

Analysis of Phytochemical Composition of Indigenous Ethiopian Kenkese Pods for Health and Food Security

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Abstract: Developing countries are both food and health insecure because of shortage, cost and nutrition related problems. Even though Benishangul-Gumuz regional state in Ethiopia is blessed with various edible fruit potentials; it is the most food and health insecure region. Among varieties of indigenous vegetable fruit pods originated in this region; an Indigenous Kenkese (Berta naming) and Andha (Gumuz naming) are widely known which paves this research. This research has been aimed to sketch a baseline research for phytochemical evaluation and medicinal activities estimation of Kenkese fruit pods of both species (*Abelmoschus esculentus* and *Abelmoschus ficulenus*) to solve food and health insecurity diet of this region by qualitative standard tests and instrumental techniques, followed by prediction of its medicinal activities. In qualitative analysis, phytochemical bioactive compounds i.e steroids, reducing sugars, triterpenoids, alkaloids, phenolic compounds, flavonoids, Saponins, tannins, anthraquinones, carbohydrates, proteins, volatile oils, carbonyls and amino acids were screened with polar and nonpolar solvents separately using standard procedures. Results showed that only glycosides and carotenoids are absent in Berta Kenkese whereas almost all bioactive compounds were found in Gumuz Kenkese in different abundance. The crude fruit pod extracts of both Berta and Gumuz Kenkese samples were undergo UV-Vis (300-800nm) and FTIR (4000-400 cm⁻¹) characterizations. Results of UV-Vis peaks at 324nm and 290nm confirmed presence of Flavonoids in both species with different intensities. The FTIR test predicted presence of functional groups vibration bands of O-H, N-H, C-H, C=O, C-O, C-N, C=C, S=O, C=N and N=C stretching. This FTIR peaks confirmed presence of alkanes, alkenes, aromatic compounds, carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, isonitriles, organic halogens and carbohydrates in both fruit pod extracts of Berta and Gumuz Kenkeses with different intensities of peaks. From results of such bioactive chemical constituents, it was concluded that fruit pods of both Kenkese species have high nutritional content and higher bio-active quantities in Berta than Gumuz Kenkeses. Moreover presence of almost all bioactive phyto-constituents confirmed fruit pods have traditional organic therapeutic properties which can treat different ailments and can produce commercial drugs. Further spectroscopic characterization studies are required to elucidate structure of bioactive compounds and to quantify individual extracted phytochemical components.

Keywords: Phytochemical, Indigenous Kenkese, Health & Food Security, Characterization

1. Introduction

According to World Food Summit definition, health and food security is maintained when all people at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and also food preferences for an active and healthy life. On the other hand, millions of people in many developing countries including Ethiopia don't have enough food to meet their daily requirements and a further more people are deficient in one

or more micronutrients [FAO, 2004] and [1]. Even though Benishangul-Gumuz is blessed with various edible vegetable fruit potentials and opportunities; remain least developed and the most food and health insecure region among the Country. There are various reasons for this; however, the most major problem faced for utilization as balanced diet is shallow scientific nutrition and medicinal activity research evidences for various indigenous vegetable fruits available in the region (www.BGR.WEP).

In recent years, increasing attention has been paid to the

role of diet in human health [2]. The high intake of food product is associated with a reduced risk of a number of chronic diseases [3]. These beneficial effects have been partly attributed to the compounds which possess antioxidant activity. Free radicals and other reactive oxygen species are those agents which are implicated in many acute and chronic diseases, like diabetes, asthma, Parkinson's, atherosclerosis, cancer, cataracts, neurodegenerative disorder, liver injury as well as humans aging [4, 5]. However, antioxidants are beneficial substances that are involved in delaying and inhibition of such agents by avoiding oxidative damage to the target sites [6]. Antioxidants have the ability to trap free radicals, such as peroxide and hydroperoxide, thereby initiating the mechanism of oxidation leading towards degenerative disorders [7]. Moreover, phytochemicals extracted from natural plants are considered to be safe and good alternatives when compared to synthetic antioxidants. They contain antioxidants in the form of flavonoids, tannins, phenols and proanthocyanidins which are involved in the reduced mortality caused by degenerative disarray [8].

Natural phytochemicals contained in plants, such as alkaloids, flavonoids, phenols and tannins, are regarded as the molecules with the ability to neutralize free radicals and have gained increased attention of researchers and consumers as potential antioxidants. In such a case, medicinal plants are important sources of biologically active antioxidants [9]. These edible plants also contribute towards an extra source of food and vegetables possessing high curative properties. Moreover, vegetables are the edible portions of herbaceous plants that are consumed as raw (main dish or as salad) and by cooking, which may be sweet, bitter, or tasteless [10]. Vegetables and fruits are vital sources of protecting the body from various diseases by elevating the normal functioning of the body system, regulating metabolic activities, tissue repair and weight maintenance which is linked to reduced risk of chronic diseases [11–13].

Among varieties of indigenous vegetable fruit pods originated from Ethiopia in Benishangul Gumuz regional state are; Kenkese (Berta naming) and Andha (Gumuz naming) which were widely known in the world with various local names commonly called Okra or ladies finger. This fruit pods attracts much attention of scientific researchers and pharmaceutical industries now adays due to their unique chemical and nutritional composition [14]. Okra (*Abelmoschus esculentus* L. Moench) is a flowering plant having better dietary value with medicinal and industrial importance that has long been a part of the diet in several countries around the world [15]. It is one of the most important vegetable crops cultivated in tropical, sub-tropical and warm temperate regions of the world. It was first found in former Abyssinia (present Ethiopia) [16, 17] and was later distributed in the Caribbean, South America, North America, Africa, India, and Eastern Mediterranean (<http://www.oshims.com/herbdirectory/o/okra>).

Considering the little contact between Ethiopia and the rest of the world within historic times, it is not surprising that

little is known about the early history, scientific investigation and distribution of okra in Ethiopia. As it can be understand from literatures, Okra is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds. Okra immature fruits, which are consumed as vegetables, can be used in salads, soups and stews, fresh or dried, fried or boiled in Ethiopia. Often the extract obtained from the fruit is added to different recipes like stews and sauces to increase the consistency [17].

Okra is good source of minerals, vitamins and nutrients that are responsible for the health benefits. Okra (*Abelmoschus esculentus*) is very popular phytomedicine for local healers in Ethiopia particularly in Benishangul Gumuz region. It is a rich source of vitamins, minerals and phytoconstituents which are used in the treatment of various types of ailments. Traditionally, the fruits are used as cooling, stomachic, astringent, and aphrodisiac purposes and used in chronic dysentery, gonorrhoea, urinary discharges, strangury, and diarrhea. Tender pods decoction is emollient, demulcent, and diuretic and used in spermatorrhea. The whole plant is emollient and demulcent, whereas the fruit pods with seed can be used in inhibition of cancer cell growth (<http://www.mpbd.info/plants/abelmoschus-esculentus.php>, n.).

The scientific community continues to unravel the mechanisms involved in disease prevention and determine how food bio-actives from such foods as lady's fruit pod can influence human health. [18]. Apart from edible use, extracts from okra fruit have been used for various applications in the food and pharmaceutical industry with significant antioxidant properties [14]. It has various reported pharmacological properties like antidiabetic, antioxidant, nootropic, eye, heart disease and neurological disorders etc. This effort is towards providing the evidence in support to encourage more scientific research to find out more pharmacological and nutritional potential of *Abelmoschus esculentus* that may be suggestive of new drug discovery [16]. To date, a number of researches have been done on these edible medicinal plants in the world, aimed to investigate and isolate the important phytochemicals present and biological activities of its extract, isolated compounds, or their derivatives with valuable drugs [19].

Even though Okra was first originated from Ethiopia particularly in Benishangul Gumuz region, which have been an indigenous food of both ethnicities Berta (named as Kenkese) and Gumuz (named as Andha), there is not scientific nutritional and medicinal research findings reported yet on this medicinal plant. Even though the indigenous people consumed Kenkese daily, there have not been adequate nutritional and phytochemical information which are not yet determined scientifically and documented in the region.

This research has been aimed to sketch a baseline research for the phytochemical evaluation and medicinal activities estimation of Kenkese fruit pod by qualitative standard tests and FTIR and UV-VIS instrumental techniques to solve food and health insecurity diet of the

region as well as nationally in Ethiopia. From the findings of this study, it is evident that Kenkese (*A. esculentus* and *A. ficulenus*) are a prominent source of nutrients and important phytochemicals. The plant fruit pond will possess various important biological effects, including antioxidant, anti-

inflammatory, antibacterial, and anticancer activities as it can be easily understood from the functionalities and bioactive components it contains. More research is necessary on this edible medicinal plant fruit pond in the context of drug discovery and development.

2. Materials and Methods

2.1. Materials

Table 1. Chemicals used in the study and their functions.

Chemicals	Function
Distilled & deionized water	For washing, volume adjustment & sample solution preparation
Ferric chloride	Precipitate the tannins for tannin & glycosides test
Hydrochloric acid	Used for the test of phylobatannins, anthraquinones & alkaloids
Chloroform	For extraction of terpenoids & anthraquinones as a solvent
Sulphuric acid	Used for carotenoids, glycosides, crude fiber tests for digestion purposes
Sodium hydroxide	For titration of reducing sugars, Flavenoids, crude fibers & nitrogen analysis
Glacial acetic acid	For the test of glycosides as a solvent
Ammonia solution	For the test of anthraquinones & alkaloids
Acetone	As a solvent for extraction purposes
Ethanol	For solubilization phytochemicals
Petroleum ether	For extraction of samples as a solvent
Acid/bas indicators	Indicating end points of acids & bases during titration
Biuret reagent	For determination of tyrosine & tryptophan protein residues
Lead acetate	As a clarifying agent in carbohydrate determination
Copper sulphate solution	For titration of reducing sugars
Methylene blue indicator	For indicating reducing sugars end point
Pure ethanol	For precipitation of fibers
100% ammonia solution	For anthraquinones determination
Picric acid solution	To check the presence of alkaloids
10% tannic solution	Added for the test of alkaloids
Mayer's reagent	A reagent for the test of alkaloids
Fehling's solution	A reagent for test of reducing sugars

Equipment's used in the study are: Grinder or blender, conical flask, test tubes, Petri plates, laminar air flow, Incubator, autoclave (AMA20N), Freeze, hot air oven (Contherm260M), water bath, heater, beaker, volumetric titration column, glass ware, crucibles, muffle furnace (BIBBY Stuart, UK), Silica crucibles, desiccators, Soxhlet extractor, sand bath, Whatmann filter paper, pestle and mortar, burette, TLC paper, funnels, volumetric flask and pipette. FTIR (Perkin Elmer analyst700), UV-Visible spectro-photo meter (254nm UV lamp), IR instrument.

2.2. Plant Kenkese Sample Preparation and Sampling Techniques

The ripe Kenkese fresh fruit pods of plant material Berta Kenkese (*Abelmoschus esculentus*) were collected from Kumruk woreda at dulshitalo kebele and Assosa woreda at tsestehalo kebele. Andiha yiza of Gumuz Kenkese (*Abelmoschus ficulenus*) fresh fruit ponds were collected from Madura woreda and Debate woreda from Metekel Zone. The fruit pods were cleaned with distilled water and slices were made using a sharp knife.

The small pieces of pods with seeds were air dried for a week away from sunlight. The samples were ground and pulverized to fine powders using a porcelain mortar and pestle followed by electrical grinder. The dried and

powdered fresh fruit pond were labelled and collected separately into different sample holders as Berta Kenkese and Gumuz Kenkese. The powders were further passed through a 2mm sieve to obtain uniform finer powders. The uniform powdered samples were stored in a clean air tight glass container at room temperature, dark and dry place until required for further analysis. For each qualitative and quantitative phytochemical metabolite constituents one sample was used per species by mixed different study areas. Sampling techniques were just weighed appropriate solvent crude extract of vegetable fruit pond powder for evaluating the bioactive components with qualitative standard and UV-Vis and FTIR techniques in triplicate tests and mean values were recorded for accurate measurement and identification.

2.3. Kenkese Crude Extract Samples Preparation

Phytochemical crude extractions of fruit pod samples of each species separately were performed using organic solvent and aqueous extraction. The organic solvent extraction was extracted by Soxhlet extraction method by weighed 200gm of dried fruit powder extracted with 600mL of different solvents separately (Methanol, ethanol, petroleum ether, acetone, n-hexane, ethyl acetate).

The extraction process was carried on till the solvent

becomes colorless. After that the extract was heated on hot water bath at 35°C until all solvent evaporated. The dried fruit powder crude extract were kept in refrigerator at 2-8°C for their future use. The aqueous extraction was done by taking 5g of each species fruit powder sample mixed with 200mL of distilled water in a beaker. The mixture was heated on a hot plate magnetic stirrer at 40°C with continuous stirring for 20 minutes. The mixture was filtered using what man number 1 filter paper. The filtrates were then evaporated under reduced pressure and dried using rotary evaporator at 55°C and the filtrate condensed extracts were used for preliminary screening of phytochemicals.

2.4. Qualitative Phytochemical Screening

Preliminary phytochemical screening tests for the detection of bioactive components of each crude extracted species such as carbohydrates, phenols, flavonoids, alkaloids, terpenoids, steroids, tannins, saponins e.t.c were conducted using standard phytochemical qualitative identification methods as described elsewhere from prepared fruit pond sample of both Berta Kenkese and Gumuz Kenkese populations.

2.5. FTIR and UV-Vis Characterizations of Kenkese Crude Extracts

2.5.1. UV-Vis Spectroscopic Analysis

UV-Vis spectrophotometric analysis was conducted for both Berta (*Abelmoschus esculentus*) and Gumuz Kenkese (*Abelmoschus ficulenus*) ethanol extract using a UV-Vis spectrophotometer (perkin elmer, USA model; Lambda 950) with a slit width of 2nm, using a 10mm cell at room temperature. The extract was examined under Visible and UV light in the wave length ranged from 300-800nm. The peak values UV-Vis were recorded.

2.5.2. FTIR Spectroscopic Analysis

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule obtained from its absorption spectrum. A small quantity of ethanol extract

of both Berta and Gumuz Kenkese separately mixed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars with in 2min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Bruker, Germany vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm⁻¹ followed by peak values were recorded.

3. Results and Discussions

3.1. Qualitative phytochemicals Screening of Berta and Gumuz Kenkese

In this study, investigations of phytochemical screening were tested with different solvents for both Berta and Gumuz crude extract Kenkese samples separately. The results revealed the presence of 20 bioactive secondary metabolites. These results of preliminary phytochemical analysis were tabulated and summarized in the following tables (tables 2 and 3). The presence of these phytochemicals are an indicators of that the plant fruit pond can be a potential sources of precursors for the development of synthetic drugs which will cure various chronic diseases including cancer and diabetes.

As shown in table 2 the qualitative analysis carried out on the various extracts of Berta Kenkese fruit pond sample which confirms the presence of active phytochemicals constituents such as tannins, saponins, flavonoids, glycosides, phenols and alkaloids intensively. From the amount of precipitate formed and degree of color change, it could be deduced that the fruit pond contains tannins, saponins, glycosides and alkaloids in high concentrations. Among all the tested phytochemicals with different solvents in test fruit pod sample, the composition arranged in the order of terpenoids, saponins, steroids, sugar, tannin, phenolic compound, amino acids, alkaloids, flavonoids and glycosides. Similar phytochemical constituents in the crude extracts were recorded in various plants by Edeoga et al., 2005. Similar report was recorded from okra fruit pond crude extract by Ganpati and Nivruti, 2010.

Table 2. Results of qualitative phytochemicals screened of Berta Kenkese (*Abelmoschus esculentus*).

Bioactive constituents	Standard tests	Extractions Solvents					
		C ₂ H ₅ OH	Petroleum ether	acetone	CH ₃ OH	n-hexane	water
Tannin	Brayer's test	++	+	++	+++	+	+++
	Gelatin test	-	-	+++	++	-	++
saponins	Foam test	++	+	+	+++	+	+++
	Lead acetate test	+	+	++	+	-	++
	Alkaline reagent test	-	-	+	+	-	++
Flavonoids	Shinoda test	+	+	+	++	-	+
	Pew's test	+	+	-	++	-	++
	Ferric chloride test	++	+	+	-	-	++
terpenoids	Salkowki's test	++	+	++	++	+	+
steroids	Chloroform test	-	-	+	++	-	++
	Ferric chloride test	++	+	+	+	+	+++
phenols	Ellagic acid test	+	+	++	-	+	+
	Lead tetra acetate test	-	-	+++	++	-	++
	Wagner's test	++	-	+	+	-	++
alkaloids	Hager's test	++	+	++	+++	+	+++
	Mayer's test	-	-	+++	++	-	++

Bioactive constituents	Standard tests	Extractions Solvents					
		C ₂ H ₅ OH	Petroleum ether	acetone	CH ₃ OH	n-hexane	water
glycosides	Keller-Kilian test	-	-	-	-	-	+
	Liebermann's test	+	+	-	+	+	-
	Bromine water test	-	-	+	++	-	+
	Alkaline test	+	+	+	+	-	+
phylobatannins	Salkowaski test	++	-	++	+	+	+
	HCl test	-	-	+	++	-	++
anthraquinones	Born Trager's test	+	-	++	+	-	+++
carotenoids	H ₂ SO ₄ test	-	-	-	-	-	-
Reducing sugar	Fehling's solution test	-	+	+	+++	+	+++
cholesterol	H ₂ SO ₄ test	+	+	-	-	+	-
proteins	Biuret test	-	-	+++	++	-	++
Free amino acids	Ninhydrin test	++	+	+	+++	+	+++
carbohydrate	Benedict's test	++	+	++	+++	+	+++
oxalate	Ethanoic acid test	++	+	+	+++	+	+++
Volatile oils	NaOH test	-	+	-	-	+	-
Carbonyls (aldehydes)	2,4-dinitrophenyl hydrazine test	-	-	+	++	-	-

(+)-low presence (++)-moderate presence (+++)-high presence abundant (-)-absent

Table 3. Results of qualitative phytochemicals screened of Gumuz Kenkese (*Abelmoschus Ficulenus*).

Bioactive constituents	Standard tests	Extractions Solvents					
		C ₂ H ₅ OH	Petroleum ether	acetone	CH ₃ OH	n-hexane	water
Tannin	Brayer's test	+	-	++	+++	-	+++
	Gelatin test	-	-	+++	++	-	++
saponins	Foam test	++	+	+	++	+	++
	Lead acetate test	++	-	++	+++	-	+++
Flavonoids	Alkaline reagent test	-	-	++	++	-	++
	Shinoda test	++	+	+	+++	+	+++
	Pew's test	++	-	+	+	+	++
terpenoids	Ferric chloride test	++	+	+	+++	+	+++
	Salkowki's test	+	+	+	++	+	++
steroids	Chloroform test	-	-	+++	+	-	++
	Ferric chloride test	++	+	-	+++	-	++
phenols	Ellagic acid test	++	+	++	++	+	+
	Lead tetra acetate test	-	-	+++	++	-	++
alkaloids	Wagner's test	++	-	+	++	+	++
	Hager's test	++	+	++	+	+	+
	Mayer's test	-	-	++	++	+	++
	Keller-Kilian test	++	+	+	++	+	+
glycosides	Liebermann's test	++	+	++	+	+	++
	Bromine water test	-	-	+++	++	-	++
	Alkaline test	++	+	+	+	+	+++
phylobatannins	Salkowaski test	++	+	++	++	+	+++
	HCl test	-	-	+++	++	-	++
anthraquinones	Born Trager's test	++	+	++	++	+	+
carotenoids	H ₂ SO ₄ test	-	-	+	+	-	-
Reducing sugar	Fehling's solution test	++	+	+	+++	+	+
cholesterol	H ₂ SO ₄ test	++	+	++	-	+	-
proteins	Biuret test	-	-	+++	++	-	++
Free amino acids	Ninhydrin test	++	+	+	+	+	++
carbohydrate	Benedict's test	++	+	++	+	+	+
oxalate	Ethanoic acid test	++	+	+	+++	-	+
Volatile oils	NaOH test	++	-	++	++	-	++
Carbonyls (aldehydes)	2,4-dinitrophenyl hydrazine test	-	-	+++	++	-	-

(+)-low presence (++)-moderate presence (+++)-high presence abundant (-)-absent

All of the phytochemicals were not detected by different standard methods with various solvents. The detection results high, moderate, average and absent the phytochemicals detected due to the difference in polarity of solvents and presence and absence of phytochemicals. From the tables 2 and 3, water is the better solvent than any other types of organic solvents for extraction of bioactive chemicals from fruit pond sample. As shown from table 3,

similar results and explanations were found for Gumuz Kenkese qualitative test.

3.2. Qualitative Identification of Phytochemicals Kenkese by FTIR Spectroscopy

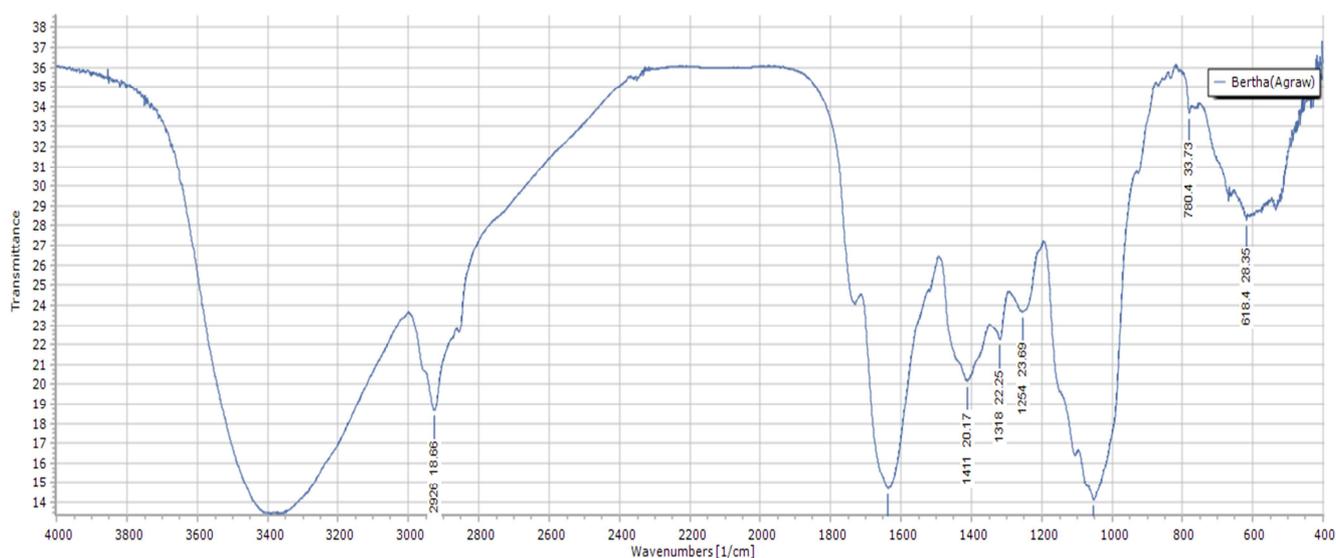
Many researchers applied the FTIR spectrum as a tool for distinguishing the medicinal plan samples based on their

chemical constituents. FTIR spectrum can be used to confirm the functional constituents present in the medicinal plant fruit pond of Kenkese. In FTIR spectroscopy powdered crude ethanol extracts of fruit ponds of both Berta and Gumuz Kenkese were loaded I spectrum Perkin Elmer 65 FTIR in the scan range of 4000-400cm⁻¹ using KBr pellets with a resolution of 4cm⁻¹. ASCII file for samples were measured and was converted into %transmittance and/or absorbance versus wave number and /wavelength FTIR spectrum graphs by spectra Gryph 1.1-spectroscopy software. The FTIR

spectrum graphs for both Berta and Gumuz Kenkese fruit pond samples are shown in the following figures 1 and 2. The major peaks and functional of dynamic compounds groups were analyzed as shown in the following tables 4 and 5 and results were compared with standard infrared chart. The results of the present studies of FTIR spectrum revealed the functional constituents present in the crude powder ethanol extract of Berta Kenkese (*Abelmoschus esculentus*) were identified as shown in table 4 based on the peak values of figure 1.

Table 4. FTIR spectrum peak values and functional group analysis for ethanol extract Berta Kenkese fruit pond (*Abelmoschus esculentus*).

S. I	Characteristic absorption wave number (cm ⁻¹)	Types of peak intensity	Type of bond vibrations	Functional groups
1	3851	weak	Aromatic O-H stretching	Alcohols and phenols
2	3600-3200	Strong, broad	N-H stretching=O stretch, asymmetric O-H vibration (S)	Amines and Amides, Sulphonic acids, alcohols
3	3000	Weak, sharp	Asymmetric C-H (s) of aromatic nucleus	Esters, carboxylic aromatic group
4	2926	Weak, sharp	Symmetric C-H (s) of aromatic nucleus, asymmetric C-H vibrations of CH ₃ (aliphatic)	Aromatic group, secondary amines, proteins, lipids, alkanes
5	2350	weak	N-N triple bond vibrations	Nitriles
6	1730	weak	Carboxylic, Carbonyl group vibrations	Aldehyde, ketone, Quinone, amino acids
7	1700-1650	strong	C=C, C=O, N-H(b) vibrations	Esters, lactones, phenols, carbonyl unsaturated Ketone, amide, alkenes
8	1500	Medium sharp	C=C, C-N vibrations	Alkenes, nitro compounds, aromatic
9	1411	Medium broad	C=C (aromatic) vibrations	aromatic
10	1350	Short, weak	C-H, C=O, C-X, S=O stretch	Alkanes, aldehydes, sulphoxides, fluorides
11	1318	Sharp, weak	N-N vibrations	Nitro compounds
12	1300	weak	-	-
13	1254	short, weak	C-X, C=O- stretch	Fluorides, alkyl halide, carboxylic acids, acid anhydrides
14	1200	Medium, sharp	COOH, COOR vibrations	Sulphonate esters
15	1100-1000	Strong, broad	C-O stretch, COOH stretch, S=O stretch	Ethers, esters, alcohol, aliphatic amines, sulphone amides
16	850	Weak, broad		Alkyl halide, aliphatic amines
17	780 and 750	weak		Aromatic compound, Primary and secondary amines
18	667	weak		Halogen compounds
19	600	weak, broad		Alkyl halide
20	550-400	Weak, overlapped		Alkyl halide, halogen, aryl disulphides



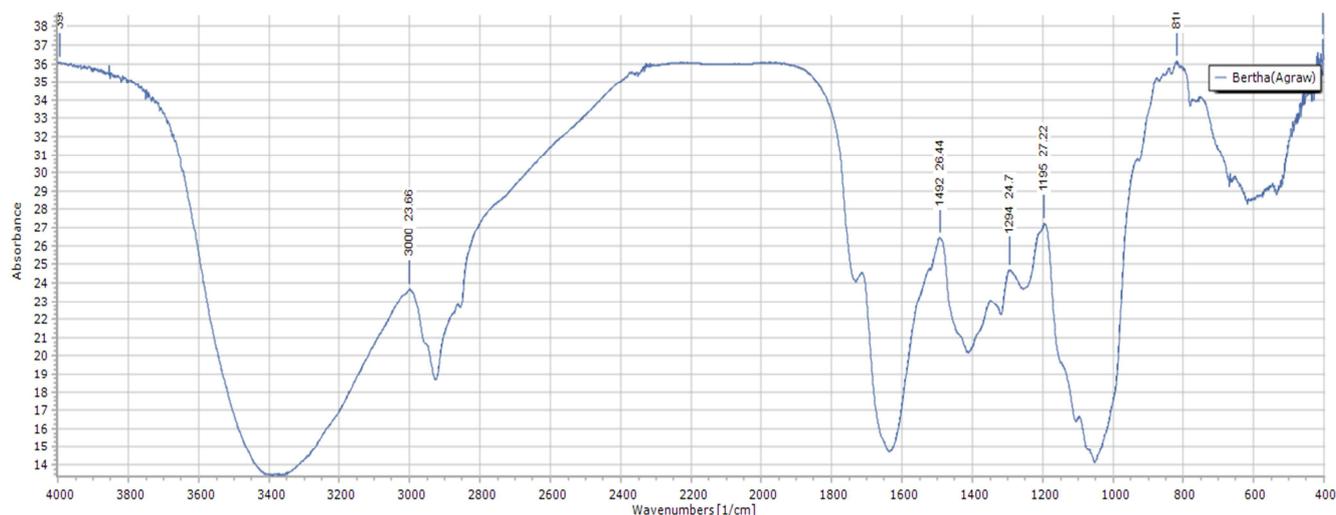


Figure 1. FTIR spectra of Berta Kenkese absorbance versus wave number (bottom) and transmittance versus wave number (top).

As the FTIR spectrum results of Berta Kenkese shown from figure 1 and Table 4, significant for the identification of the source of an absorption band are intensity of peaks (weak, medium or strong), shape (broad or sharp) and position (cm^{-1}) in the spectrum. Spectral data of most of crude extracts of Berta Kenkese fruit pod confirmed the presence of bioactive functional groups such as $-\text{OH}$, $-\text{NH}$, $-\text{CHO}$, $\text{C}-\text{O}$, $\text{C}-\text{N}$, $\text{C}=\text{C}$, $\text{S}=\text{O}$, $\text{C}=\text{N}$, $-\text{COOH}$ and $-\text{COOR}$. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines,

amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, isonitriles, organic halogens and carbohydrate could be responsible for the various medicinal activities of Berta Kenkese (*Abelmoschus esculentus*). The functional groups present in Berta Kenkese are aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids, anhydrides, esters, lactones, ethers, nitriles, isonitriles, quinones, organic halogen compounds and carbohydrates as show in table 4.

Table 5. FTIR spectrum peak values and functional group analysis for ethanol extract Gumuz Kenkese fruit pond (*Abelmoschus ficulenus*).

S. I	Characteristic absorption wave number (cm^{-1})	Types of peak intensity	Type of bond vibrations	Functional groups
1	3851	weak	Aromatic O-H stretching	Alcohols and phenols
2	3400	Strong, broad	N-H stretching, N=O stretch, asymmetric O-H vibration (S)	Aliphatic primary amines and Amides, Sulphonic acids
3	2996	medium, sharp	CH_2 -stretch	Alkanes, alkenes
4	2900	Weak, sharp	Symmetric C-H (s) of aromatic nucleus, asymmetric C-H vibrations, carbonyl	Aromatic group, secondary amines, proteins, lipids, alkanes
5	2850	weak	C-H stretch	Alkanes, carbonyl
6	2367.4	weak	$\text{C}=\text{C}$ -stretch, $\text{C}=\text{N}$ -stretch	Alkenes, carbamine
7	1700	Strong, sharp	$\text{C}=\text{C}$, $\text{C}=\text{O}$ vibrations	Aldehydes, carboxylic acids, alkenes
8	1488	Medium sharp	Aromatic $\text{C}=\text{C}$, $\text{C}-\text{N}$ vibrations	Alkenes, nitro compounds, aromatic
9	1450	Medium broad	$\text{C}=\text{CH}_2$ (aromatic), N-vibrations	Aromatic, nitrocompounds, sulfoxo compounds
10	1349.3	Short, weak	C-H, C=O, C-X, S=O, N-O stretch	Alkanes, aldehydes, sulphoxides, fluorides-O aliphatic nitro compounds
11	1300	Sharp, weak	N-N, C-N vibrations	Nitro compounds, secondary alcohols
12	1250	medium	$\text{C}=\text{O}$, COOH , COOC stretches	Aromatic amines, carboxylic acids, ethers, aliphatic organohalogen compounds
13	1193	Medium, weak	N-N stretching	Nitro groups and halogen
14	1100-1000	Strong, broad	COOH , COOC , $\text{R}-\text{CH}=\text{CH}-\text{R}$, CH -wag CH_2X alkyl halide vibrations	Sulphonate esters, carboxylic acid, primary alcohol, alkyl halides, aliphatic amines, ethers
15	859	weak	CH_2 -aromatic ring	Parabenzene, alkenyls
16	822	Weak	aromatic	Aromatic P-disubstituted compounds
17	800	strong	Aromatic rings	Aromatic compounds
18	749	weak	C-Cl stretching, metabenzene	Halogen compounds, meta halobenzenecompounds, aromatic compound
19	667	weak, broad	C-X stretching	Alkyl halide
20	550-400	Weak,	Aromatic C-X stretching	Alkyl halide, halogen, aryl disulphides

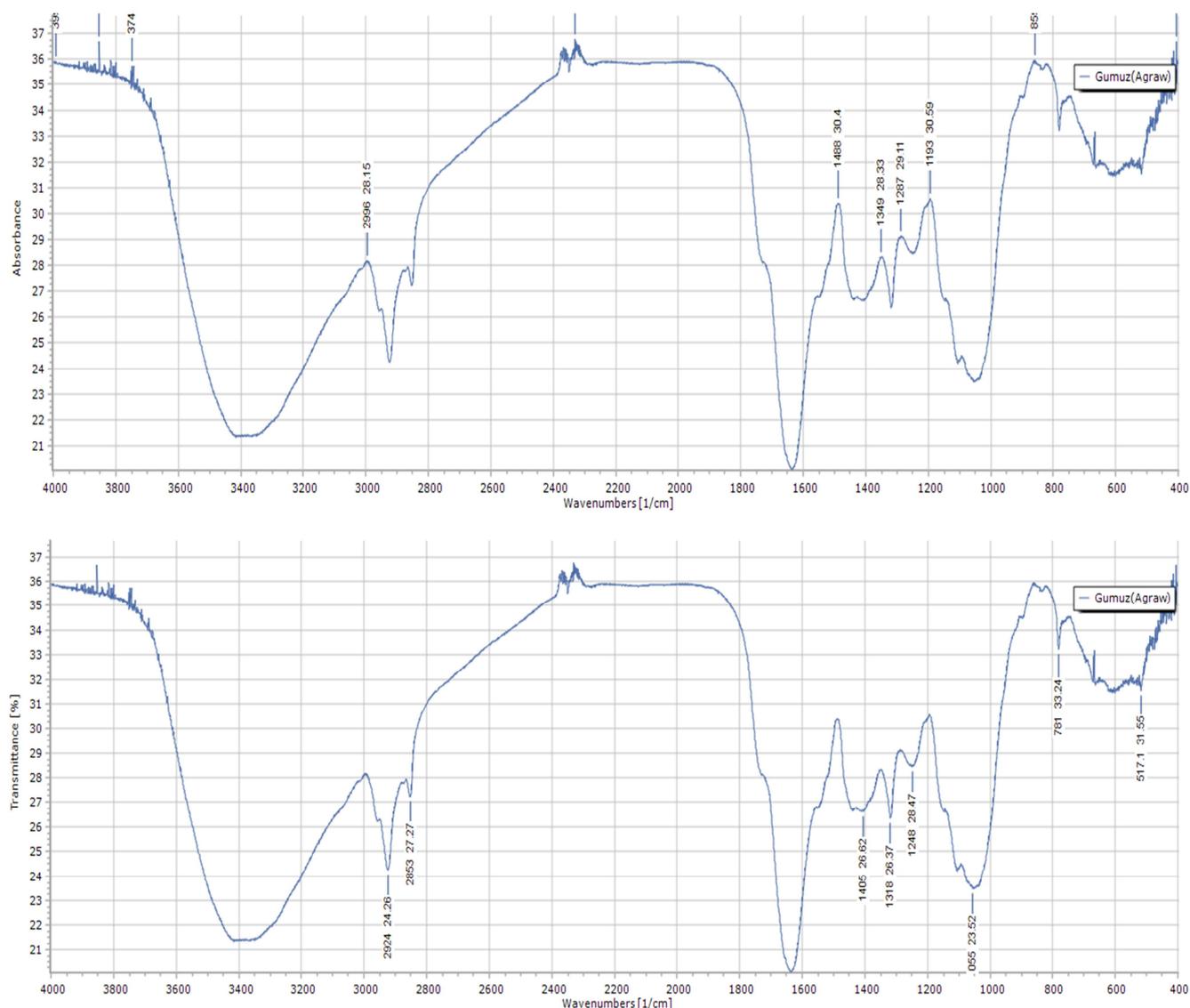


Figure 2. FTIR spectra of Berta Kenkese absorbance versus wave number (bottom) and transmittance versus wave number (top).

All of these functionalities are bioactive phytochemicals which are responsible for medicinal and pharmacological values of the fruit pond. These indicate Berta Kenkese fruit pond is rich source of phytochemicals such as glycosides, tannins, phenols, flavonoids, alkaloids, saponins, steroids and terpenoids which can solve both the nutrient and health security problems of consumers. These bioactive compounds and functional groups confirm pharmacological activities of Berta Kenkese and provide strong evidence as economic and safe alternative to treat chronic disease such as anti-cancer, anti-oxidant, anti-diabetes and anti-microbial treatments which can serve as a vital nutritional and mineral rich food for good health.

As shown in table 5 and figure 2, this study was significant for the identification of the source of an absorption band are intensity (weak, medium or strong), shape (broad or sharp), and position (cm^{-1}) in the spectrum. Spectral data of most of the crude fruit pond extracts of Gumuz Kenkese confirmed the presence of bioactive functional groups such as $-\text{OH}$, $-\text{NH}$, $\text{N}=\text{O}$, $\text{C}-\text{H}$, $\text{C}=\text{O}$, $\text{C}=\text{CH}_2$, $\text{C}-\text{X}$, CH_2X where X is halide groups, $\text{N}-\text{N}$, COOC , $\text{R}-\text{CH}=\text{CH}-\text{R}$, CHO , $\text{C}-\text{O}$, $\text{C}-\text{N}$, $\text{C}=\text{C}$,

$\text{S}=\text{O}$, $\text{C}=\text{N}$, $-\text{COOH}$, aromatic rings meta benzene and $-\text{COOR}$. The presence of characteristics functional groups of carboxylic acids, alkenes, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, quinones, nitrates, nitriles, isonitriles, organohalogen and carbohydrates could be responsible for the various pharmacological properties of Gumuz Kenkese (*Abelmoschus ficulenus*). All these compounds belong to secondary plant bioactive metabolites which can treat various chronic ailments. These indicate the Gumuz Kenkese fruit pond is rich source of phytochemicals with the presence of glycosides, tannins, phenols, flavonoids, alkaloids, saponins, steroids and terpenoids. FTIR spectra showed the presence of the functional group in extracts which have medicinal activities and can be used as antibacterial, antiviral, antidiabetic and anticancer agents. The studies also provide a strong evidence for the use of fruit extract to treat various diseases. The use of this fruit pond as a traditional medicine may be an alternative to synthetic drug and health organic diet which is both economical and safe curing system for ailments. This study

supports that traditional medicines are still used by peoples so that established with the scientific chemical composition by identified the responsible functional groups.

As shown from figure 3 below, the combined confirms almost all the peaks of Gumuz and Berta Kenkese overlaps in their peaks. This revealed both Berta and Gumuz Kenkese have almost comparable phytochemical active functional

groups and chemicals. However the only difference is the intensity of peaks of Berta Kenkese is stronger than peaks of Gumuz Kenkese confirms the quantitative phytochemicals concentration of Berta Kenkese were greater than Gumuz Kenkese. From the study it can be concluded that Berta Kenkese has more pharmacological activities than Gumuz Kenkese due to concentration of phytochemicals.

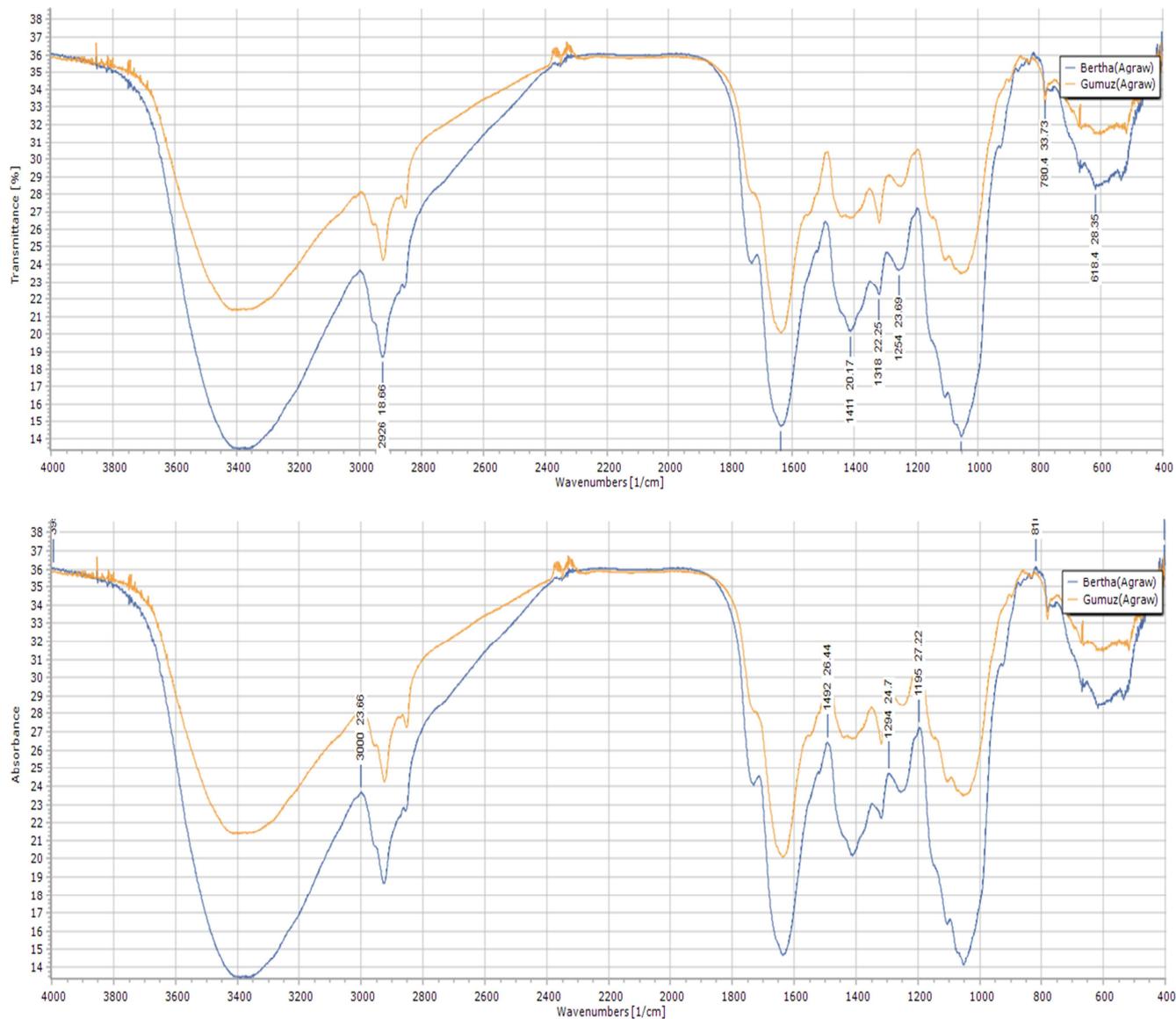


Figure 3. Combined FTIR spectra of Berta and Gumuz Kenkese absorbance versus wave number (bottom) and transmittance versus wave number (top).

3.3. Qualitative Identification of Phytochemicals of Kenkese by UV-Vis Spectroscopy

The ethanol extract of crude fruit ponds of both Berta and Gumuz Kenkese were examined by UV-Vis spectral analysis. From spectra results confirmed the presence of flavonoids and tannins in both species fruit pond crude extract samples with different intensities as shown in appendix figure 10. The flavonoids spectra typically consists of two absorption maxima in the ranges 221-600nm (band I) and 300-350nm

(band II). The precise position and relative intensities of these maxima gives valuable information on the nature of flavonoids. The result of UV-Vis spectroscopic analysis confirms the presence of tannins and flavonoids in the fruit pond extract of both Beta and Gumuz Kenkese samples. However, the peaks in the spectra of Gumuz Kenkese fruit pond was intense than Berta Kenkese fruit pond sample which revealed high concentration of flavonoids and tannins in Gumuz Kenkese fruit pond than Berta Kenkese fruit pond samples. This supports the qualitative test results of both fruit pond species.

4. Conclusion and Recommendations

Kenkese is one of the most economical, nutritional and medicinally important traditional vegetable crops cultivated in temperate and tropical regions which have been originated from our country Ethiopia in Benishangul Gumuz region. The qualitative standard experimental tests confirmed the presence of 20 active phytochemical compositions such as tannins, alkaloids, steroids, anthraquinones and flavonoids e.t.c in both Berta and Gumuz Kenkese species. The FTIR and UV-Vis characterization techniques confirmed the presence of major functional groups found in pharmacologically active phytochemicals and intense peaks of chemical functionalities peaks of in both species confirms high concentration of phytochemicals found in Kenkese. Results of both Kenkese species revealed the presence of many of secondary metabolites that is recommendable to use Kenkese as nutritious food source in line with its medicinal importance. This studied research information presented here shows the potential nutritional importance of both Berta and Gumuz Kenkese and its role in improving nutrition and health security. The data may support future multidisciplinary studies and promote rational use of the plant as a therapeutic resource. Further analytical and biological researches needs to be performed to provide compelling evidence for the direct consumption health benefits of Berta and Gumuz Kenkese.

Therefore, promoting the consumption of traditional vegetables such as the studied Kenkese could provide cheap sources of macro and micronutrients and mineral elements that can improve the nutritional status of resource-poor subsistence farmers in the area in particular and in Ethiopia in general. Furthermore, this vegetable can also be used as an indispensable tool when it comes to reducing the prevalence of malnutrition, especially among resource constrained urban households in addition to rural household in the regional and national level. Consumption of Kenkese by both low-income and high-income groups can also use as a means of dietary diversification approach. Evidenced from this research finding and from similar giant researchers across the world, it was

concluded both Berta and Gumuz Kenkese (*A. esculentus* and *A. ficulenus*) are best sources of pharmacologically active compounds for treatment of chronic ailments i.e. as an antioxidant, anti-inflammatory, antibacterial, & anticancer activities. However; result provides more scientific research is necessary to find out pharmacological & nutritional potential of Kenkese i.e. quantitative nutritional constituents, phytochemicals, vitamins, minerals & structural elucidation of each specific compounds.

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Conflicts of Interest

The author declares no conflict of interest.

Appendix

Title of Journal: Analysis of phytochemical composition of Kenkese (*Abelmoschus esculentus* and *Abelmoschus ficulenus*) indigenous food of Berta and Gumuz for the sake of health and food security in BGR, Ethiopia.



Figure 4. Kenkese fruit pod Sampling & Sample preparation.

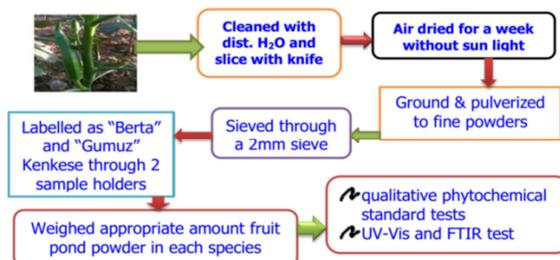


Figure 5. Kenkese organic solvent crude extract samples preparation.

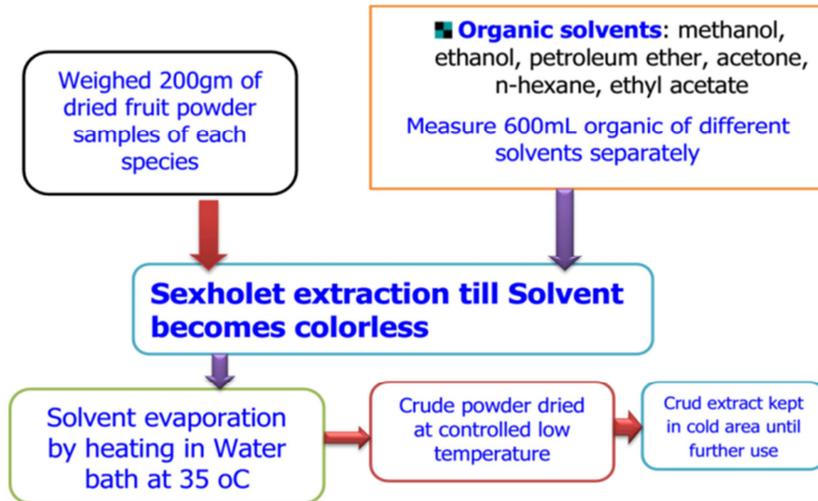


Figure 6. Kenkese aqueous solvent crude extract samples preparation.

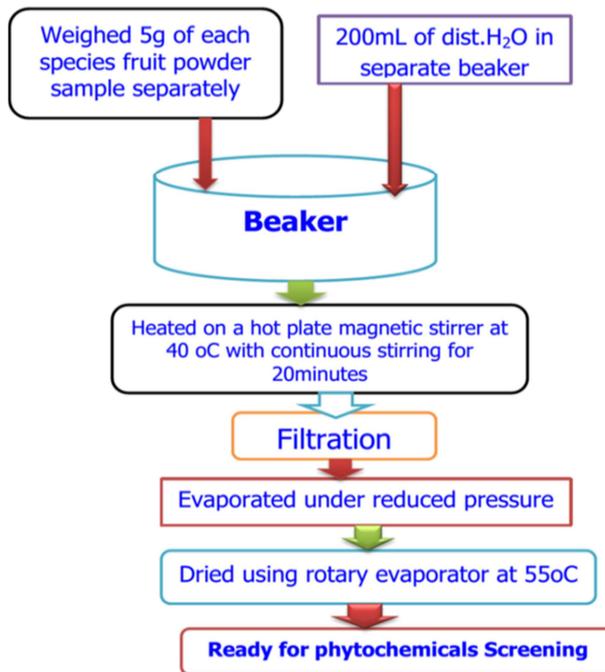


Figure 7. Sample preparation for phytochemical screening.

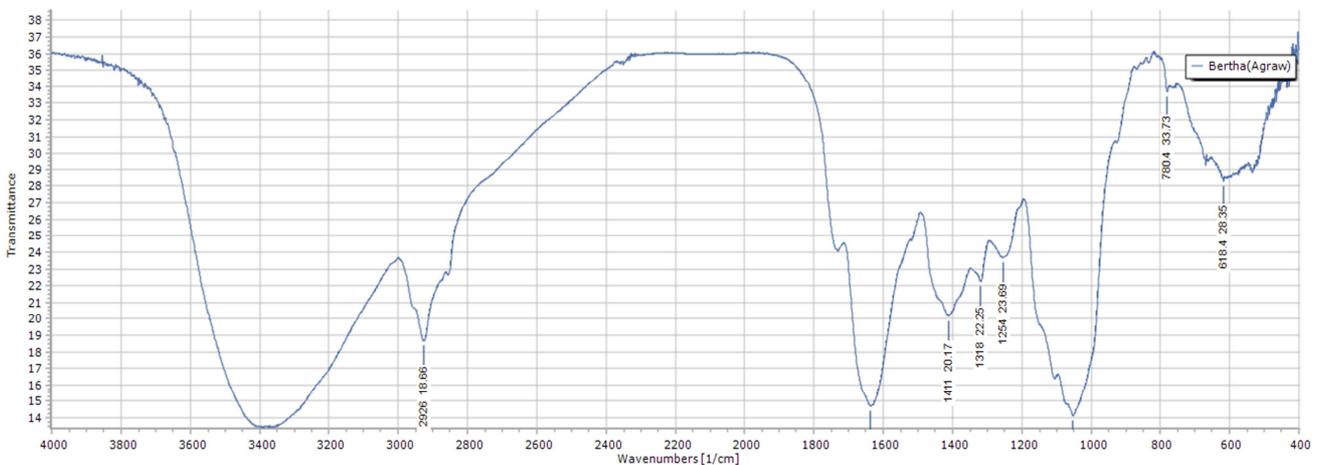


Figure 8. FTIR spectra of Berta Kenkese: transmittance versus wave number.

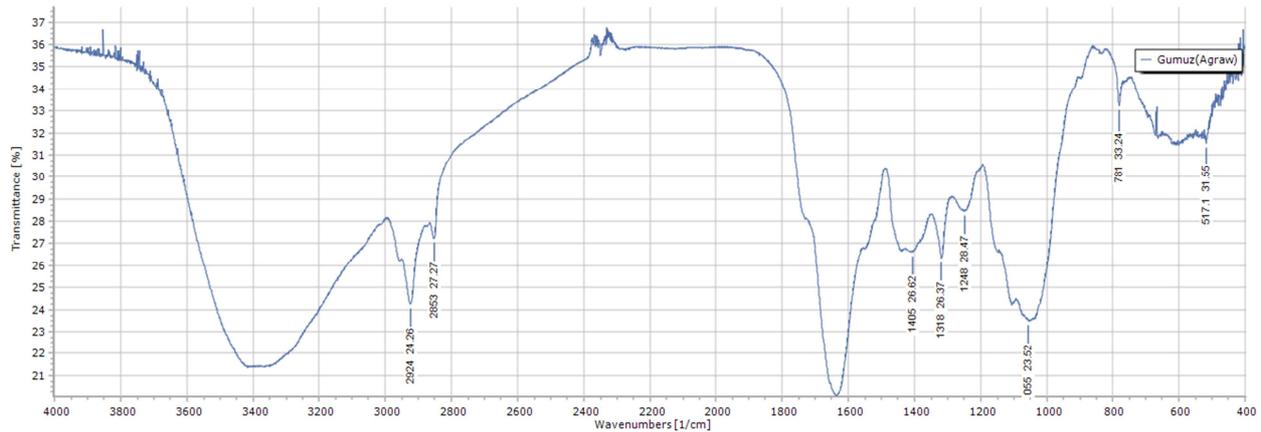


Figure 9. FTIR spectra of Gumuz Kenkese: transmittance versus wave number.

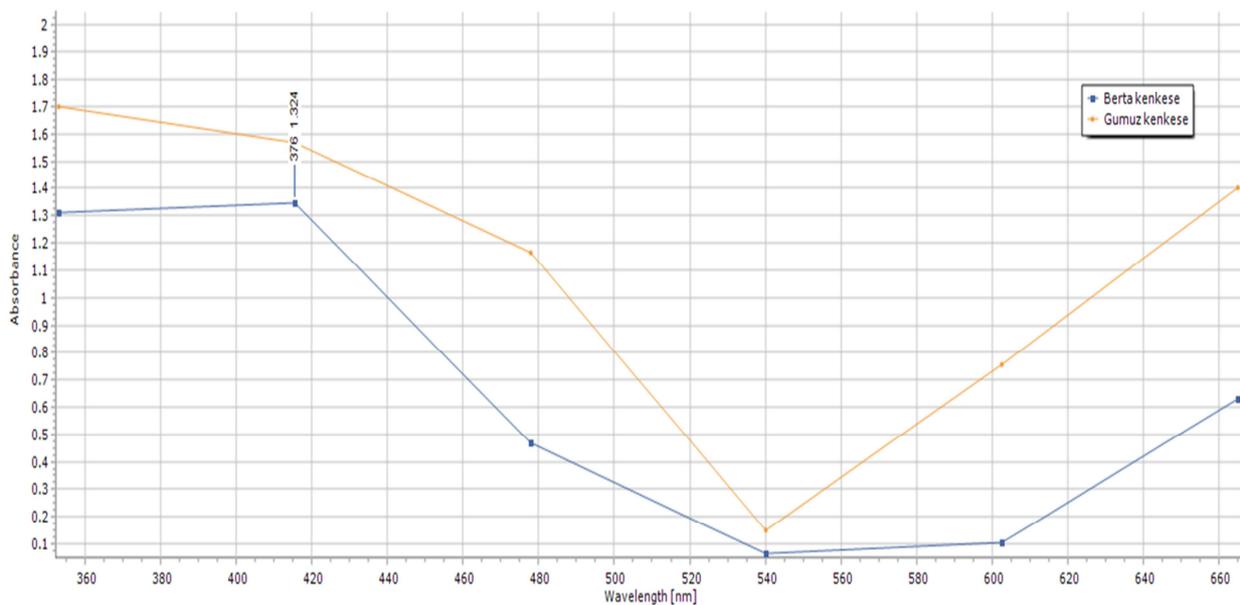


Figure 10. Combined UV-Vis spectra of Berta & Gumuz Kenkese samples in ETOH.

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