysis

All the statistical analyses were performed using SAS version 9.3 and significance difference was considered at $p \leq 0.05$. *Fisher's* least significant difference (LSD) was used for mean comparison tests to identify significant differences among means. The result was reported as a mean ± standard deviation (SD). Correlation between the phytochemical contents and antioxidant activity were conducted using the Pearson's correlation method.

3. Results and discussion

3.1. Phytochemical contents

The results of phytochemical contents such as total flavonoids, total anthocyanins, β-carotene, and L-ascorbic acid contents are given in Table 1.

Among the seven edible plant parts studied, brown calyces of Karkade had significantly ($p < 0.05$) highest amount of total flavonoids content (TFC). Similar results were reported by Shruthi et al. (2017). The TFC content found from the seeds of Karkade was higher than *Hibiscus cannabinus* seed extracts (1.64 to 2.96 mg/g) (Yusri et al., 2012). The main variation might be due to nature of crop, growing condition, solvent and method used during extraction. The TFC in the leaves of Figl and Girgir were comparable with TFC reported for the leaves of *Moringa oleifera* (9.9 mg/g) (Geetha et al., 2018). The TFC of roots of Figl was comparable with TFC reported for wild root vegetables (*Achyranthes aspera* L, *Eclipta alba* L and *Vitex negundo* L). Therefore, the finding showed that calyces of Karkade, and leaves of Figl and Girgir can be used as an important ingredient during food and beverage formulation due to their rich sources of bioactive compounds with potential to impact on the nutrition and health of consumers.

The result indicated that there was a significant difference ($p < 0.05$) in the total anthocyanins content (TAC) between brown and brown-red calyces of Karkade. The finding showed significant ($p < 0.05$) difference in the TAC between brown and brown red calyces of Karkade. But, there was no significant difference ($p > 0.05$) in the TAC between leaves and roots of Figl, leaves of Figl and Girgir, brown and brown red Karkade seeds. This might be because of presence of coloring compounds if calyces of Karkade as compared to other edible plant parts. The calyces of Karkade had the highest TAC when compared with other studied edible plant parts. The TAC in brown calyces of Karkade was similar with the same edible plant parts grown in India (2.8 mg/g) (Zaman et al., 2017). The TAC in the seeds of Karkade was comparable with the TAC in soybean black seeds (0.19–14.20 mg/g) (Lee et al., 2016). The leaves of Figl and Girgir were similar in the TAC with leaves of *Rutachalepensis* (0.28 mg/g) reported by Ereifejet al. (2015). The TAC in the leaves of Figl and Girgir was similar with the TAC from the leaves of *Ruta chalepensis* (0.28 mg/g) (Ereifej et al., 2015). The TAC found in the white roots of Figl was lower than red roots of radish (Figl) (0.30 mg/g) reported by Khedr and El Sheikh (2016). This might be due to somewhat reddish pigments nature of the red roots of radish as compared to the sample used in this study. The finding suggested that calyx of Karkade can be used as an excellent potential source of dietary polyphenol compounds and as a natural colorant for food and pharmaceutical industries.

The β-carotene contents in the leaves of Figl and Girgir were significantly ($p < 0.05$) greater than the β-carotene contents found in the roots of Figl, calyces and seeds of Karkade in this study, but was similar to the β-carotene contents reported from leaves of *Moringa oleifera* (0.39 mg/g) (Djuikwo et al., 2011). The β-carotene content found in this study was higher than the values reported for leaves of radish (Figl) (0.25–0.31 mg/g) dried at 50–90 °C (Ankita, 2015). This might be in part contributed from difference in the drying temperature used (i.e., in this study dried at 45 °C). The roots of Figl were lower in the β-carotene content than the roots of carrot (0.32 mg/g) (Tiwari and Sarkar, 2018). This could be due to carrot root contains the major pigments like orange and yellow that serve as an intermediate product in the carotenoids path (Koch and Goldman, 2005). The β-carotene contents found from seeds of Karkade were similar to the value reported for brown grain teff flour (0.19 mg/g) (Mezgebo et al., 2018). The β-carotene contents found in the calyces of Karkade were similar with the value reported for the same edible plant part reported from Nigeria (0.28 mg/g) (Chinatu et al., 2016).

The difference in L-ascorbic acid contents between leaves and roots of Figl and among leaves of Girgir, calyces, and seeds of Karkade was statistically significant ($p < 0.05$). In terms of the L-ascorbic acid contents,

Table 1. Phytochemical (total flavonoids, total anthocyanin, β-carotene and L-ascorbic acid) contents of edible parts of Figl, Girgir and Karkade (mg/g db).

Edible part	Total flavonoids	Total anthocyanin	β- carotene	L-ascorbic Acid
Leaves Figl	11.76 ± 1.71^{c}	0.22 ± 0.02^{cd}	0.42 ± 0.02^{a}	1.22 ± 0.10^{b}
Roots Figl	5.28 ± 0.70^{d}	0.01 ± 0.01^{d}	0.21 ± 0.02^{e}	0.69 ± 0.10^{e}
Leaves Girgir	14.03 ± 1.13^{b}	0.25 ± 0.02^{c}	0.36 ± 0.01^{b}	1.49 ± 0.07^{a}
Brown calyces Karkade	35.97 ± 1.47^{a}	2.53 ± 0.04^{a}	0.25 ± 0.02^{d}	1.09 ± 0.07^{c}
Brown red calyces Karkade	13.13 ± 1.39^{bc}	1.31 ± 0.10^{b}	0.33 ± 0.02^{c}	0.95 ± 0.06^{d}
Brown seeds Karkade	6.39 ± 0.58^{d}	0.15 ± 0.01^{de}	0.16 ± 0.02^{f}	0.38 ± 0.02^{f}
Brown red seeds Karkade	5.41 ± 0.65^{d}	0.13 ± 0.02^{e}	0.15 ± 0.02^{f}	0.28 ± 0.02^{f}

Means with different letters across a column are significantly different ($p < 0.05$).

leaves of Figl and Girgir was better than other plants part studied (roots of Figl, calyces and seeds of Karkade), but with similar values what reported from the leaves of *Moringa oleifera* (1.35 mg/g) (Mathiventhan and Ramiah, 2015). The L-ascorbic acid contents found from the roots of Figl were two times lower than the value reported from the roots of radish (Figl) from Pakistan (1.23 mg/g) (Khattak and Rahman, 2017). The variation may be due to the roots of Figl grown in Pakistan that were dried in the shade which probably reduce the L-ascorbic acid loss, since L-ascorbic acid is heat sensitive. The L-ascorbic acid contents found in the calyces of Karkade was comparable with the calyces of Karkade from Indonesia (0.81–0.85 mg/g) (Muslihatin et al., 2015).

3.2. In vitro antioxidant activity

Antioxidants are substances that prevent an autoxidation process of other compounds or neutralize free radicals and widely used in food processing industries to limit oxidation, enhance flavor, aroma and color (Kebede and Admassu, 2019). In line to this assertion, the percent of DPPH at different concentration (0.20–0.56 mg/mL) for leaves and roots of Figl, leaves of Girgir, calyces and seeds of Karkade were evaluated (Figure 2), and %DPPH,.inhibition concentrations (IC_{50}) of DPPH scavenging and FRAP are given in Table 2.

Among concentration used for IC_{50} calculation (0.20, 0.33, 0.43, 0.50 and 0.56 mg/mL), the medium concentration (0.43 mg/mL) was selected to indicate the potential of scavenging activities. Additional medium concentration also selected to balance between lower and higher concentration and maximum and minimum scavenging activities of free radical by samples extract. The result revealed that roots of Figl had significant ($p < 0.05$) different IC_{50} value and percentage of inhibition of free radicals when compared to the leaves of Figl, leaves of Girgir, calyces and seeds of Karkade. The result showed that calyces of Karkade had a better DPPH scavenging ability with low IC_{50} value when compared to the extract used from leaves of Girgir, leaves and roots of Figl and seeds of Karkade. The roots of Figl were lower in its percent of DPPH scavenging ability with high IC_{50}. Specifically at 0.43 mg/mL concentration, calyces of Karkade contain high in % DPPH (78.85–79.18%) with low IC_{50} (0.139–0.145) and also high in ferric reducing antioxidant power (FRAP) (392.11–680.89 $mMFe^{2+}/g$) at 2 mg/mL of concentration.

The result also indicated that as concentration of the plant extracts increased the percentage of free radical scavenging activities increased from 0.2 to 0.56 mg/mL (Figure 2). Specifically, at 0.43 mg/mL concentration, calyces of Karkade showed high percentage DPPH (78.85–79.18%) with low IC_{50} (0.139–0.145) and high FRAP (392.11–680.89 $mMFe^{2+}/g$ at 2 mg/mL of concentration). The calyces of Karkade extract was also showed highest free radical scavenging effect when compared to synthetic antioxidants BHT (76.07%), but was lower than L-ascorbic acid (84.21%). This is attributed to the existence of highest total flavonoids and total anthocyanins content in the calyces of Karkade extract. The high antioxidant activities of calyces of Karkade may convey novel natural products for the food industry with safer and improved antioxidants that has potential for protection against the oxidative damage, which occurs both in the body and our daily diets. In

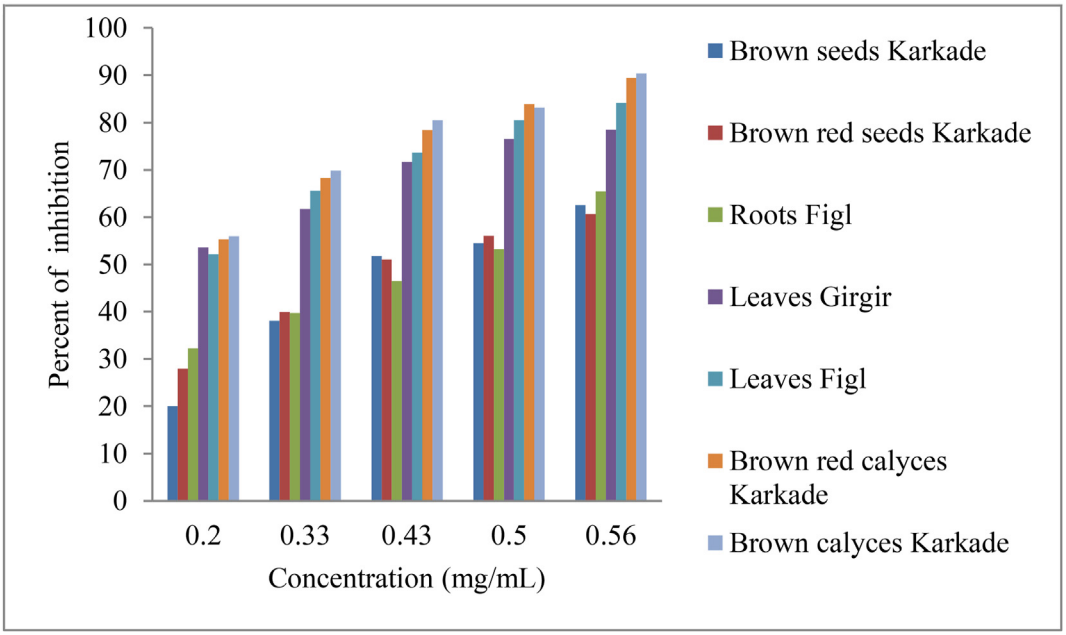

Figure 2. Free radical scavenging activities of methanolic extract of edible of Figl, Girgir and Karkade

Table 2. Total antioxidant capacity and IC_{50} values of edible parts of Figl, Girgir and Karkade

Edible parts	IC_{50} (mg/mL)	%DPPH(0.43 mg/mL)	FRAP (mM of Fe^{2+}/g)
Leaves Figl	0.16 ± 0.02^{c}	73.66 ± 0.94^{ed}	194.27 ± 3.95^{c}
Roots Figl	0.45 ± 0.02^{a}	46.50 ± 0.67^{g}	78.22 ± 7.15^{e}
Leaves Girgir	0.15 ± 0.01^{dc}	71.70 ± 1.69^{e}	123.16 ± 8.22^{d}
Brown calyces Karkade	0.14 ± 0.01^{d}	79.18 ± 1.46^{b}	680.89 ± 15.06^{a}
Brown red calyces Karkade	0.150 ± 0.004^{d}	78.85 ± 1.44^{bc}	392.11 ± 6.55^{b}
Brown seeds Karkade	0.44 ± 0.01^{ba}	51.78 ± 1.66^{f}	$113.00 + 10.82^{d}$
Brown red seeds Karkade	0.430 ± 0.004^{b}	51.01 ± 0.81^{f}	125.89 ± 5.50^{d}
BHT	0.010 ± 0.003^{e}	76.07 ± 0.97^{cd}	ND
L-Ascorbic Acid	0.002 ± 0.001^{e}	84.21 ± 0.80^{a}	ND

ND: Not determined. Means with different letters across a column are significantly different.

contrary, white roots of Figl had relatively low DPPH scavenging ability (46.50%) with high IC_{50} (0.45 mg/mL) values and low FRAP (78.22 mM of Fe^{2+}/g) at the same concentration. This result was similar with the percentage of free radical scavenging activity difference obtained between white roots and red roots of radish (Figl) from Iraq (Rathi et al., 2019). This could be because of the limited pigmented compounds in the white roots and also the TFC and TAC were obtained to be low.

3.3. Correlation between antioxidant capacity and phytochemicals

The correlation results between antioxidants and phytochemicals contents evaluated for the seven edible parts in this study are shown in Table 3. The correlation strengths were classified according to Evans (1996) as: r = 0.00–0.19: "very weak", r = 0.20–0.39: "weak", r = 0.40–0.59: "moderate", r = 0.60–0.79: "strong", r = 0.80–1.0: "very strong". The IC_{50} of DPPH had a significant ($p < 0.05$) and very strong negative correlation with the β-carotene (r = -0.83) and L-ascorbic acid (r = -0.87) contents because the higher these chemical concentration the lower the concentration amount required to achieve IC_{50}. The total flavonoids and total anthocyanins content in this study had a highly significant ($p < 0.01$) and very strong positive correlation with their FRAP values (r = 0.92; r = 0.99, respectively). This finding suggests that total anthocyanin and total flavonoids compounds are the major providers in their antioxidant activities of FRAP. The IC_{50} of DPPH had a significant ($p < 0.05$) and very strong negative correlation with β-carotene (r = - 0.83) and L-ascorbic acid (r = -0.87) contents because the higher these chemical concentration the lower amount concentration are required to achieve IC_{50}.

3.4. Functional properties

Functional properties are the primary physical properties that reflect the complex interaction between the composition, structure, molecular conformation and physical properties of food components together with the nature of environment in which these are associated and measured (Siddiq et al., 2009). The functional properties (bulk density, water/oil absorption capacity, solubility and swelling power) for leaves and roots of Figl, leaves of Girgir, calyces and seeds of Karkade evaluated in this study are shown in Table 4.

Bulk density (BD) indicates flour heaviness, type of packaging material to be used to handle and transport products (Abe-Inge et al., 2020). It is also helps to choose target customer groups since food products of with high BD is not suitable for children and infants. In this study, there was no significant difference ($p > 0.05$) in the BD between calyces of Karkade and leaves of Figl. The roots of Figl had the highest BD as compared to edible leaves of Figl, leaves of Girgir, calyces and seeds of Karkade, but were similar to the finding reported for tuber of untreated of sweet potato (0.8 g/mL) reported by Ngoma et al. (2019). A higher bulk density is desirable for a greater ease of dispersibility and a reduction of paste thickness. In view of this, the high bulk density contents of roots of Figl indicates that they would serve as a potential to prepare thickener food products in food industry, but might not suitable to formulate children food. The BD of leaves of Girgir was similar to leaves of spinach powder BD (0.41 g/mL) (Ankita, 2013), but was higher when compared to leaves BD of Figl. Osundahunsi and Aworh (2002) reported that the lower BD implies less quantity of the food samples which could be packaged in a constant volume, ensuring an economical packaging and could be used to formulate children diets to exploit their potential benefits. The finding showed leaves of Figl with low BD would allow incorporation of other additives like fat and protein rich ingredients which are important in the formulation of complementary foods for children. The BD value for Karkade seeds were similar with the BD reported for the same crop from Sudan (0.50 g/mL) (Salah and Hayat, 2009).

The water absorption capacity (WAC) is an indicator of the maximum amount of water that a food product could absorb and retain (Ijarotimi and Keshinro, 2012). Among the samples in this study, roots of Figl had the highest significance ($p < 0.05$) difference in WAC. This result was also two times higher than for roots of carrot flours (5.43 g/g) and beetroots (5.04 g/g) reported (Sahni and Shere, 2017). The variation may be because of mainly greater number of hydroxyl groups which exist in the fiber structure and allow more water interaction through hydrogen bonding (Adegunwa et al., 2017). The WAC of leaves of Figl and Girgir were higher than reported for moringa leaf powder (3.17 g/g) (Mune et al., 2016). This might be due to different nature of the leaves plant, location and time of harvesting. The WAC for the seeds of Karkade were similar with WAC of Karkade seeds reported by Suliman et al. (2017) (2.95 g/g). The result indicated that the seeds of Karkade flour contain

Table 3. Correlation between phytochemical contents (total flavonoids, total anthocyanin, β-carotene and L-ascorbic acid) and antioxidant activity.

Phytochemical parameters	Antioxidant activities		
	%DPPH(0.43 mg/mL)	IC50 of DPPH	FRAP
Total flavonoids content	0.72 (0.07)	-0.67 (0.10)	0.92 (<0.01)
Total anthocyanins content	0.64 (0.12)	-0.54 (0.20)	0.99 (<0.01)
β-carotene content	0.75 (0.05)	-0.83 (0.02)	0.16 (0.73)
L-ascorbic acid content	0.78 (0.04)	-0.87 (0.01)	0.33 (0.48)

Numbers between brackets correspond to the p-value of the Pearson test.

Table 4. Bulk density (BD), oil absorption capacity (OAC), water absorption capacity (WAC), swelling power (SP) and solubility (SO) functional properties of edible parts of Figl, Girgir and Karkade (db).

Edible parts	BD (g/mL)	OAC(g/g)	WAC(g/g)	SP(g/g)	SO (%)
Leaves Figl	0.20 ± 0.02^{d}	3.48 ± 0.11^{b}	6.80 ± 0.27^{c}	7.77 ± 0.11^{d}	43.51 ± 0.93^{a}
Roots Figl	0.77 ± 0.05^{a}	5.50 ± 0.40^{a}	10.53 ± 0.37^{a}	11.37 ± 0.43^{a}	46.12 ± 1.80^{a}
Leaves Girgir	0.37 ± 0.01^{c}	3.71 ± 0.13^{b}	5.31 ± 0.17^{d}	8.81 ± 0.36^{c}	43.34 ± 4.24^{a}
Brown calyces Karkade	0.19 ± 0.02^{d}	2.85 ± 0.10^{c}	8.37 ± 0.23^{b}	9.91 ± 0.04^{b}	34.75 ± 1.49^{b}
Brown red calyces Karkade	0.17 ± 0.02^{d}	2.75 ± 0.04^{dc}	7.94 ± 0.44^{b}	9.32 ± 0.81^{bc}	34.08 ± 1.17^{b}
Brown seeds Karkade	0.53 ± 0.02^{b}	2.86 ± 0.02^{c}	2.79 ± 0.07^{e}	3.62 ± 0.08^{e}	21.76 ± 0.04^{c}
Brown red seeds Karkade	0.55 ± 0.01^{b}	2.46 ± 0.15^{d}	3.20 ± 0.17^{e}	3.56 ± 0.04^{e}	15.40 ± 2.10^{d}

Means with different letters across a column are significantly different.

comparatively low water absorption capacity than the leaves and roots of Figl, leaves of Girgir and calyces of Karkade in this studied. This shows the lower WAC is vital in the baby food formulations with high energy density per unit volume (Omueti et al., 2009).

Oil absorption capacity (OAC) is the physical entrapment of oil in foods, especially by proteins which plays a major role in flavor retention by interacting with hydrophobic groups of flavors and fat-soluble nutrient compounds and trapping them in the food matrix (Chandra and Samsher, 2013). The result reveals that roots of Figl had significantly ($p < 0.05$) high OAC followed by leaves of Girgir and Figl. This result is also higher than the value reported (Sahni and Shere, 2017) for roots of carrot (2.44 g/g) and beetroots (2.21 g/g). A high OAC in the flours of roots of Figl is valuable in the preparation of doughnuts, pancakes and baked foods (Naiker et al., 2019). The OAC for leaves of Figl and Girgir were more than two times higher than the OAC of *Moringa olifeira* leaves flour (1.46 g/g) (Alain et al., 2016). This might be because of the studied edible plant may contains different nature and the existence of non-polar side chain that may bind hydrocarbon chain between the oil and flours (Jan et al., 2015). The OAC reported in seeds of Karkade was five times lower than for Karkade seed reported from Malaysia (Nyam et al., 2014). This probably could be due to protein content found in the seeds of Karkade shows lower hydrophobic properties (Choonhahirun, 2010).

Swelling power (SP) is an indication of the hydration capacity of flour and is measured as weight of water occluded by swollen flour components when flour sample is soaked in water (Ocloo et al., 2010). The data in this study showed that roots of Figl had the highest SP when compared to the leaves of Figl and Girgir, calyces and seeds of Karkade of studied plants. The higher value of SP recorded in roots of Figl is probably due to higher amount of soluble component like for example from starch granules and hydrophilic fiber that are expected to bind sufficient amount of water and thereby contributes to increased swelling property (Dhankhar et al., 2019). The SP result found for the roots of Figl was similar with roots of cassava flour (13.80 g/g) (Kusumayanti et al., 2015). The SP of leaves of Figl and Girgir were similar to the SP reported for leaves of Amaranthus cultivar (6–8 g/g) (Kong et al., 2009). The seeds of Karkade were comparable with the SP of chickpea flour (4.7 g/g) reported by Dhankhar et al. (2019).

Water solubility can be an indication of the strength of interaction between starch chains with in the amorphous and crystalline domains (Kumoro et al., 2012). On dry matter basis (db) no significant difference ($p < 0.05$) was showed in water solubility of leaves and roots of Figl and leaves of Girgir. Relatively the highest solubility was recorded in roots of Figl when compared to leaves of Figl and Girgir, calyces and seeds of Karkade studied. This is probably due to the weak non-covalent bonding forces between molecules within the flours (Aryee et al., 2006). This result was similar with dried roots flour of anchote (*Coccinia Abyssinica*) (41.55–48.10%) (Shebabaw, 2013). The water solubility of leaves of Figl and Girgir was comparable with leaves of spinach flour (41.65%) (Ankita, 2015). The water solubility from seeds of Karkade flour (15.40–21.76%) were comparable with solubility of mung bean flour (18.80 g/g) and red bean flour (24.21 g/g) (Ratnawati et al., 2019).

4. Conclusions

Exploitation of underutilized plants for food and medicinal properties are important for food and nutrition security and for future sustainability of foods system. In this study, leaves and roots of Figl, leaves of Girgir, calyces and seeds of Karkade for phytochemical, antioxidant capacity and functional properties were studied. The finding showed that calyces of Karkade can be used as a candidate to substitute synthetic antioxidants and food colorant in food, beverage and pharmaceutical industries due to their desired color, high antioxidant capacity and as a good source of phytochemicals. The study also indicated that the leaves of Figl and Girgir were found to exhibit good sources of vitamin C, β-carotene with low BD, because of these properties they can be regarded as good candidate to supplement micronutrients particularly for vulnerable groups like infants and young children. However, flour of roots of Figl with high BD and oil holding capacity an be used as a potential to prepare thickener food products in food industries. Therefore, these underutilized plants can offer a great potential in various food applications in Ethiopia and elsewhere in the world.

Declarations

Author contribution statement

Ebisa Olika Keyata: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yetenayet B. Tola, Geremew Bultosa, Sirawdink Fikreyesus Forsido: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Abe-Inge, V., Asaam, E.S., Agbenorhevi, J.K., Bawa, N.M., Kpodo, F.M., 2020. Development and evaluation of African palmyra palm (*Borassusaethiopum*) fruit flour–wheat composite flour noodles. Cogent Food & Agriculture 6 (1), 1749216.

Adegunwa, M., Adelekan, E., Adebowale, A., Bakare, H., Alamu, E., 2017. Evaluation of nutritional and functional properties of plantain (*Musa paradisiaca L.*) and tigernut (*Cyperus esculentus L.*) flour blends for food formulations. Cogent Chemistry 3 (1), 1–15.

Alain, M.M.M., Nyobe, E.C., Bakwo Bassogog, C., Minka, S.R., 2016. A comparison on the nutritional quality of proteins from *Moringa oleifera* leaves and seeds. Cogent Food and Agriculture 2 (1), 1213618.

Ankita, P.K., 2013. Studies on spinach powder as affected by dehydration temperature and process of blanching. Int. J. Agric. Food Sci. Technol. 4 (4), 309–316.

Ankita, K.P., 2015. Characterization of dehydrated functional fractional spinach powder. Indian J. Biotechnol. 11 (11), 426–435.

AOAC, 2005. International (2007) Official methods of analysis, 2005. In: Current through Revision 2, 2007 (On-Line), eighteenth ed. AOAC International, Gaithersburg, MD. Official method 967.21.

Aryee, F.N.A., Oduro, I., Ellis, W.O., Afuakwa, J.J., 2006. The physicochemical properties of flour samples from the roots of 31 varieties of cassava. Food Contr. 17 (11), 916–922.

Beuchat, L., 1977. Functional and electrophoretic characteristics of succinylated peanut flourprotein. J. Agric. Food Chem. 25, 258–261.

Burits, M., Bucar, F., 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res. 14, 323–328.

Chandra, S., Samsher, L., 2013. Assessment of functional properties of different flours. Afr. J. Agric. Res. 8 (38), 4849–4852.

Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., 2002. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10 (3), 178–182.

Chinatu, L.N., Akpan, A.U., Echereobia, C.O., Harriman, J.C., 2016. Assessment of chemical composition of flower, variability, heritability and genetic advances in *Hibiscus sabdariffa*. J. Biol. Gen. Res. 2 (2), 15–23.

Chipurura, B., Muchuweti, M., Kasiyamhuru, A., 2013. Wild leafy vegetables consumed in Buhera district of Zimbabwe and their phenolic compounds content. Ecol. Food Nutr. 52 (2), 178–189.

Choonhahirun, A., 2010. Proximate composition and functional properties of pra (*Elateriospermun tapos Blume*) seed flour. Afr. J. Biotechnol. 9 (36), 5946–5949.

Dhankhar, J., Vashistha, N., Sharma, A., 2019. Development of biscuits by partial substitution of refined wheat flour with chickpea flour and date powder. J. Microbiol. Biotechnol. Food Sci. 8 (4), 1093–1097.

Djuikwo, V.N., Ejoh, R.A., Gouado, I., Mbofung, C.M., Tanumihardjo, Sh.A., 2011. Determination of major carotenoids in processed tropical leafy vegetables indigenous to Africa. Food Nutr. Sci. 2, 793–802.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., Mérillon, J., 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. 57 (5), 1768–1774.

Ereifej, K.I., Feng, H., Rababah, T., Almajwal, A., Alu'datt, M., Gammoh, S.I., Oweis, L.I., 2015. Chemical composition, phenolics, anthocyanins concentration and antioxidant activity of ten wild edible plants. Food Nutr. Sci. 6 (7), 581–590.

Evans, J.D., 1996. Straight Forward Statistics for the Behavioral Sciences. Thomson Brooks/Cole Publishing Co.

Geetha, K., Savita, H., Shivaleela, H., 2018. Effect of cooking on total antioxidant activity, polyphenols and flavonoids content in commonly consumed vegetables. Int. J. Curr. Microbio. Appl. Sci. 7 (2), 1459–1466.

Gemede, H.F., Haki, G.D., Beyene, F., Rakshit, S.K., Woldegiorgis, A.Z., 2018. Indigenous Ethiopian okra (*Abelmoschus esculentus*) mucilage: a novel ingredient with functional and antioxidant properties. Food Sci. Nutr. 6 (3), 563–571.

Gupta, S., Parvez, N., Sharma, P.K., 2015. Extraction and characterization of *Hibiscus rosasinensis* mucilage as pharmaceutical adjuvant. World Appl. Sci. J. 33 (1), 136–141.

Handa, S., Khanuja, S., Longo, G., Rakesh, D., 2008. Extraction Technologies for Medicinal and Aromatic Plants. United Nations Industrial Development Organization and the International Centre for Science and High Technology, p. 260.

Ijarotimi, O.S., Keshinro, O.O., 2012. Formulation and nutritional quality of infant formula produced from germinated popcorn, Bambara groundnut and African locust bean flour. J. Microbiol. Biotechnol. Food Sci. 9 (4), 1358–1388.

Jan, U., Gani, A., Ahmad, M., Shah, U., Baba, W.N., Masoodi, F.A., Wani, S.M., 2015. Characterization of cookies made from wheat flour blended with buckwheat flour and effect on antioxidant properties. J. Food Sci. Technol. 52 (10), 6334–6344.

Khattak, K.F., Rahman, T.U., 2017. Analysis of vegetable's peels as a natural source of vitamins and minerals. Int. Food Res. J. 24 (1), 292–297.

Khedr, A., El Sheikh, N., 2016. Antidiabetic and antiatherosclerotic activity of dried red radish roots (*Raphanus sativus L*) on hypercholesterolemic diabetic rats. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt 47 (2), 1–31.

Kebede, M., Admassu, S., 2019. Application of antioxidants in food processing industry: options to improve the extraction yields and market value of natural products. Adv. Food Techn. Nut. Sci. Open J. 5 (2), 38–49.

Keyata, E.O., Tola, Y.B., Bultosa, G., Forsido, S.F., 2020. Proximate, mineral, and anti-nutrient compositions of underutilized plants of Ethiopia: Figl (*Raphanus sativus L.*), Girgir (*Eruca sativa L*) and Karkade (*Hibiscus sabdariffa*): implications for in-vitro mineral bioavailability. Food Res. Int. 137, 109724.

Kirby, A.J., Schmidt, R.J., 1997. The antioxidant activity of Chinese herbs for eczema and of placebo herbs I. J. Ethnopharmacol. 56 (2), 103–108.

Koch, T.C., Goldman, I.L., 2005. Relationship of carotenoids and tocopherols in a sample of carrot root-color accessions and carrot germplasm carrying Rp and rp alleles. J. Agric. Food Chem. 53 (2), 325–331.

Kong, X., Bao, J., Corke, H., 2009. Physical properties of amaranthus starch. Food Chem. 113 (2), 371–376.

Kumoro, A.C., Retnowati, D.S., Budiyati, C.S., 2012. Water solubility, swelling and gelatinization properties of raw and ginger oil modified gadung (*Dioscorea hispida Dennst*) flour. Res. J. Appl. Sci. Eng. Technol. 4 (1), 2854–2860.

Kusumayanti, H., Handayani, N.A., Santosa, H., 2015. Swelling power and water solubility of cassava and sweet potatoes flour. Procedia Environmental Sciences 23, 164–167, 0.

Lee, J., Durst, R., Wrolstad, R., 2005. Determination of total monomeric anthocyanins pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method. J. AOAC Int. 88 (5), 1269–1278.

Lee, K.J., Lee, J.R., Ma, K.H., Cho, Y.H., Lee, G.A., Chung, J.W., 2016. Anthocyanins and isoflavones content in Korean black soybean landraces and their antioxidant activities. Plant Breed. Biotechn. 4 (4), 441–452.

Mathiventhan, U., Ramiah, S., 2015. Vitamin C content of commonly eaten green leafy vegetables in fresh and under different storage conditions. Tropical Plant Res. 2 (3), 240–245.

Mazzucotelli, C.A., González-Aguilar, G.A., Villegas-Ochoa, M.A., Domínguez-Avila, A.J., Ansorena, M.R., Di Scala, K.C., 2018. Chemical characterization and functional properties of selected leafy vegetables for innovative mixed salads. J. Food Biochem. 42 (1), e12461.

Mezgebo, K., Belachew, T., Satheesh, N., 2018. Optimization of red teff flour, malted soybean flour, and papaya fruit powder blending ratios for better nutritional quality and sensory acceptability of porridge. Food Sci. Nutr. 6 (4), 891–903.

Mikore, D., Mulugeta, E., 2017. Determination of proximate and mineral compositions of *Moringa oleifera* and *Moringa stenopetala* leaves cultivated in Arbaminch Zuria and Konso, Ethiopia. Afr. J. Biotechnol. 16 (15), 808–818.

Mune, M.A., Bakwo Bassogog, C.B., Nyobe, E.C., René Minka, S.R., 2016. Physicochemical and functional properties of *Moringa oleifera* seed and leaf flour. Cogent Food & Agriculture 2 (1), 1220352.

Muslihatin, W., Daesusi, R., Kuncoro, E.P., 2015. Influence of photopheriod to red roselle (*Hibiscus sabdariffa* L.) calyx phytochemical content. J. Chem. Pharmaceut. Res. 7 (11), 154–157.

Naiker, T.S., Gerrano, A., Mellem, J., 2019. Physicochemical properties of flour produced from different cowpea (*Vignaunguiculata*) cultivars of Southern African origin. J. Food Sci. Technol. 56 (3), 1541–1550.

Narzary, H., Islary, A., Basumatary, S., 2016. Phytochemicals and antioxidant properties of eleven wild edible plants from Assam, India. Mediterr. J. Nutr. Metabol. 9 (3), 191–201.

Ngoma, K., Mashau, M.E., Silungwe, H., 2019. Physicochemical and functional properties of chemically pretreated ndou sweet potato flour. Int. J. Food Sci. 2019, 1–9. Article ID 4158213.

Niaz, K., Shah, M.A., Khan, F., Saleem, U., Vargas, C., Panichayupakaranant, P., 2020. Bioavailability and safety of phytonutrients. In: Phytonutrients in Food. Woodhead Publishing, pp. 117–136.

Nyam, K.L., Leao, S.Y., Tan, C.P., Long, K., 2014. Functional properties of roselle (*Hibiscus sabdariffa L.*) seed and its application as bakery product. J. Food Sci. Technol. 51 (12), 3830–3837.

Ocloo, F.C.K., Bansa, D., Boatin, R., Adom, T., Agbemavor, W.S., 2010. Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds. Agric. Biol. J. N. Am. 1 (5), 903–908.

Oladele, A., Aina, J., 2007. Chemical composition and functional properties of flour produced from two varieties of tigernut (*Cyperus esculentus*). Afr. J. Biotechnol. 6 (21), 2473–2476.

Omueti, O., Otegbayo, B., Jaiyeola, O., Afolabi, O., 2009. Functional properties of complementary diets developed from soybean (*Glycine Max*), groundnut (*Arachis Hypogea*) and crayfish (*Macrobrachium Spp*). Electron. J. Environ. Agric. Food Chem. 8 (8), 563–573.

Osundahunsi, O.F., Aworh, A.C., 2002. A preliminary study on the use of tempe-based form ula as a weaning diet in Nigeria. Plant Foods Hum. Nutr. 57 (3-4), 365–376.

Parmar, A., Gebre, B.A., Legesse, A., Demelash, Y., Fladung, K., Hensel, O., 2017. Nutritional comparison of white and red *Coccinia abyssinica* (Lam.) Cong. accessions: an under-utilised edible tuber of the Ethiopian highlands. Foods 6 (8), 1–8, 71.

Rathi, H., Molan, L., Ismail, H., 2019. The free radical scavenging activities and total phenolic contents of extracts from leaves and roots of two radish varieties grown in Iraq. Biochem. Cell. Arch. 19 (1), 1313–1317.

Ratnawati, L., Desnilasari, D., Surahman, D.N., Kumalasari, R., 2019. March). Evaluation of physicochemical, functional and pasting properties of soybean, mung bean and red kidney bean flour as ingredient in biscuit. In: IOP Conference Series: Earth and Environmental Science, 251. IOP Publishing, 012026, 1.

Sadler, G., Davis, J., Dezman, D., 1990. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. J. Food Sci. 55, 1460–1461.

Sahni, P., Shere, D.M., 2017. Comparative evaluation of physico-chemical and functional properties of apple, carrot and beetroot pomace powders. Int. J. Food Ferment. Technol. 7 (2), 317–323.

Salah, E.M., Hayat, Z.E., 2009. Proximate composition of Karkadeh (*Hibiscus sabdariffa*) seeds and some functional properties of seed protein isolate as influenced by pH and NaCl. Int. J. Food Sci. Nutr. 60 (3), 183–194.

Sarikurkcu, C., Targan, S., Ozer, M.S., Tepe, B., 2017. Fatty acid composition, enzyme inhibitory, and antioxidant activities of the ethanol extracts of selected wild edible plants consumed as vegetables in the Aegean region of Turkey. Int. J. Food Prop. 20 (3), 560–572.

Shebabaw, H., 2013. Effect of Processing on Physicochemical and Antinutritional Factors of "anchote"(*Coccinia Abyssinica*) and Development of Value Added Biscuit (Doctoral Dissertation. Addis Ababa University, pp. 1–102.

Shruthi, V.H., Ramachandra, C.T., Nidoni, U., Hiregoudar, S., Naik, N., Kurubar, A.R., 2017. Physico-chemical, nutritional and functional properties of roselle (*Hibiscus sabdariffa L.*). Int. J. Curr. Microbiol. Appl. Sci. 6 (12), 1–7.

Siddiq, M., Nasir, M., Ravi, R., Dolan, K.D., Butt, M.S., 2009. Effect of defatted maize germ addition on the functional and textural properties of wheat flour. Int. J. Food Prop. 12, 860–870.

Suliman, G.M., Babiker, S.A., Eichinger, H.M., 2017. Effect of *Hibiscus* seed-based diet on chemical composition, carcass characteristics and meat quality traits of cattle. Indian J. Anim. Res. 51 (4), 694–699.

Tiwari, S., Sarkar, N., 2018. Development and evaluation of carotene rich carrot powder. Int. J. Res. Biosci. Agricul. Techn. 6 (1), 123–131.

Yusri, N.M., Chan, K.W., Iqbal, S., Ismail, M., 2012. Phenolic content and antioxidant activity of *Hibiscus cannabinus L.* seed extracts after sequential solvent extraction. Molecules 17 (11), 12612–12621.

Zaman, H., Das, P., Das, P., Sahu, N., 2017. Analysis of physicochemical, nutritional and antioxidant properties of fresh and dried roselle (*Hibiscus sabdariffa* Linn.) calyces. Int. J. Pure Appl. Biosci 5 (1), 261–267.