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Research Article

Characterization of Roselle (*Hibiscussabdariffa L.*) Fruit Extract in Some Parts of Nothern Nigeria

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Abstract

This study explored the valuable components from the different varieties of Roselle (zoborodo) extract, it also focuses on the assessments of the nutritional value of the extract due to its constant utilization as soft drinks in localities to replace conventional soft drinks such as coca cola and similar drinks. Analysis showed that the dried red sample contain 0.08 mg/g reducing sugar while the dried white sample showed 0.098 mg/g as compared to fresh red sample that showed 0.096 mg/g, but fresh white sample has only 0.081 mg/g. Organic acids of the extract showed the dried red and white samples has 15.5 mg/g and 15.25 mg/g ascorbic acid, and the fresh red and fresh white showed 14.3 mg/g and 14.0 mg/g of ascorbic acids respectively. Citric acid content of dried red showed 1.88 mg/g > 0.58 mg/g dried white samples. Tartaric acid of dried red sample showed 2.0 mg/g > 0.88 mg/g in dried white samples. Other component such as pectin showed that both fresh red and dried red samples have the same values of 0.6mg/g > fresh white 0.58mg/g > dried white sample with 0.40mg/g. Tartaric acid showed 2.0 mg/g and 0.67 mg/g for dried red and dried white samples, while citric acid values of dried red and dried white showed 1.88mg/g and 0.58mg/g accordingly. This study indicated that the values of ascorbic acid, citric acids and other essentials components of Roselle extract are within the recommended food supplements for body metabolism and it should be utilized as common drinks.

Keywords: Roselle; Organic acids; Reducing sugar; Tartaric acid; Ascorbic acid; Pectin

Introduction

Hibisccussabdariffa fruit extract known as Sorrel or Roselle is widely consumed as a vegetable drinks. It is commonly known as Zobo drink in tropical countries especially Nigeria. The fruit extract of sorrel serves as a cheaply utilized drinks that substituted soft drinks like Coca Cola, Mirinda etc. These were due to its nutritional values of Reducing Sugars, Vitamin C (Ascorbic Acid) and pectin content (Janick, 1974).

Roselle extract drinks contain phosphoric acid, malic acid, citric acid, and tartaric acid which corrode the surface of teeth and cause many dental problems and osteoporosis. These shortcomings can be avoided by taking naturally blended fruit juice or beverages such of Roselle. Foods and beverages that originated from plants have been reported by (Serifat and Anthony 2020) to be free from saturated fats, sugars, and salts and therefore prevent the build-up of some chronic disease conditions. Roselle drink has been gaining popularity globally as a refreshing medicinal drink. Calyces of *Hibiscus sabdariffa* are the major raw material for the drink (Serifat and Anthony, 2020).

The red acid in Roselle known as *Varsabdariffa* are boiled with water, sugar and flavor was added to produce sorrel drink locally known as Zobo drink. The flowers are used in jellies and confectionaries while its seeds are roasted for meals as they contain oil which also used as medicine (Purseglove, 1968).

Composition of the Fruit Extract of Roselle (Zobo Drink)

The fruit extract contains approximate amount of foods substances which include water 84.5%, protein 1.7%, lipids/ fats and oil 1.0% and carbohydrates 12%. However, certain organic acids are about 4%. Citric Acids are present in edible calyx extract of the fruit as reported by (Serifat and Anthony, 2020). Nutritional values of Roselle vegetables showed that they are used as a major diet for young children as they contain active growing tissues due to presence of protein, calcium, iron, carotene, ascorbic acids and many others important constituents (Janick, 1974).

Recent research reported that 100g of the calyces had 49 J energy, 84.5% water, 1.99 mg protein, 0.1g fat, 12.3g carbohydrate, 2.3g fiber, 1.2g ash, 1.72mg calcium, 57mg phosphorus, 2.9mg iron, 300µg vitamin A, and 14mg of vitamin C (Serifat et al., 2020). It was also found that Roselle calyces contain a high amount of vitamins (especially vitamin C), carbohydrate, protein, antioxidants, and also minerals (Serifat et al., 2020). In addition, the extracts of Roselle have been reported to contain phytochemicals, vitamins, and several minerals (Okereke et al., 2015)

Important uses of the Fruit Extract of Roselle (Zobo Drink)

Fresh or dried Roselle fruit extract of *H. sabdariffa* are used traditionally in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, flavoring agents, puddings and cakes as reported by (Bakoet al., 2009). Significant amount of the Roselle fruit extract is used to produce liters of soft drink (Zobo drink) as 100g of the fruit can produce 4 or more litters of hibiscus tea (Serifat and Anthony, 2020). The constant rate of consuming the Zobo drink is because of its nutrient and medicinal values, and it is cost-effective, readily available, easy to prepare, and also because of its good taste, aroma, palatable and attractive colour (Shruthi and Ramachandra, 2019). Roselle leaves and calyx are used for animal fodder and fiber. The seeds can be used to feed poultry as well as sheep, and the residue from the seeds oil extraction can also be used to feed cattle and chicks (Plotto, 2004).

Materials and Methods

Sample Collection

Eight different samples of red and white sorrel fruits were collected. Four samples from Gombe (a state in north eastern part of Nigeria) and four samples from Maiduguri (A capital city of Borno State also in north eastern part of Nigeria). Two samples of dried red and white were collected at the outskirts of Gombe, while the other two red and white samples were collected in the town center about 10km apart. Also, the same method was adopted to Maiduguri fresh red and fresh White samples. The two common samples from difference locations were mixed together to form represented fractions which was used for investigations of valuable components as adopted by (Usman et al, 2020).

Steps in preparation of dry *Hibiscussabdariffa*.

i. First, collect the hibiscus fruits and wash them clean, and air dry or dry them in an oven at 70 degree C for 3days.
ii. Peel off the calyx and store them in air-tight containers.
iii. To make it dry, simply take 2grams of the dried calyx, and crash them into small pieces using a wooden roller.
iv. Put them in a net, bring out your favorite mug, add 8 drop of boiling water, steep it for 2-4minutes, add sugar if desired, or add other flavors of your choice such as few drops of lemon juice.
v. You can also refrigerate it and make it dried.

Source: Serifat, et. al. (2020).

Preparation of Water extract of Roselle Fruit Samples

Thirty grams of both dried and fresh samples were separately placed in 400cm^3 beakers. About 200cm^3 of distilled water was added to the sample. The mixture was boiled for 1hr over hot plate at several time, lost molecules were replaced during evaporation. The solution was the allowed to cooled and transferred to a volumetric flask and stored in refrigerator for further analysis of constituents (Neish, 1952).

Pectin Determination from Roselle extract

About 100 cm^3 of the Roselle extract sample was transferred into a beaker and place over hot plate that evaporated some portion of water on heating. The solution was then allowed to cool then 3cm^3 of 0.5 M H_2O was added immediately, followed by 100 cm^3 of alcohol with constant stirring to precipitate pectin. The precipitate of pectin formed was allowed to settle. Filtration and washing with alcohol were carried out. The washed precipitate of pectin was transferred into a tired small beaker and was dried in an oven at 100^{o}C at constant weight. The filtrate was reserved for organic acids determinations. Amount of pectin was calculated from 100g of the original sample and recorded (Janick, 1974).

Organic Acids Determination from Roselle extract

About 10 cm^3 of the alcoholic filtrate was pippeted into a conical flask and then titrated to end point with 0.1 M N_aOH using phenolphthalein indicator. The solution from end point was then mixed up with another 200cm^3 of alcoholic filtrate and 2 cm^3 of 1.0 M N_aOH solution was added and placed on steam bath for 30 minutes. It was cooled at room temperature and 5 cm^3 of 1.0 M acetic acid was added, 0.6g of powdered lead acetate were added. This was the required amount to form Pb (lead) salt of Organic acids in 100cm^3 of the filtrate. The mixture formed precipitated immediately.

The precipitate of the Pb salt formed was dispersed with a 50cm^3 H_2O and diluted to about 150 cm^3, then saturated with H_2S to liberate the organic acids when inserted into the mixture. The Pb precipitate became Pb salt. The solution was shaken for 1 minute and rinse into a 250cm^3 volumetric flask and was diluted to mark with water. Filtration was carried out to recover the darker colour of the solution. Twenty fivecm^3 of the clear filtrate which contain the organic acid was pippeted into a conical flask and was treated with 0.1 M N_aOH using phenolphthalein to end point. Three titrations were carried out considering the mean titer value for the two duplicate readings which were recorded.

The amount of organic acids were determined to calculate the content of pectin, reducing sugar, ascorbic acid (Vitamin C), tartaric acid and many others using the formula.

$$10^2XV1200 \text{ cm}^3 \text{ of 0.1 M } N_aOH = V1V220 \text{ cm}^3 \text{ of 0.1 M } N_aOH$$

Where V1= 200cm^3 of water extract of the sample V2 = mean titer value (Neish, 1952).

Reducing Sugar Determination from Roselle extract

Reducing sugars can be determined as glucose by any of the reduction methods. Volumetric methods were carried out as 5 cm^3 of the test solution was mixed with 3 g of glucose D then 5 cm^3 of volumetric copper reagent was added and then heated in a water bath for 15 minutes. The solution was removed and cooled in a water bath. Then 2 cm^3 of KI solution and 1 cm^3 of 1.5 M H_2SO_4 were added. The liberated iodine was titrated with standard 0.1 M Thiosulphate solution with a starch indicator to end point. The blank solution was carried out in the same way using 5 cm^3 of H_2O instated of the test solution as adopted by (Neish, 1952). Calculated amount of reducing sugar was determine using the formula.

$$WV^2{-}V1 \text{ mg of glucose}$$

V1 = Thiosulphate (cm^3) for blank.
V2 = Thiosulphate (cm^3) for standard solution.
W = weight of glucose standard tube.

Therefore 1cm^3 of Thiosulphate solution = $WV^2 – V1$ mg of glucose (Neish, 1952).

Ascorbic acid (Vitamin C) Determination from Roselle extract

The process was carried out in three stages as oxidation, extraction and colour development. Oxidation was carried out where 25cm^3 of organic acid was measured then placed in a beaker and 3.0 cm^3 of 1.1 H_2SO_4 solution was added. Some anti-bumping granules were added and boiled that reduce the volume to about half and some interfering substances were decomposed and cooled at room temperature. An excess bromine was added and left for 20 minutes that precipitated. The content was transferred to a separating funnel for separation. Then 2cm^3 of 1.0 M KBr solution and 10cm^3 of 0.3 M $KMnO_4$ was also poured into the separating funnel. The mixture was left to stand for 10minutes, later 3% H_2O_2 solution was carefully added that decolorized the excess permanganate. Thus, citric acid has now been converted to Penta-bromo acetone as adopted by Shruthi and Ramachandra (2019).

Oxidized mixture was extracted upon constant shaken in the separating funnel with 25cm^3 of petroleum ether, the aqueous layer was withdrawn after washing the ethereal layer with 25cm^3 portion of water. The aqueous layer was then transferred to another separating funnel and re extracted as before with 25cm^3 of petroleum ether. The petroleum ether extracted was combined and washed two times with 10cm^3 water. The petroleum ether contain Penta-bromo acetone.

Colour development was done by shaken petroleum ether of Penta-bromoacetone with freshly filtered 4% aqueous solution of sodium Sulphate. The Sulphide solution was not more than two days old. Successive withdrawal of 3cm^3, 2cm^3 and 1cm^3 quantities into 10 cm^3 measuring cylinder containing 3.5 cm^3 of redistilled pyridine (Pb 112-117°C) was made to 10 cm^3 with 1:1 dilution of the same pyridine with water. Later spectrophotometric reading was recorded within 30 minutes and the wave length of maximum absorption was also recorded to determined amount of ascorbic acid present as reported by (Shruthi and Ramachandra, 2019).

Tartaric Acid Determination from Roselle Extract

About 10cm^3 of the extract of organic acids was placed in a beaker and pH value of the extract was adjusted using addition of dilute acids as the case may be. Then 0.5 g of 1% ferrous sulphate solution and 0.5cm^3 of 3% H_2O_2 solution were mixed thoroughly. Yellow solution formed was transferred quantitatively to a 50cm^3 volumetric flask. It was left to stand for few minutes until the solution became brownish and was placed in an ice bath. The brown colour disappeared and the solution colour left turned to pinkish and purple. At this instance 15cm^3 of 1.0 M N_aOH solution was added and made to mark with water. It was then placed in a stopper flask with several shaken. The flask was then placed in ice bath again for 10 minutes and the solution was then mixed and inverted and filtered to remove the precipitate and finally absorbance at maximum wavelength was recorded, the concentration of the tartaric acid was determined from the standard curve (Janick, 1974).

Results and Discussion

The parameters determined from the Roselle extract of samples after evaporation of water from the extract was shown on the table 1.

Amount of Organic Acids Recovered

The result of titrations of 0.1 M N_aOH carried out from the alcoholic filtrate which was precipitated with H_2S, liberating organic acids gave brownish solution end point and has a mean titer value of 12.25cm^3 and 8.50cm^3 for dried red and dried white samples respectively. The values of organic acids from white samples was 16.17cm^3 as shown on Table 1.

	Dried Red	*Dried White*	*Fresh Red*	*Fresh White*
Mean Titer				
Pectin	1.9g	1.7g	23.7g	23.7g
Organic Acids	16.17cm^2	8.50 cm^2	ND	ND
Reducing sugar	0.25mg/g	0.29 mg/g	0.98 mg/g	0.084 mg/g
Ascorbic Acids	14.1 mg/g	15.25 mg/g	14.3 mg/g	14.1
Citric Acids	1.88 mg/g	0.58 mg/g	ND	ND
Tartaric Acids	2.0 mg/g	0.67 mg/g	ND	ND

Table 1: Parameters determined in Roselle Extract samples.

Amount of Reducing Sugars Recovered in Dry samples

The results also show that the titrations was carried out using $Na_2S_2O_4$ solution and alcoholic filtrate of both dried white and red Roselle extract and the result revealed that the dried red has mean titer value of 12.30cm^3 while the dried white sample showed 10.30cm^3. However, the blank solution was also titrated against $Na_2S_2O_4$ solution has a mean titer value of 0.2cm^3 this values are corresponded with findings of Okereke, et. al, (2015).The total amount of reducing sugar recovered in dry red sample Roselle extract was calculated to be 0.25 mg as shown on Table 1. Initially 30g of dry sample was dissolved in 1000cm^3, then 100cm^3 is equal to 3g of dry sample. Thus, every 3g of dry weight when dissolved utilized 0.25mg of thiosulphate recovered 0.098mg/gm of reducing sugar from the original sample.

On the other side total amount of reducing sugar in dry white sample was calculated as 0.29mg as indicated on Table 1. This shows that 1cm^3 of thiosulphate produces 0.29 mg/g of reducing sugar, since 30 g of dry sample was dissolved in 1000 cm^3, then 100 cm^3 is equivalent to 3g of dry sample. Thus, every 3g of dry weight when dissolved recovered 0.29mg of reducing sugar that is 0.098 mg/g of original sample this findings is to the report of (Neish, 1952).

Amount of Reducing Sugars Recovered in Fresh samples Roselle

The extract of both fresh samples of white and Red Roselle extract were reflux for two hours with 100cm^3 of water and later 100cm^3 of HCl were added to the extract before Titrations was carried out against $Na_2S_2O_4$ solution for reducing sugar determination. The result showed that the titer values of fresh red has 11.65cm^3, while fresh white has mean titer value of 12.00cm^3. However, the blank sample glucose was titrated against $Na_2S_2O_4$ and has a titer value of 0.20cm^3. The total amount of reducing sugar recovered in fresh red sample was calculated showed 0.86mg as indicated on Table 1.

Three gram of fresh red sample when dissolved and calculated gave 0.254mg of reducing sugar that formed 0.098mg/g from original sample. The composition of reducing sugar was lower than carbohydrates contents of Roselle of 12.3 mg/g this agreed with the finding of Serifat and Anthony, 2020 which was also less than 3-5% sugars content as similar to the report of Ines et al., 2014. The calculated amount of reducing sugar in fresh white sample was 0.254 mg/g which was related to the amount of 0.23 mg/g this tallies with the work of (Neish, 1952).

Thus, 3g of fresh white sample when dissolved yield 0.254mg of reducing sugar that is 0.084mg/g from the original fresh white Roselle sample. This shows that the amount of reducing sugar is less than 3-5% in Roselle extract and this coincides with the report of (Ines et al., 2014).

Amount of Ascorbic acid (Vitamin C) Recovered in Dried samples Roselle

Standard indophenol solution was titrated with ascorbic acid (vitamin c) which gave a titer value of 0.1cm^3. Alcoholic filtrate of both dried white and red Roselle extract were also titrated against indophenol solution and the result obtain showed that dry red sample

has a mean titer value of 4.43cm^3 while dry white sample has a mean tier value of 4.60cm^3. The total amount of ascorbic acid in dry red sample was calculated as 14.1mg/g as adopted by (Neish, 1952).

Moreover, 4.30cm^3 of Indophenol neutralized 2mg of standard ascorbic acid, then 1cm^3 of dried red sample produced 0.465cm^3. Therefore, 100cm^3 water extract of dried red sample produced 46.5 mg/g of original sample. Thus, in every 100cm^3 of original dried red sample extract produced 46.5 mg of ascorbic acid. Also 3g of original dried red sample produced 15.5mg/g from original sample this is related to the report Ines et al., 2014.

Highest amount of ascorbic acid determined by other studies found that in genotype BUM-004 of Roselle calyx has (424.19 µg/g) > BUM-003 (321.35 µg/g) > BUM-007 (200.30 µg/g) were also less significant, as the same study showed that the lowest amount of ascorbic acid found in genotype with ascorbic acid in samples of 4561 (26.20 µg/g) < BUM-002 (41.35 µg/g)< 1740 (47 µg/g) this findings is the same with the report of (Jaminiet al., 2019).

On the other hand, the amount of ascorbic acid in dry white sample was calculated as 0.4577 mg. This shows that in every 100cm^3 of dried white Roselle extract sample can produce 4.577 mg of ascorbic acid. 1.0cm^3 of Indophenol is equivalent to 0.477 times 100mg produces 45.77mg of Ascorbic acid. Every 100 cm^3 of water dissolved 3g of dried white Roselle extract sample produces 45.77 mg of ascorbic acid is present in every 3g of original dried white sample. Thus 15.25mg/g of original sample was higher than the vitamin C content of (14 mg/100 g), as reported by Ines et al., 2014.

Amount of Ascorbic acid (Vitamin C) Recovered in Fresh samples Roselle

Alcoholic filtrate of both fresh white and red Roselle extract were also titrated against indophenol solution and the result obtain revealed that dry red sample has a mean titer value of 4.90cm^3 while dry white sample has a mean tier value of 4.35cm^3. The total amount of Ascorbic acid in fresh red sample was calculated to be14.3 mg/g while total amount of Ascorbic acid in fresh white sample was calculated to be 14.1 mg/g. This shows that 4.25cm^3 Indophenol neutralized 1.827 mg Roselle extract produces 14.1 mg/g of ascorbic acid was higher than vitamin C content of (14 mg/100 g) this equally the same with the reported by (Ines et al., 2014). The determined value of the ascorbic content of fresh white sample Roselle was 14.1 mg/g as indicated on Table 1 which was similar to the amount of Ascorbic acid of 14m g/g as revealed by (Serifat and Anthony 2020).

Amount of Citric Acid Recovered in Dried samples Roselle

The concentrations of citric acid from Roselle extract was calculated from the standard curve at 350nm by graphical extrapolations showed dried red sample has 0.58mg/g while dried white sample has 1.88mg/g these values corresponds with the work of Usman, et.al, (2020). The amount of citric acid in dried red and dried white Roselle extract was not determine as indicated on Table 1.

Amount of Tartaric Acid Recovered in Fresh samples Roselle

The concentrations of tartaric acid from Roselle extract was calculated from the standard curve at 350nm by graphical extrapolations showed dried red sample showed 2.0mg/g tartaric acid while dried white sample has a value of 0.67mg/g tartaric acid, these value agreed with findings of Jamini, et, al. (2019). The concentrations of tartaric acid in fresh red and fresh white Roselle extract was not determine as indicated on Table 1.

Conclusion

Hibiscussabdariffa or "Roselle" is medicinal plant with a worldwide fame. Roselle, having various medically important compounds called phytochemicals, is well known for its nutritional and medicinal properties. Seeds, leaves, fruits and roots of the plant are used as food and herbal medicine. Extracts from Roselle plays a crucial role in treating different medical problems including many cardiovascular disorders and cancer but further researches are required to know its exact mechanism of action and to formulate food products using Roselle with locally grown food items. Obesity is a growing problem, affecting not only adults but also children. The effectiveness

of Roselle extract for metabolic disorders like type II diabetes should be examined further, as previous clinical studies have shown encouraging effects on hyperlipidemia and hypertension, conditions strongly correlated with type II diabetes or metabolic syndrome.

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