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ORIGINAL ARTICLE

Nutraceutical, phytochemical, and antioxidant activities of selected wild edible fruits as natural foods

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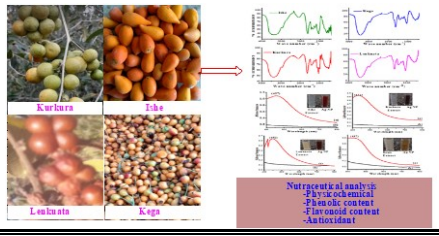
Graphical abstract



Highlights

- Wild edible fruits are rich sources of nutraceuticals, phytochemicals and polyphenols.
- FT-IR analysis confirms, fruits have various surface functional groups.
- These fruits showed significant antioxidant potential of their biological activities.
- Antioxidant activity was evaluated using DPPH assay and Ag nanoparticle synthesis.

ABSTRACT

The present study aimed to analyze the nutritional value, phytochemicals content and antioxidant activities of four wild edible fruits: ''Ishe'' (*Mimusops kummel Bruce* ex A.DC), ''Kega'' (*Rosa abyssinica* R.Br.), ''Kurkura'' (*Ziziphus spina-christi* L. Desf.), and ''Lenkuata'' (*Grewia ferruginea* Hochst. ex A) which are commonly consumed as natural food sources. The results of nutritional properties showed that fruits have rich in nutrients due the presence of various surface functional groups. The total phenolic and flavonoid content in fruits were ranged from 61 ± 1.02 to 98.3 ± 0.44 and 24.1 ± 0.10 to 87.1 ± 0.44 mg QE/100g of fruit paste, respectively. Antioxidant activity of fruits were analyzed in terms of free radical scavenging potential, DPPH assay (IC50%: 108.31 to 401.7 µg/mL of fruit paste and reducing/stabilizing ability via synthesis of silver nanoparticles confirmed by color change and λmax value. Therefore, fruits showed important nutritional, phytochemical, and antioxidant properties with biological interest, and can be a potential source of functional ingredients and nutraceuticals.

Keywords: Wild edible fruit; Nutraceutical; Phytochemical; Antioxidant activity, Natural food.

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1. Background

The long history of humans' ability adapt to natural environments; and interact with nature and social circumstances is profoundly devoted to wild edible plants [1]. Wild edible plants serve as alternatives to staple food during periods of food deficit; they are valuable supplements for a nutritional balanced diet; have medicinal properties; and are primary alternative sources of income for food-insecure families living in poverty in many developing countries [2-4]. The efficacy of wild food resources, that is, fruits, seeds, roots, leaves, tubers, barks, vegetables, mushrooms, nuts, and grains, are substantial to introducing functional foods and novel drugs and its consumption is closely related to health, spiritual, cultural, and socio-economic aspects of human life [5-7].

Wild fruits have received increasing attention due to their medicinal properties and high nutritional and nutraceutical values, being rich in organic/inorganic sources such as carbohydrates, proteins, lipids, vitamins, minerals, and polyphenols [6, 8]. Polyphenols such as phenolic acids, flavonoids, proanthocyanidins, stilbenes, and lignans are the most abundant phytochemicals in fruits and exhibit multifold health benefits of antioxidant, anticancer, antidiabetic, antiaging, neuroprotective effects and prevent other diseases [9, 10]. Therefore, understanding and knowing the phytochemical profile, nutritional value, and bioactivities of wild edible fruits can provide a scientific basis for their human intake of critical nutrients and health benefits.

The Ethiopian flora has approximately 6000 species of higher plants of which about 10% are endemic [11]. Ethiopia is a global biodiversity hot-spot and center of origin for many food plants and deep traditional knowledge concerning the use of those plants [7]. Though many more wild edible plant species are believed to be undocumented to date, about 413 species of wild/semi-wild species used by the people in Ethiopia have been recorded [12]. The indigenous knowledge, practice, and skill associated with wild edible plants are highly developed, but it is poorly investigated and documented [12]. The ethno-botanical study and its importance of wild edible plants as a source of food and medicine in rural populations are very widely recognized through various studies [1, 7, 11-15]. However, information regarding its physicochemical analysis, chemical composition, phytochemicals, antioxidant potential and other studies, which can assist in the decision making for its application as the food source, is not available as well.

The food nutritional contribution and the medicinal value of wild edible fruits have not been studied fully in Ethiopia [1, 16-19]. Therefore, the present study attempts to: (i) screen phytochemicals, (ii) asses the nutritional value, (iii) estimate polyphenols (phenolics and flavonoids), and (iv) evaluate health-promoting antioxidant activity using DPPH assay and nanoparticle synthesis of four commonly used wild edible fruits such as ''Ishe'' (*Mimusops kummel Bruce ex* A.DC), ''Kega'' (*Rosa abyssinica* R.Br.), ''Kurkura'' (*Ziziphus spina-christi L.* Desf.), and ''Lenkuata'' (*Grewia ferruginea* Hochst. ex A).

1. Materials and methods

2.1. Chemicals

In this experiment the following reagents: Gallic acid (Sigma Aldrich, Germany), ascorbic acid (Sigma Aldrich, Germany), methanol (Sisco research laboratories Pvt. Ltd. India), aluminum chloride (Loba chemie Pvt. Ltd, India), sodium nitrite (Sigma Aldrich), siliver nitrate (Resreach lab chem industries, India), sodium carbonate (Abron Exports-133001, India), quercetin, 2, 2-diphenyl-1-picrylhydrazyl (Sigma Aldrich, Germany), and sodium hydroxide

(Alphax chemical industry, India) were used. All other reagents and chemicals used were of analytical grade. Distilled water was used in all the experiments.

2.2. Collection and preparation of fruits

Four wild edible fruits: ‘‘Kega’’ (*Rosa abyssinica* R.Br.), ‘‘Kurkura’’ (*Ziziphus spina-christi (L.)* Desf.), and ‘‘Lenkuata’’ (*Grewia ferruginea* Hochst. ex A.) used as traditional diet have been selected based on previous ethno-botanical study [15]. Currently, these fruits are used as a source of food and income for the local communities. They were collected from local areas of Woldia, Ethiopia, except ‘‘Ishe’’ (*Mimusops kummel Bruce ex* A.DC.) from Bahir Dar, Ethiopia, in December 2020 during their fruiting period. Taxonomic identification of the fruits were made according to the previous literature [15]. Fruits were collected when they were ready for consumption according to traditional usage, and analysis was performed on three fruit samples picked from different sides of edible parts (100 g) per plant from each of ten plants. After harvesting, the fruits were stored at room temperature (~25 °C), until complete maturation (a uniform skin color and equal stage of ripeness). Fruit samples were selected by visual inspection (removing the damaged units) and underwent a washing process with distilled water. The fruits were manually separated into fruit flesh (edible part) and central core (seed) and their flesh used for all analysis were refrigerated at 4°C to prevent oxidation up to analysis.

2.3. Physicochemical analysis

Manually separated fruit fleshs (edible parts) from each fruits were chopped in a juice blender to yields a paste. Then the flesh pastes obtained were immediately analyzed. The pH, titeratable acidity and total soluble solids were determined according to the previous report [20]. For pH determination, the fruit pastes were diluted 1/10 (w/v) in distilled water and the supernatant was directly measured by a pH meter (PHS-3B, China). Total soluble solids (TSS) were determined by measuring the °Brix of about 2 drops of the paste at room temperature using a portable pocket digital refractometer (Atago-PAL-33S, Japan). Titratable acidity (TA) was determined by titration method; briefly, about 10 g of fruit paste was mixed with 100 mL distilled water, homogenized, and titrated with 0.1 N NaOH up to pH 8.2, and the results were expressed as percentage of citric acid. Moisture content was determined by drying in an oven at 105°C, (drying oven/incubator-PH-030A, China), and total ash content (550°C, Nabertherm GmbH, Germany) were obtained by gravimetry and protein content was estimated using the Kjeldahl method (N × 6.25) [21]. Crude fat (or lipid) content was determined using Soxhlet extraction method using petroleum ether as a solvent and crude fiber content by gravimetry after H_2SO_4 and NaOH digestion. Available carbohydrates were calculated as difference: 100 – (g moisture + g protein + g fibre + g fat/lipid + g ash) [22]. Total energetic value (kcal/100 g) = (% protein * 3.36 kcal/g) + (% lipids * 8.37 kcal/g) + (% carbohydrates * 3.6 kcal/g) [23]. Total phosphorus was determined via spectrophotometrically.

2.4. Extraction of Phytochemicals

Frozen fruits were ground up/chopped in a juice blender to yield a paste and extracted overnight using shaker (Heidolph unimax 2010, Gemany) in the dark at room temperature with fruit peel paste to methanol ratio of 1:5w/v [20]. The supernatants were collected after centrifugation at 4000 rpm for 20 min, filtered through a Whatman filter paper No. 1 and kept at 4 °C prior to analysis. The extracts were used for phytochemical screening, determination of phytochemicals (total phenolics and flavonoids) and antioxidants (DPPH assay).

2.5. Phytochemical screening

Crude extracts of fruits were phytochemically evaluated to determine the presence of alkaloids (Wagner's test), flavonoids (alkaline test), phenols and tannins (ferric chloride test), saponins (frothing test), steroids and terpenoids (Salkowski test), glycosides (Keller-Killiani test), sugars (Fehling's test), and amino acids (ninhydrin test) according to standard methods [24-26]. Any change of colors or the precipitate formation was used as indicative of positive response to these tests.

2.6. Spectroscopic analysis

The sample extracts were diluted to 1:10w/v with respective solvents and scanned in the wavelength ranging from 400 to 800 nm using UV-Vis spectrophotometer (Perkin Elmer Lambda 35) in order to detect the characteristic peaks [27]. Functional groups of chemical compounds in fruits were characterized by Fourier transform infrared spectrophotometer, FT-IR (Jasco FT/IR-6600A). Approximately 1 mg of the fruit pastes was mixed with 100 mg of KBr and made a pellet via mechanical press. The spectrum was recorded within 4000-400 cm^{-1} with resolution of 0.4 cm^{-1}.

2.7. Determination of polyphenols

2.7.1. Determination of total phenolic content (TPC)

The total phenolic content of extracts was determined with the Folin–Ciocalteu assay, according to the previous findings [20]. Briefly, 100 µL of sample extract was added to 500 µL of Folin–Ciocalteu (10-fold with distilled water). The mixture was shaken for 2 min and 400 µL of 7.5% Na_2CO_3 was added, adjusting with distilled water to a final volume of 5 mL, and mixed thoroughly. After 60 min of incubation in the dark at room temperature, the absorbance was measured at 765 nm against blank. A standard calibration curve was plotted using Gallic acid as a standard in the range of 50–1000 µg/mL (in methanol) and results will be expressed as Gallic acid equivalents (mg GAE/100g fruit peel paste).

2.7.2. Determination of total flavonoid content (TFC)

The total flavonoid content of the extracts were determined using aluminum trichloride ($AlCl_3$) colorimetric method [10] with slight modifications. Briefly, 0.5 mL of extract was mixed with 2 mL of distilled water and 0.15 mL of $NaNO_2$ (5%) and allowed to stand for 6 min. Thereafter, 0.15 mL of $AlCl_3$ (10%) and 1 mL of NaOH (1 M) was added to the solution and then made up to 5 mL with distilled water. After 60 min of incubation in the dark at room temperature, the absorbance was measured at 510 nm against blank. A standard calibration curve was plotted using Quercetin as a standard in the range of 50-1000 µg/mL and results were expressed as Quercetin equivalents (mg QE/100g fruit peel paste).

2.8. Determination of antioxidant activity

2.8.1. DPPH assay

The scavenging activity of DPPH radical was measured according to the method described in the previous report [20]. Briefly, 0.1 mL of the prepared extract paste was mixed with 3.9 mL DPPH solution (6×10^{-5} M, prepared by dissolving 5.9 mg DPPH with 250 mL methanol) and then samples were shaken vigorously, kept in the dark for 60 min and the absorbance of samples was read spectrophotometrically at 517 nm against blank. A standard calibration

curve was plotted using ascorbic acid as a standard in the range of 20-100 µg/mL and results were expressed as percent inhibition and IC50. The percent of reduction of DPPH was calculated based on [28].

2.8.2. Synthesis of silver nanoparticles (Ag NPs)

Antioxidant capacity of all the extracts was measured in terms of reduction ability of silver salt to Ag nanoparticles (Ag NPs) based on the previous findings [29, 30]. About 10 g fruit pastes were added to 50 mL distilled water and heated at 80 °C for 20 min in order to get a suitable concentration of antioxidant phenolics (e.g. flavonoids, tannins and glycosides) as highly soluble compounds in water. Then the mixture was allowed to cool to room temperature and aqueous extracts were easily obtained by centrifugation at 4000 rpm followed by filtration. The biosynthesis of Ag NPs; an aqueous solution of silver nitrate (10 mM, 90 mL) was mixed with each fruit extract (10 mL). On exposure to sunlight, the solution changes its color within 10 min. The formation of Ag NPs through bio-reduction process by extracts were confirmed by color change and recording the absorbance at regular intervals in UV-Vis spectrophotometer at the wavelength scan range of 200-800 nm.

2.9. Statistical analysis

All experimental data were taken three times (n=3), and the measured results were expressed as the mean ± standard deviation (SD). Statistical analysis was subjected to one-way analysis of variance (one-way ANOVA) and means were separated by Tukey's test at $p \leq 0.05$ significance level using Origin software (Origin Pro 8).

3. Result and discussion

3.1. Physicochemical analysis

The physicochemical analysis of the four selected wild edible fruits was summarized in **Table 1**. The fresh fruit samples were subjected to the proximate analysis since these were believed to be more relevant to the perspective of consumers. The moisture content values were observed in different fruits, with total content varying from 31.76±0.21% (Lenkuata) to 67.49±0.10% (Kurkura). The amount of dry matter is an important criterion in terms of the determination of the consumption status of the fruits. The dry matter content of Lenkuata was very high, on average 68.24±0.11%, while for the other fruits, it varied between 32.51±0.01% (Kurkura), 37.96±0.09% (Ishe), and 76.30±0.05% (Kega). The dry matter content of the analyzed fruits were obtained in the same range as has been reported in the previous reports [31, 32].

The crude fat/lipid content of Kega (2.41±0.01) is similar with Kadamba (*Neolamarckia cadamba*, 2.39%) fruit [33], Lenkuata (0.64±0.01%) compared favourably with Ziziphus mauritiana [34]; Ishe (3.34±0.01%) and Kurkura (1.16±0.01%) also compared with wild edible plants [35]. The crude fat/lipid content of all fruits was comparable with the previous report [21]. Fiber usually consists of cellulose present in plant parts which is indigestible and passed through the digestive system [34]. The crude fiber content ranged between 1.75±0.01-9.57±0.08% in analyzed fruits. The Kega had the highest (9.57±0.08%) crude fiber content amongst the wild edibles fruits under study followed by almost similar values in Lenkuata (8.54±0.07) and Ishe (8.52±0.01%), and Kurkura (1.75±0.01%). The crude protein values were ranged from 3.81% (Lenkuata) to 8.00±0.26% (Kega). Crude protein values of Ishe (3.88%) and Lenkuata (3.81%) fruits were close to that of spondias purpura (3.13%), plum (3.68%), mango (3.3%) [36]; Kurkura (6.56%) are close to *Elaeocarpus sikkimensis* (6.5%), *Ziziphus mauritiana* (6.67%) and Kega (8.00%) close to Morus alba

(8.9%) of wild edible fruits [34, 37]. The carbohydrate content of the wild edible fruits was found between 12.79±0.20% and 48.08±0.28%, which was lowest in Ishe and highest in Lenkuata. The carbohydrate contents of the wild edible fruits are comparable with the native fruits from southern Brazi reported in [21]. According to the results obtained for the caloric value, Kega had the highest energy content (192.52±0.53 kcal/100 g), almost similar with Lenkuata (191.26±0.94 kcal/100g) and lowest energy content in Kurkura (84.91±0.37 kcal/100g), almost similar with Ishe (87.02±0.77 kcal/100 g) represented in Table 1. The phosphorus content of Kega was very high, on average 7.99±0.04%, while for the other fruits, it varied between 6.55±0.02% (Kurkura), 3.85±0.03% (Ishe), and 3.81±0.01% (Lenkuata).

The pH and titratable acidity (TA) are two interrelated concepts in food analysis that deal with acidity and presented in **Table 1**. Each of these quantities is analytically determined in separate ways and each provides its own particular insights on food quality [38, 39]. The pH values were ranged from 2.91±0.04 (Kega) to 4.01 (Ishe), and the acidity level/titeratable acidity ranged from 0.64% (Kurkura) to 3.31±0.01% (Kega). The fruits pH values are close to that of apple (2.9-3.8), grape (2.8-3.8), orange (3.6-4.3), peach (3.1-4.2), and passion fruit (2.94). All the evaluated fruits presented higher pH values than lemon (2.0-2.4) [40, 41]. According to the previous report [41], Kega (2.91±0.04) is considered very acidic (pH<3.7) and Kurkura (3.78±0.01), Lenkuata (3.85±0.03), and Ishe (4.01) are classified as acidic (pH 3.7 to 4.6) fruits. Regarding the titeratable acidity/acidity index, the results found for Ishe (0.74%) fruit was close to acerola (0.71%) [41], Hawthorn (C.pentagyna, 0.75%) values [42]. The Lenkuata (1.92±0.04%) fruit obtained value close to the Cape gooseberry fruit (1.85%) [22]. The Kega (3.31±0.01%) fruit obtained value close to the Kadamba (3.32%) fruit [33].

The total soluble solids (TSS) values indicated the total amounts of soluble constituents of the frit samples. These soluble constituents were mainly sugars, with smaller amounts of organic acids, free amino acids, proteins, vitamins, essential oils, and glucosides [43]. The total sugar content, which is an important aspect when correlated to the fruit sweetness, and sweet fruits are more likely to be accepted by consumers [41]. In other words, higher Brix^{o} values implied a superior nutritional content of the respective fruit samples. The Lenkuata fruit presented the highest total soluble solids (8.50±0.09 Brix^{o}) and the Kurkura had the lowest total soluble solid (3.75±0.01 Brix^{o}). Therefore, it can be proposed with respect to the TSS that Lenkuata had the highest quality, followed by Ishe and Kega, which had almost equal TSS levels, and finally with Kurkura with the least amount of TSS. At the 0.05 level ($p<0.05$), the population means are significantly different (**Table 2**).

Table 1: Physicochemical composition of wild edible fruits (triplicate, mean ± SD)

Fruit samples	Physicochemical Parameters										
	Moisture content	Dry matter	Ash content	Titeratable acidity	Total soluble solids	Crude fat	Crude fiber	Crude protein	Phosphorus	Carbohydrate	Energy
Ishe	62.04 ±0.15	37.96 ±0.09	9.44 ±0.06	0.74 ±0.00	7.73 ±0.03	3.34 ±0.01	8.52 ±0.01	3.88 ±0.00	3.85 ±0.03	12.79 ±0.20	87.02 ±0.77
Kega	33.70 ±0.06	76.30 ±0.05	5.91 ±0.02	3.31 ±0.01	7.03 ±0.02	2.41 ±0.01	9.57 ±0.08	8.00 ±0.26	7.99 ±0.04	40.41 ±0.38	192.52 ±0.53
Lenkuata	31.76 ±0.21	68.24 ±0.11	7.17 ±0.00	1.92 ±0.04	8.50 ±0.09	0.64 ±0.01	8.54 ±0.07	3.81 ±0.00	3.81 ±0.01	48.08 ±0.28	191.26 ±0.94
Kurkura	67.49 ±0.10	32.51 ±0.01	8.28 ±0.05	0.64 ±0.00	3.75 ±0.01	1.16 ±0.01	1.75 ±0.01	6.56 ±0.05	6.55 ±0.02	14.77 ±0.12	84.91 ±0.37

Moisture content, dry matter, ash content, titeratable acidity, crude fat, crude fiber, crude protein, phosphorus, carbohydrate (%), total soluble solids (Brix^{o}), and energy (kcal/100g).

Table 2: Multivariate ANOVA for physicochemical composition of wild edible fruits

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	Moisture Content (%)	28512.900	1	28512.900	1438834.330	.000	1.000
	Dry matter (%)	34691.974	1	34691.974	10459891.625	.000	1.000
	Ash content (%)	711.480	1	711.480	479649.438	.000	1.000
	pH	158.850	1	158.850	307450.903	.000	1.000
	Total Acidity (%)	32.736	1	32.736	91356.372	.000	1.000
	Total Suspended Solid (Brix^{o})	547.425	1	547.425	241511.121	.000	1.000
	Crude fat/lipid (%)	42.752	1	42.752	394632.692	.000	1.000
	Crude fiber (%)	603.643	1	603.643	221520.248	.000	1.000
	Crude protein (%)	371.519	1	371.519	20441.233	.000	1.000
	Phosphorus (%)	369.741	1	369.741	583801.592	.000	1.000
	Carbohydrate (%)	10101.282	1	10101.282	143586.100	.000	1.000
	Energy (kcal/100 g)	231629.653	1	231629.653	489074.277	.000	1.000

3.2. Phytochemical screening analysis

The identification of phytochemicals in each fruits is a pivotal onset point for evaluating their biological and nutritional facets [44]. Increasing evidence has shown that health-promoting properties of fruits are mainly due to their

phytochemical constituents; especially phenolic compounds [45]. The results in **Table 3** confirm the presence of a variety of phytochemicals including; alkaloids, flavonoids, phenolics, tannins, terpenoids, steroids, sugars, amino acids and absence of saponnins, and glycosides in all fruits.

Table 3: Preliminary phytochemical screening analysis for methanolic extracts of wild edible fruits

Phytochemicals	**Tests**	**Observation**	**Results**			
			Ishe	Kega	Lenkuata	Kurkura
Alkaloids	Wagner's test	Brown-red ppt	+	+	+	+
Flavonoids	Alkaline test	Yellow to colorless	+	+	+	+
Phenols	$FeCl_3$ test	Bluish green color	+	+	+	+
Tannins	$FeCl_3$ test	Dirty green ppt	+	+	+	+
Saponnins	Frothing test	Stable foam	-	-	-	-
Terpenoids	Salkowski test	Reddish-brown	+	+	+	+
Steroids	Salkowski test	Reddish-brown	+	+	+	+
Glycosides	Keller-Killiani	Green-blue color	-	-	-	-
Sugars	Antherone test	Bluish green color	+	+	+	+
Amino acids	Ninhydrin test	Deep blue/purple	+	+	+	+

(+)-Present, (-)-absent, (ppt)-precipitate.

3.3. FT-IR analysis

The FTIR spectra of the fruit samples are shown in **Fig. 1** and **Table 4**. The Fourier Transform Infrared (FT-IR) spectroscopy is an important method to identify compounds or functional groups (chemical bonds) present in fruit materials [46]. The functional groups: -O-H or N-H stretching (3424-3406cm^{-1}), -C-H stretching (2937-2920 cm^{-1}), -C=O and COO−stretching vibrations (1640-1624 cm^{-1}), -N-H, C-N stretching and O-H bending (1449-1402 cm^{-1}), -C-O stretching (1257-1228 cm^{-1}), and C-OH, C-O-C and C-C stretching (668-521 cm^{-1}) were presented in sample extracts of wild edible fruits [47-51]. The detailed interpretation of functional groups presented in fruits is presented in **Table 4**.

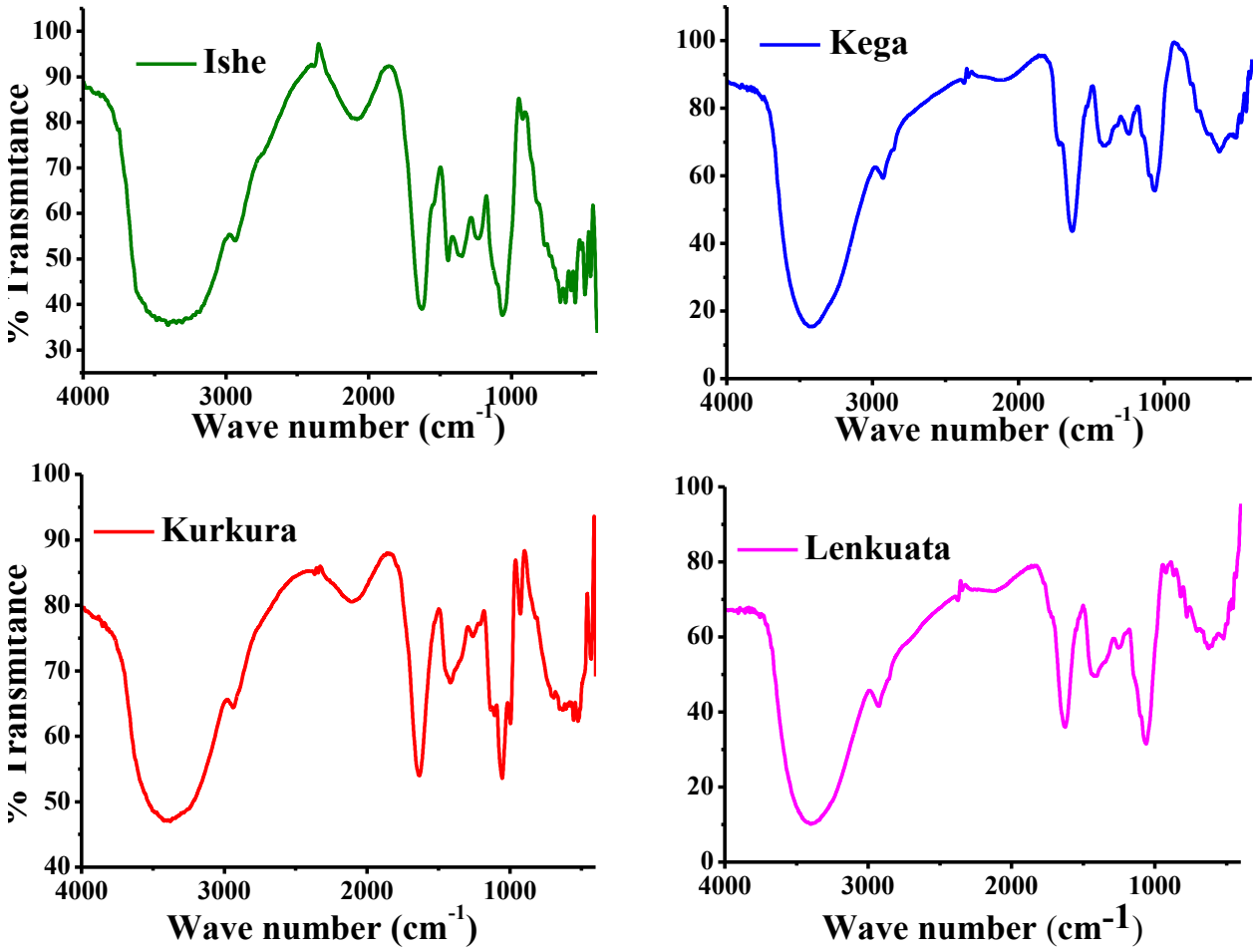

Figure 1: FTIR spectra of wild edible fruits

Table 4: Functional groups and absorption bands for wild edible fruits from FTIR spectra

No	Absorption bands (cm^{-1})				Functional groups	References
	Ishe	Kega	Lenkuata	Kurkura		
1	3409	3421	3406	3424	O-H or N-H stretching vibrations from water, alcohol, phenols, carbohydrates, peroxides, polysaccharides and proteins	[47, 48]
2	2937	2920	2934	2937	C-H stretching vibrations of CH, CH_2 and CH_3 groups, mainly unsaturated lipid and little contribution from proteins, lipids, carbohydrates, nucleic acids	[47, 49]
3	2083	-	-	2112	Unknown	-
4	1640	1638	1624	1625	Stretching vibration of the C=O bond for the methyl esterified carbonyl groups and the ionic carboxyl groups (COO−). -Protein peaks	[50, 52]
5	1449	1402	1417	1420	N-H, C-N from proteins and O-H bending from phenyl groups	[48]
6	1346	-	-	-	CH_3 bending vibrations, lipids and proteins	[47]
7	1228	1240	1240	1257	Stretching vibration of C-O group	[48]
8	1066	1063	1063	1051	C-OH, C-O-C and C-C stretching vibrations, indicating the presence of pyran ring structure	[49]
9	668	621	621	521	C–H bending vibrations	[48]

(-) in row 3 and 6 represents, absorption peaks are not clearly identified.

3.4. Determination of polyphenols

3.4.1. Determination of total phenolic content (TPC)

The content of total polyphenols in the analyzed wild edible fruits were obtained from a standard curve of Gallic acid ranging from 50 to 1000 µg/mL ($y = 0.0004x+0.072$; $R^2 = 0.993$) as shown in **Table 5** and **Fig. 2**. The amount of total phenolic content varied in methanolic extracts of wild edible fruits and ranged from 61.0±1.02 to 98.3±0.44 mg of GAE/100g of fruit paste. According to these results, TPC (mg GAE/100 g fruit paste) is decreased as Kurkura>Ishe>Kega>Lenkuata. These differences may be due to change in growing environment, harvesting time, and species [5]. The findings of this study are in comparable with some previous results observed for pineapple (61 mg GAE/100 g), *Monstera deliciosa* fruit (32.69±11.2 mg GAE/100 g) [20], *Juglans regia* fruit (95.09 ± 0.51 mg GAE/100g FW), *Grewia optiva* (91.47±0.86 mg GAE/100g) and *Berberis lyciumat* (90.57±0.77 mg GAE/100g),

Rubus ellipticus (83.33 ± 0.37 mg GAE/100g) [5], lemon (66.3±3.4 mg GAE /100g), peach (65.3±0.4 mg GAE /100g), orange (56.8±0.9 mg GAE /100g), banana (56.1±2.8 mg GAE /100 g), pear (53.6±2.5 mg GAE/100 g), and Grapefruit (30.7±0.9 mg GAE /100 g) [53, 54]. This confirms that wild edible fruits are rich in health promoting bioactive compounds compared to cultivated fruits, vegetables, and other crops.

Table 5: Total phenolic and flavonoid content of wild edible fruits

Wild edible fruits	TPC (mg GAE/100g fruit paste)	TFC (mg QE/100g fruit paste)
Ishe	71.2±1.15	45.0±0.54
Kega	62.8±0.83	87.1±0.44
Kurkura	98.3±0.44	24.1±0.10
Lenkuata	61.0±1.02	26.5±0.17

The data are presented as Mean ± SD.

3.4.2. Determination of total flavonoid content (TFC)

The content of total flavonoids in the analyzed wild edible fruits were obtained from a standard curve of Quercetin ranging from 50 to 1000 µg/mL ($y = 0.0013x + 0.0182$; $R^2 = 0.9972$). The TPC was expressed as mg Quercetin equivalents per hundred gram of sample (mg QE/100g fruit paste) as shown in **Table 5** and **Fig. 2**. Total flavonoids ranged from 24.1± 0.10 to 87.1± 0.44 mg QE/100g fruit paste. Total flavonoid content was recorded higher in Kega (87.1± 0.44 mg QE/100g), followed by Ishe (45.0±0.54 mg QE/100g), Lenkuata (26.5±0.17 mg QE/100g) and lowest in Kurkura (24.1±0.10 mg QE/100g). The order of total flavonoids content of four species analyzed was as follows: Kega>Ishe>Lenkuata>Kurkura. Our findings are in agreement/comparable with some previous results observed for Lovely Pink (78.64 mg/100 g) [55], pinosa (68.21 mg QE/100 g), P. domestica (10.13 mg QE/ 100 g), rose hips /*R. canina* hips (41 mg/100 g), R. canina fruits (23.6 mg QE/g), C. monogyna (31.37 mg QE/100g), C. mas (17.27 mg QE/100 g) and R. fruticosus (55.82 mg QE/100g) [56]. As in the case of total phenol content, the authors believe that this difference between the species used in terms of total flavonoid content could be explained by genetic factors and by the different ability of species in synthesizing secondary metabolites.

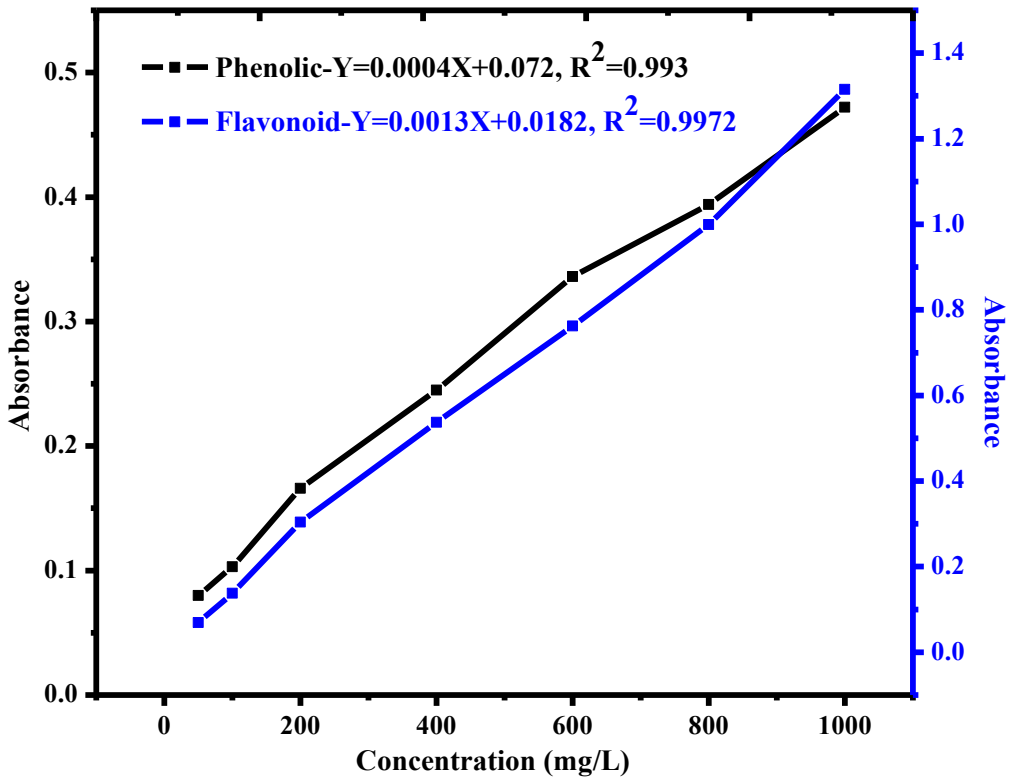

Figure 2: Calibration curve for phenolic and flavonoid content determination

3.5. Antioxidant activity of wild edible fruits

Currently, the study of antioxidant activity has increased in the field of nutrition and food sciences. Antioxidant capacity is an estimate of the nutraceutical quality of a food and constitutes the biological activity responsible in part for the preventive effect against oxidative damage [20]. The scavenging effect of different concentration of fruits extract on the DPPH free radical was compared with standard antioxidant, ascorbic acid as shown in **Table 6**. At 100 mg/L concentration, Kega fruit extract (85.4±0.72%) showed higher percentage of inhibition followed by Lenkuta (84.6±0.53%), Kurkura (84.4±1.9%), and Ishe (83.22±0.1%), While standard ascorbic acid showed 86.34±1.06% of inhibition at the same concentration. The IC50 value was calculated to estimate the concentration of the extract required to inhibit 50% of a radical. The IC50 values of Kega, Ishe, Kurkura, Lenkuata, and ascorbic acid obtained in DPPH assay were 108.31µg/mL, 286.47 µg/mL, 318.26 µg/mL, 401.7 µg/mL, and 34.37 µg/mL, respectively. The lower the IC50 value, the higher is the antioxidant activity of the test sample [57, 58]. Therefore, those fruit samples having the minimum/comparable amount of IC50 values compared to [59, 60] could have high antioxidant activity DPPH free radical scavenging.

Table 6: Free radicals scavenging activity of wild edible fruits

Concentration (µg/mL)	% Inhibition				
	Ishe	Kega	Lenkuata	Kurkura	Ascorbic acid
20	75.4±0.63	68.1±1.05	76.8±0.26	76.9±0.79	41.88±0.47
40	80.9±0.98	82.4±0.20	81.9±0.36	82.2±0.61	52.6±0.62
60	81.6±0.62	83.7±0.59	83.06±0.32	83.06±0.85	65.04±1.39
80	83.06±0.85	84.4±0.36	84.4±0.69	84.2±0.96	76.35±0.52
100	83.22±0.1	85.4±0.72	84.6±0.53	84.4±1.9	86.34±1.06
$IC_{50\%}$ Value	286.47	108.31	401.7	318.26	34.37

The data are presented as Mean ± SD.

3.6. Biosynthesis of silver nanoparticle

The color change, UV–Visible spectra of silver nanoparticles with its surface Plasmon resonance are shown in **Fig. 3**. When each fruit extract was mixed with silver nitrate solution at room temperature upon exposure to sunlight; the solution changes its color from colorless to deep red (*Mimusops kummel Bruce ex* A.DC), colorless to brown (*Ziziphus spina-christi (*L.*)* Desf.), yellow to reddish brown (*Grewia ferruginea* Hochst. ex A), and light yellow to light red (*Rosa abyssinica* R.Br.), which is a clear indication of silver nanoparticle formation. In the UV-visible spectroscopic observations of silver nanoparticles synthesized using fruit extracts, a maximum absorbance were observed from 431-457 nm (**Fig. 3**). According to previous studies, it can be said that the spectrum values for Ag nanoparticles vary between 420-480 nm depending on the particle size, plant extraction concentration, chemical environment, and dielectric medium [29, 30]. Phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles using biological methods [61]. Therefore, formation of silver nanoparticles can be rationalized by the involvement of phytochemicals (polyphenols, flavonoids and terpenoids) present in the aqueous extract of fruits as reducing and stabilizing agent.

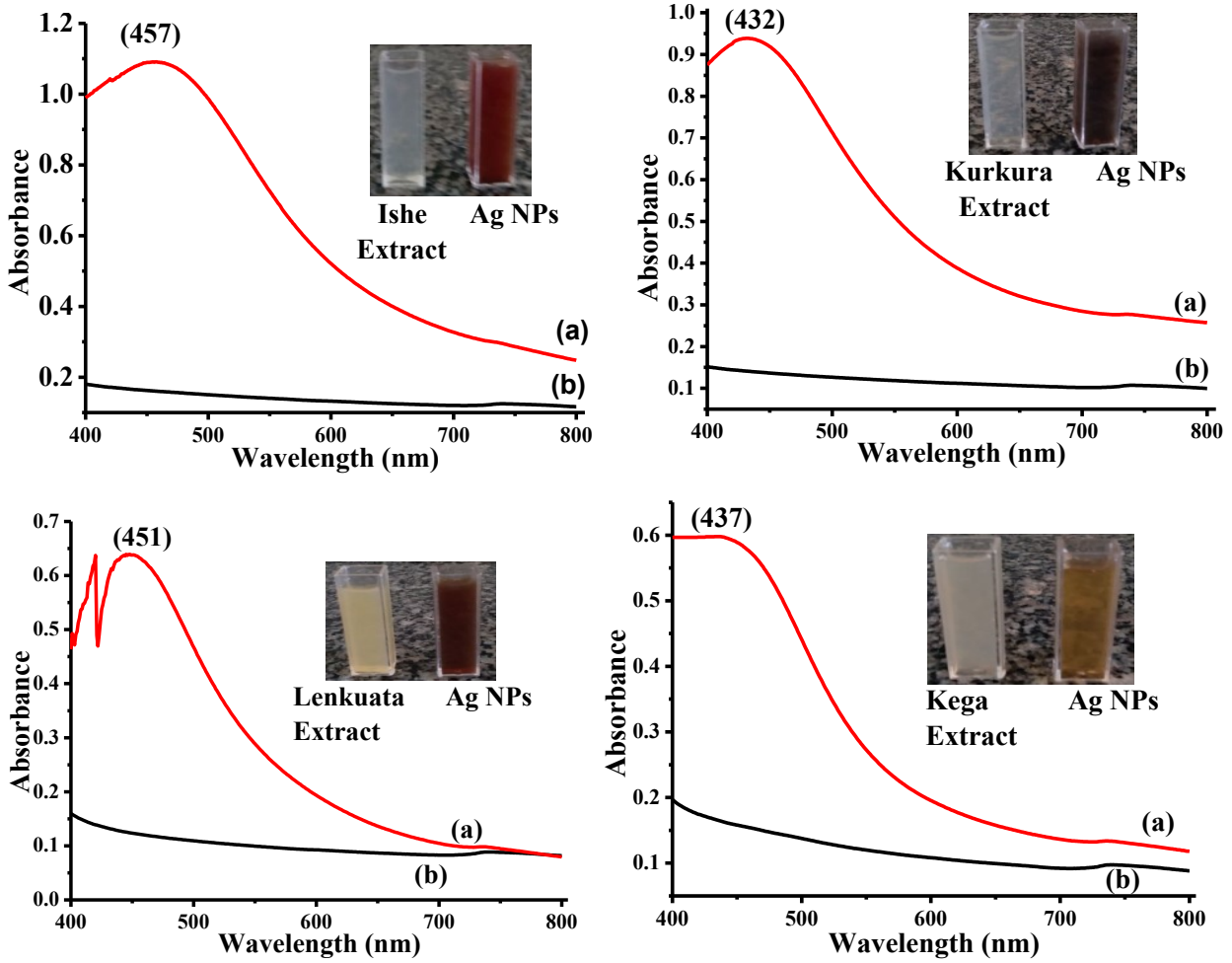

Figure 3: UV–Visible spectra of (a) Ag NPs, (b) fruit extracts and its color changes

4. Conclusion

Results indicated that the wild edible fruits are the rich source of essential nutrients and other beneficiary phytochemicals with extraordinary antioxidant potentials. Therefore, utilization of these wild edible fruits could serve as material for dietary supplementation and functional food ingredients and may have a great role in nutraceutical development. The study revealed that these fruits are a very rich source of antioxidant polyphenols and could become an interesting raw material for the nutraceutical industry in addition to gaining acceptance as health-promoting food. Therefore, awareness of nutritional content and medicinal values of these fruits need to be created among communities to ensure appropriate exploitation and usage of these fruits in solving nutritional problems in the society.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Duguma, H.T., *Wild edible plant nutritional contribution and consumer perception in Ethiopia.* International Journal of Food Science, 2020. **2020**.
2. Hazarika, T. and T.S. Singh, *Wild edible fruits of Manipur, India: associated traditional knowledge and implications to sustainable livelihood.* Genetic resources and crop evolution, 2018. **65**(1): p. 319-332.
3. Hazarika, T. and M. Pongener, *Potential wild edible fruits of Nagaland, North-east India and its significance in the livelihood and nutritional security of rural, indigenous people.* Genetic resources and crop evolution, 2018. **65**(1): p. 199-215.
4. Ong, H.G. and Y.-D. Kim, *The role of wild edible plants in household food security among transitioning hunter-gatherers: evidence from the Philippines.* Food security, 2017. **9**(1): p. 11-24.
5. Shan, S., et al., *Evaluation of polyphenolics content and antioxidant activity in edible wild fruits.* BioMed research international, 2019. **2019**.
6. Bayba, K.B., et al., *Chemical Composition of Urtica simensis Grown in Different Regions of Ethiopia.* Journal of Chemistry, 2020. **2020**.
7. Tebkew, M., et al., *Uses of wild edible plants in Quara district, northwest Ethiopia: implication for forest management.* Agriculture & Food Security, 2018. **7**(1): p. 12.
8. Natić, M., et al., *Nutraceutical properties and phytochemical characterization of wild Serbian fruits.* European Food Research and Technology, 2019. **245**(2): p. 469-478.
9. Patle, T.K., et al., *Phytochemical screening and determination of phenolics and flavonoids in Dillenia pentagyna using UV–vis and FTIR spectroscopy.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2020. **242**: p. 118717.
10. Yang, Q.-Q., et al., *Phytochemicals, essential oils, and bioactivities of an underutilized wild fruit Cili (Rosa roxburghii).* Industrial Crops and Products, 2020. **143**: p. 111928.
11. Berihun, T. and E. Molla, *Study on the diversity and use of wild edible plants in Bullen District Northwest Ethiopia.* Journal of Botany, 2017. **2017**.
12. Ashagre, M., Z. Asfaw, and E. Kelbessa, *Ethnobotanical study of wild edible plants in Burji District, Segan area zone of southern nations, nationalities and peoples region (SNNPR), Ethiopia.* Journal of ethnobiology and ethnomedicine, 2016. **12**(1): p. 32.
13. Teklehaymanot, T., *An ethnobotanical survey of medicinal and edible plants of Yalo Woreda in Afar regional state, Ethiopia.* Journal of ethnobiology and ethnomedicine, 2017. **13**(1): p. 40.
14. Dejene, T., et al., *Ethnobotanical Survey of Wild Edible Fruit Tree Species in Lowland Areas of Ethiopia.* Forests, 2020. **11**(2): p. 177.
15. Gebru, M., et al., *Ethiopia's Food Treasures: Revitalizing Ethiopia's underutilized fruits and vegetables for inclusion in the Food-Based Dietary Guidelines for improved diet diversity, nutrition and health of the population.* 2019.

16. Kidane, L. and A. Kejela, *Food security and environment conservation through sustainable use of wild and semi-wild edible plants: a case study in Berek Natural Forest, Oromia Special Zone, Ethiopia.* Agriculture & Food Security, 2021. **10**(1): p. 1-16.
17. Yimer, A., et al., *Ethnobotanical study of wild edible plants used by Meinit Ethnic Community at Bench-Maji Zone, Southwest Ethiopia.* 2021.
18. Aragaw, H.S., D. Nohr, and D. Callo-Concha, *Nutritional potential of underutilized edible plant species in coffee agroforestry systems of Yayu, southwestern Ethiopia.* Agroforestry Systems, 2021. **95**(6): p. 1047-1059.
19. Bazezew, A.M., S.A. Emire, and M.T. Sisay, *Bioactive composition, free radical scavenging and fatty acid profile of Ximenia americana grown in Ethiopia.* Heliyon, 2021. **7**(6): p. e07187.
20. Barros, T., L. Galego, and P. Pires-Cabral, *Monstera deliciosa fruit: physicochemical characterization and potential for distillate production.* Journal of Food Measurement and Characterization, 2018. **12**(4): p. 2874-2882.
21. Rockett, F.C., et al., *Native fruits from southern Brazil: Physico-chemical characterization, centesimal composition, and mineral content.* Journal of Food Processing and Preservation, 2020: p. e14582.
22. Pereda, M.S.B., M.A. Nazareno, and C.I. Viturro, *Nutritional and antioxidant properties of Physalis peruviana L. fruits from the Argentinean northern Andean region.* Plant Foods for Human Nutrition, 2019. **74**(1): p. 68-75.
23. Barbosa, A.P.P., A.F. Moraes, and R.C. Chisté, *Physicochemical characterization and quantification of bioactive compounds of Antrocaryon amazonicum fruits cultivated in Brazilian Amazonia.* CyTA-Journal of Food, 2020. **18**(1): p. 616-623.
24. Ginovyan, M., et al., *Phytochemical Screening and Detection of Antibacterial Components from Crude Extracts of Some Armenian Herbs Using TLC-Bioautographic Technique.* Current Microbiology, 2020: p. 1-10.
25. Ghany, T.A., et al., *Antioxidant, Antitumor, Antimicrobial Activities Evaluation of Musa paradisiaca L. Pseudostem Exudate Cultivated in Saudi Arabia.* BioNanoScience, 2019. **9**(1): p. 172-178.
26. Phuyal, A., et al., *Phytochemical screening, metal concentration determination, antioxidant activity, and antibacterial evaluation of Drymaria diandra plant.* Beni-Suef University Journal of Basic and Applied Sciences, 2019. **8**(1): p. 16.
27. Dwivedi, M.K., et al., *Antioxidant, antibacterial activity, and phytochemical characterization of Carica papaya flowers.* Beni-Suef University Journal of Basic and Applied Sciences, 2020. **9**: p. 1-11.
28. Hassanzadeh, Z. and H. Hassanpour, *Evaluation of physicochemical characteristics and antioxidant properties of Elaeagnus angustifolia L.* Scientia Horticulturae, 2018. **238**: p. 83-90.
29. Sasidharan, D., et al., *Synthesis of silver and copper oxide nanoparticles using Myristica fragrans fruit extract: Antimicrobial and catalytic applications.* Sustainable Chemistry and Pharmacy, 2020. **16**: p. 100255.

30. Korkmaz, N., et al., *Biogenic silver nanoparticles synthesized via Mimusops elengi fruit extract, a study on antibiofilm, antibacterial, and anticancer activities.* Journal of Drug Delivery Science and Technology, 2020. **59**: p. 101864.
31. Magaia, T., et al., *Proximate analysis of five wild fruits of Mozambique.* The Scientific World Journal, 2013. **2013**.
32. Eroğlu, A.Y., et al., *Bioactive Characteristics of Wild Berberis vulgaris and Berberis crataegina Fruits.* Journal of Chemistry, 2020. **2020**.
33. Pandey, A., et al., *Proximate and mineral composition of Kadamba (Neolamarckia cadamba) fruit and its use in the development of nutraceutical enriched beverage.* Journal of food science and technology, 2018. **55**(10): p. 4330-4336.
34. Mohd Jailani, F.N.A., et al., *Evaluation of constituents and physicochemical properties of Malaysian underutilized Ziziphus mauritiana (Bidara) for nutraceutical potential.* International Journal of Fruit Science, 2020. **20**(3): p. 394-402.
35. Datta, S., et al., *Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants.* Heliyon, 2019. **5**(3): p. e01431.
36. Satpathy, G., Y.K. Tyagi, and R.K. Gupta, *Preliminary evaluation of nutraceutical and therapeutic potential of raw Spondias pinnata K., an exotic fruit of India.* Food Research International, 2011. **44**(7): p. 2076-2087.
37. Bhutia, K.D., et al., *Nutraceutical potential of some wild edible fruits of Sikkim, Himalaya, India.* Ethno Med, 2018. **12**(2): p. 106-112.
38. Tyl, C. and G.D. Sadler, *pH and titratable acidity*, in *Food analysis*. 2017, Springer. p. 389-406.
39. Maraei, R.W. and K.M. Elsawy, *Chemical quality and nutrient composition of strawberry fruits treated by γ-irradiation.* Journal of Radiation Research and Applied Sciences, 2017. **10**(1): p. 80-87.
40. de Oliveira, T.A., et al., *Avaliação da qualidade físico-química de polpas de frutas congeladas na cidade de Mossoró, RN.* Revista Verde de Agroecologia e Desenvolvimento Sustentável, 2014. **9**(2): p. 34.
41. Schiassi, M.C.E.V., et al., *Fruits from the Brazilian Cerrado region: Physico-chemical characterization, bioactive compounds, antioxidant activities, and sensory evaluation.* Food chemistry, 2018. **245**: p. 305-311.
42. Alirezalu, A., et al., *Physicochemical Characterization, Antioxidant Activity, and Phenolic Compounds of Hawthorn (Crataegus spp.) Fruits Species for Potential Use in Food Applications.* Foods, 2020. **9**(4): p. 436.
43. Abdul-Hamid, N.A., et al., *Physicochemical characteristics, nutritional composition, and phytochemical profiles of nine Algerian date palm fruit (Phoenix dactylifera L.) varieties.* Journal of Food Biochemistry, 2018. **42**(6): p. e12663.
44. Farag, R.S., et al., *Phytochemical screening and antioxidant activity of some medicinal plants' crude juices.* Biotechnology Reports, 2020. **28**: p. e00536.
45. Aly, A.A., H.G. Ali, and N.E. Eliwa, *Phytochemical screening, anthocyanins and antimicrobial activities in some berries fruits.* Journal of Food Measurement and Characterization, 2019. **13**(2): p. 911-920.

46. Thakur, N., A. Thakur, and P. Kumar, *Effect of different drying modes on phenolics and antioxidant potential of different parts of wild pomegranate fruits.* Scientia Horticulturae, 2020. **274**: p. 109656.
47. Christou, C., A. Agapiou, and R. Kokkinofta, *Use of FTIR spectroscopy and chemometrics for the classification of carobs origin.* Journal of advanced research, 2018. **10**: p. 1-8.
48. Thummajitsakul, S., et al., *Study on FTIR spectroscopy, total phenolic content, antioxidant activity and anti-amylase activity of extracts and different tea forms of Garcinia schomburgkiana leaves.* LWT, 2020. **134**: p. 110005.
49. Li, Q., et al., *Physicochemical properties and functional bioactivities of different bonding state polysaccharides extracted from tomato fruit.* Carbohydrate polymers, 2019. **219**: p. 181-190.
50. Zhu, R., et al., *Characterization of polysaccharide fractions from fruit of Actinidia arguta and assessment of their antioxidant and antiglycated activities.* Carbohydrate Polymers, 2019. **210**: p. 73-84.
51. He, C., et al., *Fabrication and characterization of oat flour processed by different methods.* Journal of Cereal Science, 2020. **96**: p. 103123.
52. Ying, D., et al., *Physical properties and FTIR analysis of rice-oat flour and maize-oat flour based extruded food products containing olive pomace.* Food Research International, 2017. **100**: p. 665-673.
53. Sun, J., et al., *Antioxidant and antiproliferative activities of common fruits.* Journal of agricultural and food chemistry, 2002. **50**(25): p. 7449-7454.
54. Chu, Y.-F., et al., *Antioxidant and antiproliferative activities of common vegetables.* Journal of agricultural and food chemistry, 2002. **50**(23): p. 6910-6916.
55. Yang, H. and Y. Shin, *Antioxidant compounds and activities of edible roses (Rosa hybrida spp.) from different cultivars grown in Korea.* Applied Biological Chemistry, 2017. **60**(2): p. 129-136.
56. Cosmulescu, S., I. Trandafir, and V. Nour, *Phenolic acids and flavonoids profiles of extracts from edible wild fruits and their antioxidant properties.* International Journal of Food Properties, 2017. **20**(12): p. 3124-3134.
57. Bizuayehu, D., M. Atlabachew, and M.T. Ali, *Determination of some selected secondary metabolites and their invitro antioxidant activity in commercially available Ethiopian tea (Camellia sinensis).* SpringerPlus, 2016. **5**(1): p. 1-9.
58. Zeghad, N., et al., *Antioxidant activity of Vitis vinifera, Punica granatum, Citrus aurantium and Opuntia ficus indica fruits cultivated in Algeria.* Heliyon, 2019. **5**(4): p. e01575.
59. Bardakci, H., et al., *Phytochemical characterization and antioxidant activities of the fruit extracts of several Crataegus taxa.* South African Journal of Botany, 2019. **124**: p. 5-13.
60. Muniyandi, K., et al., *Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activities of Rubus fruits from Western Ghats, India.* Food Science and Human Wellness, 2019. **8**(1): p. 73-81.
61. Mekonnen, A., M. Atlabachew, and B. Kassie, *Investigation of antioxidant and antimicrobial activities of Euclea schimperi leaf extracts.* Chemical and Biological Technologies in Agriculture, 2018. **5**(1): p. 1-12.