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Phytochemical Evaluation of *Hibiscus Sabdariffa* Powder, Jam and Yoghurt

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ABSTRACT

Background

Hibiscus sabdariffa is popularly known as food and herbal drink with numerous health benefits. The phytochemical compounds present in *Hibiscus sabdariffa* calyces are important in developing nutraceutical foods. In this study hibiscus jam and yoghurt were produced from dried hibiscus calyces' powder.

Methods

The phytochemical content and antioxidant activity of these products were then analysed in terms of Total phenolic content (TPC), Total flavonoid content (TFC), Condensed tannins (CT) and DPPH Scavenging activity.

Results

The results showed the presence of all phytochemical compounds (TPC, TFC, CT) and antioxidant activity in all *Hibiscus sabdariffa* products. The hibiscus calyces powder showed the highest phytochemical contents of 35.24 mg GAE. g^{-1}, 0.91 mg QE. g^{-1}, 2.85 mg CAE. g^{-1} and 48.2 % inhibition for TPC, TFC, CT and DPPH Scavenging activity, respectively. Hibiscus jam and Hibiscus yoghurt had phytochemical contents of 6.44 and 4.81 mg GAE. g^{-1}, 0.19 & 0.24 mg QE. g^{-1}, 1.40 & 0.66 mg CAE. g^{-1} and 26.2 & 39.3 % inhibition for TPC, TFC, TC and DPPH Scavenging activity, respectively.

Conclusions

The results of the current study showed that there is potential in using *Hibiscus sabdariffa* to develop functional foods.

Key Words: *Hibiscus sabdariffa*, phytochemicals, antioxidants, calyces, therapeutic

BACKGROUND

Since ancient times, plant-based traditional medicine has played a major role in the therapy of many diseases (1). The use of herbal extracts as medicine for the treatment of many disease is well documented (2,3). Currently, the food market encourages industries to develop products that have functional, nutritional, and therapeutic properties (4). In efforts to meet food market demands, *Hibiscus sabdariffa or "*Mutete*"*, has gained popularity as a miracle plant with potential medicinal benefits (5). This is mainly due to the therapeutic effects it has against many diseases including cancer (6).

Hibiscus sabdariffa, is an annual herbaceous subshrub that belongs to the *Malvaceae* family (Formaggio *et al*., 2015). The plant is native to India but also grown in many parts of the world including Africa (3). It is widely cultivated for its strong fibre (3), and popular for the edibility and therapeutic effects of its leaves and calyces (8,9). The calyces and leaves have been used to make food (juice, jam, jellied confectionaries, ice cream, chocolates and flavouring agents) and herbal medicine (herbal tea to sooth colds, clear a blocked nose, clear mucous, as an astringent, promoting kidney function, aiding digestion, as a general tonic, diuretic, and antipyretic)(8,10–12). The calyces and leaves contain bioactive molecules including flavonoids, anthocyanins, alkaloids, saponins, steroid, sterols and tannins (3,10,11). The seeds are a great source of lipid-soluble antioxidants, particularly γ-tocopherol (1). These bioactive molecules have choleretic, febrifugal, hypertensive and diuretic effects, decreasing blood viscosity, stimulating intestinal peristalsis and reducing blood pressure (5). The *Hibiscus sabdariffa* extract has been effective in treating abscesses, bilious conditions, cancer, coughs, kidney stones and *Mycobacterium Tuberculosis*

(1,13). The tea has been used to lower blood pressure (Chopra et al., 1986), cholesterol level, and to prevent cardiovascular disease (1).

The *Hibiscus sabdariffa* plant deserves attention especially in Namibia where it is underutilised. The plant's tremendous health benefits also present an opportunity for the development of potential nutraceutical and functional food products. The aim of this study was, therefore, to evaluate the potential of using *Hibiscus sabdarrifa* to develop foods that have nutraceutical and functional properties

MATERIALS AND METHODS

Collection of samples

Hibiscus sabdariffa calyces were used as the plant material in the study. Air-dried *Hibiscus sabdariffa* calyces were purchased from a commercial farm 20 km Northeast of Otavi, Namibia. The calyces were transported to the University of Namibia's Food Science and Technology Department and kept at 7˚C before use.

Preparation of H. sabdariffa calyces' powder

Dried *Hibiscus sabdariffa* calyces were ground using pestle and mortar. Following this, the ground calyces were sieved using a 0.5 mm aperture sieve to obtain a fine powder.

Preparation of Mutete Jam

The jam was prepared by boiling the clean hibiscus pulp with enough sugar and pectin to a thick consistency (Figure 1).

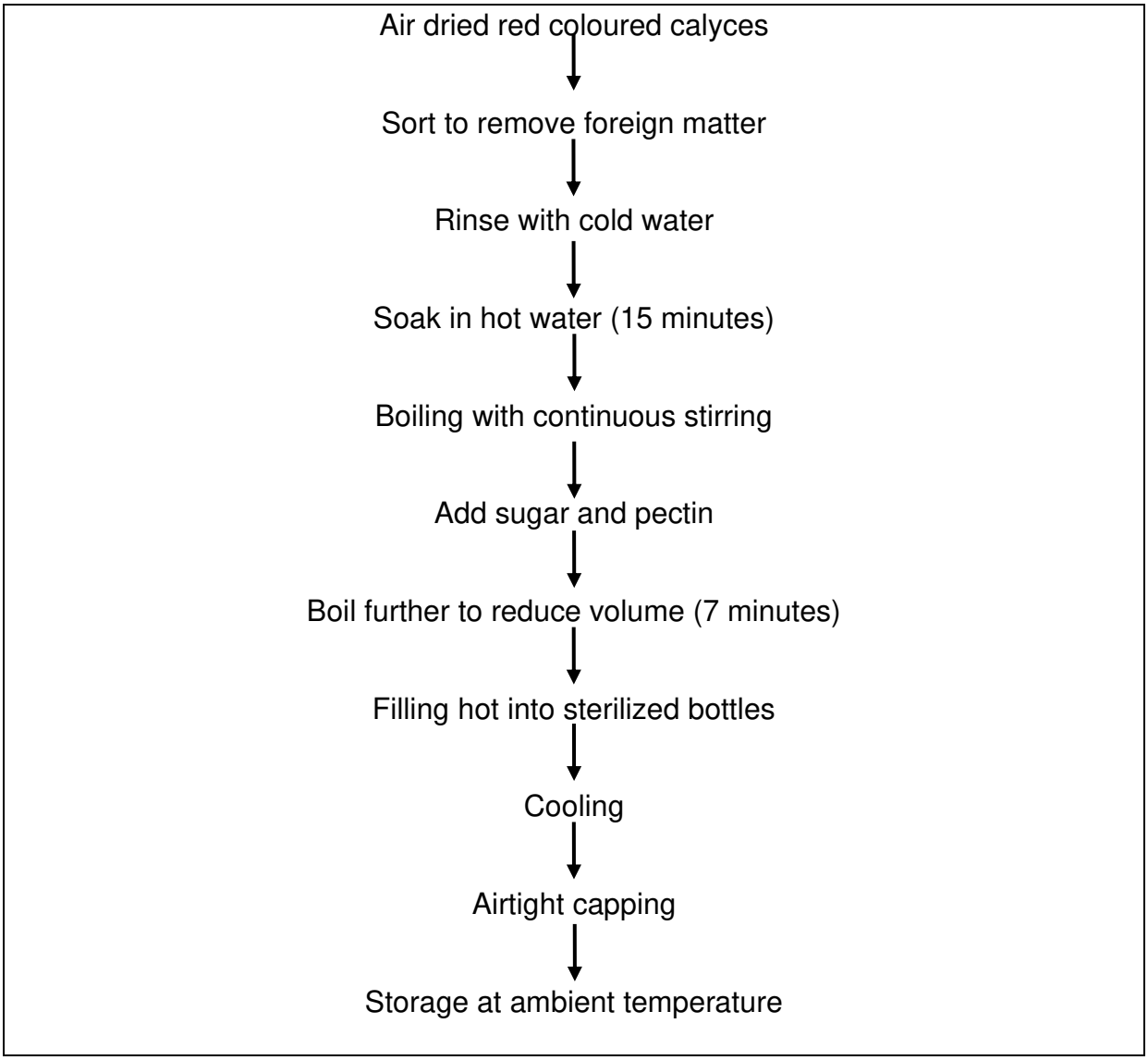

Figure 1. Preparation of Hibiscus jam

Preparation of Mutete Yoghurt

Yoghurt was prepared using commercial thermophilic starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophiles.* In brief, fresh good quality cow milk was pasteurised at 85-90^{o}C for 30 minutes to kill unwanted microorganisms

as well as denature whey proteins (Albumins and Globulins). Stabilizers were subsequently added at the rate of 5-10 millilitre per Litre of milk after which, 6% white sugar was added and mixed while hot. It was then cooled to 43-45^{o}C. The starter culture was then added, mixed, and incubated to 43^{o}C for 6-8 hrs until coagulation. The yoghurt was cooled to 5-7^{o}C overnight to set. The yoghurt was then gently agitated to obtain a smooth and thick texture before 15% of the hibiscus powder was added

Phytochemical analysis

Phytochemical analysis in terms of total phenolic content, total flavonoid content, condensed tannins, and antioxidant activity were done using spectrophotometric techniques.

Sample extraction

Sample extraction was done following a method outlined by Rooney and Waniska (2004) using two extraction solvents, 1% Hydrochloric acid (v/v) in methanol. About 0.5 g of the sample was weighed into 50 ml centrifuge tubes. To the tubes, 5 ml of 1% HCl in methanol was added. The mixture was sonicated at 25°C for 10 minutes. After sonication, the tubes were centrifuged at 4000 rpm for 5 minutes. The supernatant was decanted in a separate centrifuge tube and extraction was repeated using another 5 ml of 1% HCl in methanol. The extracts were stored at -4°C until further analysis. Extraction was done in duplicates and determinations were done in triplicates.

Determination of Total Phenolic Content (TPC)

Total phenolic contents were determined using the Folin-Ciocalteu method described by Mohd-esa et al., (2010). Extract sample of 0.5 ml was mixed with 0.1 ml of 0.5N

Folin-Ciocalteu reagent (2.5 ml of the 2N Folin-Ciocalteu reagent original bottle mixed with 7.5 ml of distilled water) and gallic acid stock solution (50 mg of gallic acid into 100 ml of methanol in a volumetric flask). The mixture was then incubated for 15 minutes at room temperature in the dark after which 2.5 ml of sodium carbonate (20 g/100 ml) was added to the test tubes and incubated once more for 30 minutes at room temperature in the dark. The absorbance of the sample extract was measured at 760 nm using UV/VIS spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of the sample. It was calculated using the equation $y = 1.3016x - 0.0275$ with $R^2 = 0.9925$, generated from the gallic acid standard curve. Where Y is the absorbance of the sample extract and X is the unknown concentration of the sample extract.

Determination of Total Flavonoids Content (TFC)

Total flavonoids content was determined using Aluminium Chloride method described by Chang, Yang, Wen, and Chern (2002). For the standard curve, quercetin stock solution was prepared by dissolving 100 mg of quercetin into 100 ml of methanol in a volumetric flask. Volumes of quercetin stock solution (0, 0.0315, 0.0625, 0.125, 0.25, 0.5, 1, 2, and 4 ml) were pipetted into separate volumetric flasks and made up to 50 ml with methanol to have concentrations of 0, 0.000625, 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04 and 0.08 mg/ml. The appropriate number of test tubes were prepared for each sample to replicate, blank and standard solutions. In those test tubes, 0.5 ml of 1.2% (w/v) aluminium chloride and 0.5 ml of 120 mM potassium acetate (1.1778 g into 100 ml distilled water) were added. One ml of sample extracts or blank (extracting solvent) or standard solutions was added to the test tubes containing aluminium chloride and potassium acetate solution. The mixture was incubated at room

temperature for 30 minutes. The absorbance of the samples, blank and standard solutions were read at 415 nm using a UV/VIS spectrophotometer. The total flavonoids content of the sample extracts was calculated using the equation y = 31.046x +0.0311 with R^2 = 0.9975, generated from the quercetin standard curve. Where Y is the absorbance of the sample extracts and X is the unknown concentration of the sample extract.

Determination of Condensed Tannins (CT)

Condensed tannins were determined using the Vanillin-HCl method described by Price, Van Scoyoc and Butler (1978). For the standard curve, the catechin stock solution was prepared by dissolving 10 mg of catechin into 100 ml of methanol in a volumetric flask. Volumes of catechin stock solution (0, 1, 2, 3, 5 and 10 ml) were pipetted into separate volumetric flasks and made up to 10 ml with methanol to have concentrations of 0, 0.01, 0.02, 0.03, 0.05 and 0.1 mg/ml. The standard solutions, sample extract and vanillin reagent (4% HCl (v/v) in methanol and 0.5% (w/v) vanillin in methanol) were kept in the water bath at 30°C for 20 minutes before mixing the reactants. One ml of sample extract or blank (extracting solvent) or standard solutions was mixed with 5 ml of vanillin reagent in test tubes and maintained at 30°C in the water bath for 20 minutes. The absorbance of the sample extract, blank and standard solutions was read at 500 nm using a UV/VIS spectrophotometer. The condensed tannins of the sample extracts were calculated using the equation y = 0.222x - 0.0011 with R^2 = 0.9987, generated from the catechin standard curve. Where Y is the absorbance of the sample extracts and X is the unknown concentration of the sample extract.

Determination of Antioxidant Capacity

The antioxidant activity of hibiscus products (hibiscus calyces' powder, hibiscus jam and hibiscus yoghurt) was evaluated by the free radical scavenging activity of the products using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by McCune and Johns (2002). One millilitre of 0.3 mM DPPH solution (0.012 g into 100 ml of methanol) was added to test tubes containing 5 ml hibiscus product. For the blank sample, 1.0 ml of extracting solvent was added to a test tube containing 1.0 ml of 0.3 mM DPPH solution and 1.0 ml of methanol. Quercetin was used as positive control and standard concentration solutions (0.1, 0.2, 0.4 and 0.6 mg/ml) of quercetin were prepared. From each standard concentration solution, 1.0 ml was added to test tubes containing 1.0 ml of 0.3 mM DPPH solution and 1.0ml of methanol. The tubes were incubated for 10 minutes at room temperature in the dark for the reaction to take place. The absorbance of the sample extract, blank, standard solutions were read at 517 nm using a UV/VIS spectrophotometer. The radical scavenging activity was calculated as percentage inhibition of DPPH discolouration according to the following equation:

$$\% \, inhibition = \frac{\mathrm{A_0} - A_s}{\mathrm{A_0}} \, \mathrm{x} \, 100$$

Where A_s is the absorbance of the sample extract or standard and A_o is the absorbance of the negative control, which is the blank. Quercetin was used as a standard for comparison in antioxidant activities of all infusions

Data Analysis

All determinations were done in triplicates. The results were reported as mean ± standard deviation that was analysed using SPSS software version 21.

Results and discussion

Studies have shown the presence of various phytochemicals in *Hibiscus sabdariffa* calyces (Formaggio et al., 2015; Ghodke and Mane, 2017; Okereke et al., 2016). To evaluate its potential in developing functional foods, this study, determined the phytochemical contents and antioxidant activity of products (powder, jam, and yoghurt) made from dried *Hibiscus sabdariffa* calyces. The results are summarised in Table 1.

Total phenolic compounds

The total phenolic content varied between the three hibiscus products. Comparatively, the hibiscus powder showed the highest total phenolic content of 35.42 ± 0.28 mg GAE. g^{-1} (Table 1). Hibiscus jam and yoghurt showed lower total phenolic content of 6.44 ± 0.20 mg GAE. g^{-1} and 4.81 ± 0.57 mg GAE. g^{-1} respectively. The total phenolic content of calyces powder in this study is slightly lower than those obtained by Hassan, (2014) and Oloumi, Shakeri, & Behzadi,(2016) of 41.07 mg GAE. g^{-1} and 59 mg GAE. g^{-1} in hibiscus extract, respectively. The yoghurt results (4.81 ± 0.57 mg GAE. g^{-1}) corresponds with findings from Dabija et al., (2018)'s study. In their study, the total phenolic content was 5.12 mg GAE/g and 4.17 mg GAE/g for yoghurt samples inoculated with hawthorn (*Crataegus monogyna*) and sage (*Salvia officinalis* L.) respectively. The variation in total phenolic content among the products is possibly due to processing. High temperature has been reported to negatively affect the phenolic compounds present in medicinal plants and herbs such as hibiscus (2). In this study, the hibiscus powder was minimally processed using pestle and mortar, while the processing of jam and yoghurt involved thermal treatment. The impact of temperature might have caused degradation of phenolic compounds which resulted in lower phenolic content observed in hibiscus jam and yoghurt (Table1). The presence

of phenolic compound is attributable to the therapeutic properties of the plant. Phenolics present in plants have been popular mainly because of their potential antioxidant activity (16). They may therefore be responsible for the protective effect against the risk of many disease processes, such as cancer, cardiovascular and circulatory diseases (10).

Total flavonoid compounds

The flavonoid content of the products differed and ranged between 0.19 ± 0.01 QE. g^{-1} and 0.91 ± 0.03 QE. g^{-1}. Hibiscus powder showed the highest flavonoid content, while the hibiscus jam had the lowest flavonoid content. The total flavonoid content of calyces powder observed in this study is comparable to those observed by other researchers. Oloumi et al., (2016) reported flavonoid content of 0.97 ±0 .05 QE. g^{-1} in hibiscus extract. Formagio, ASN.a*, Ramos, DD.a, Vieira, MC.a, Ramalho et al., (2015) reported flavonoid content of 1.18 ± 2.51 QE. g^{-1} in hibiscus calyces. The total flavonoid content of jam in this study was higher compared to jam from other products. Farida, (2018) observed flavonoid content 0.08 QE. g^{-1} in melon jam. This illustrates that *Hibiscus sabdariffa* is a good source of flavonoid content. Flavonoids are produced as natural secondary metabolites in plants and contain high antioxidant properties (16). These compounds are capable of scavenging free radicals. They can therefore be effective against many human disorders (16). The flavonoids in *hibiscus sabdariffa* showed desirable effects on peroxidase and protease activity in human blood (19), which confirmed its potential as an antioxidant and anti-aging plant. Flavonoids have also been shown to protect against coronary heart disease (8).

Condensed Tannins

The condensed tannins content of the hibiscus products followed the trend observed with total phenolic content (Table 1). Again, the calyces powder showed the highest condensed tannins of 2.85 ± 0.06 mg CAE. g^{-1} followed by hibiscus jam with flavonoid content of 1.40 ± 0.05 mg CAE. g^{-1}. Despite the small amounts of tannins present in hibiscus jam and yoghurt, tannins found in *hibiscus sabdariffa* extract have shown antioxidant effects (19). The presence of tannins in food product is advantageous because they have cardioprotective actions (3), may reduce the risk of cancer and may prevent menopausal symptoms (20). Tannins also have anti-microbial activity by precipitating protein content of the outer wall of the microbes, thereby forming a complex with the proteins and stop their activities (10).

DPPH scavenging activity

The results of the antioxidant activity expressed as DPPH scavenging activity correlates with that of total flavonoid content (Table 1). The highest antioxidant activity of 48.2 ± 0.2% was observed in calyces' powder. The lowest antioxidant activity of 26.2 ± 0.3% was observed in jam. Although, hibiscus jam had the lowest antioxidant activity, this percentage was higher than the antioxidant activities observed in other fruit jams. Farida, (2018) found antioxidant activity of 4.95% in melon jam. While Rababah *et al.* (2011) found an antioxidant activity of 10.06%, 9.95% and 8.96%, for cherry, apricot and fig jams respectively. These suggest that hibiscus Jam has good antioxidant activity, with potential health benefits. The higher antioxidant activity in the calyces' powder may be attributed to presence of higher polyphenolic compounds (total phenolic, total flavonoid and tannins) in the product (Table 1).

Table 1 Phytochemical content of *Hibiscus sabdariffa* products

	Hibiscus products		
Phytochemical compound	Calyces powder	Jam	Yogurt
TPC (mg GAE/g	35.42 ± 0.28	6.44 ± 0.20	4.81 ± 0.57
TFC (mg QE/g	0.91 ± 0.03	0.19 ± 0.01	0.24 ± 0.03
CT (mg CAE/g	2.85 ± 0.06	1.40 ± 0.05	0.66 ± 0.06
DPPH scavenging activity (% inhibition	48.2 ± 0.2	26.2 ± 0.3	39.3 ± 0.2

CONCLUSIONS

The results showed the presence of phenolic, flavonoids, tannins and antioxidant activity in all three hibiscus samples (powder, jam and yoghurt). This indicate that *Hibiscus Sabdariffa* is a good source of phytochemical compounds and provides high antioxidant activity based on scavenging the DPPH radicals. This demonstrates its potential as a nutraceutical food, providing many health benefits. The results also show higher phytochemical compounds and antioxidant activity in hibiscus calyces compared to hibiscus jam and yoghurt. This suggests that, when higher phytochemical content is required, minimally processed hibiscus product such as hibiscus extract or powder should be used. Likewise, when thermally processed hibiscus products such as hibiscus jam and yoghurt are consumed, it can be expected to result in less phytochemical compound acquired. The presence of phytochemical compounds and antioxidant activity in hibiscus jam and yoghurt, however, implies that hibiscus calyces' powder can be successfully processed or incorporated into products such as yoghurt obtain a product with health benefits.

DECLARATIONS

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author's contributions

Conception design: P.H. , C.S.

Data acquisition and Analysis: H.S., K.H., P.H.

Interpretation of Data: K.H., E.S., C.S.

Drafting: E.S., C.S.

Reading and approving final manuscript: All authors

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REFERENCES

1. Islam M. Open Journal of Nutrition and Food Sciences Food and Medicinal Values of Roselle (Hibiscus sabdariffa L . Linne Malvaceae) Plant Parts : A Review. Open J Nutr Food Sci. 2019;(June).
2. Nguyen Q, Chuyen H. Processing of Herbal Tea from Roselle (Hibiscus sabdari ff a L .): E ff ects of Drying Temperature and Brewing Conditions on Total Soluble Solid , Phenolic Content , Antioxidant Capacity and Sensory Quality. 2020;(2002).
3. Okereke C, Iroka F, Chukwuna M. Phytochemical analysis and medicinal uses of Hibiscus. Int J Herb Med. 2016;(January 2015):16–9.
4. Kermiche F, Boulekbache L, Felix M, Harkat-Madouri L, Remini H, Madani K, et al. Effects of the incorporation of cantaloupe pulp in yogurt : Physicochemical , phytochemical and rheological properties. Food Sci Technol Int. 2018;35–89.
5. Guardiola S, Nurla M. Therapeutic potential of Hibiscus sabdariffa : A review of the scientific evidence &. 2014;61(5).
6. Guardiola S, Mach N. Therapeutic potential of Hibiscus sabdariffa : A review of the scientific evidence &. Endocrinol y Nutr. 2014;61(5).
7. Formagio A., Ramos D, Vieira M, Ramalho S, Silva M, Zárate N, et al.

Phenolic compounds of Hibiscus sabdariffa and influence of organic residues on its antioxidant and antitumoral properties. 2015;75(1):69–76.

8. Ajala LO, Ibiam A, Polytechnic F, Eo O. Nutritional assesment of Juice extract from Hibiscus sabdariffa. Itnernational J Biol Pharm Allied Sci. 2014;(May):10–9.

9. Islam A, Jamini TS, Islam M, Yeasmin S. Roselle : A Functional Food with High Nutritional and Medicinal Values. 2016;2021(2):44–9.

10. Da-costa-rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. Hibiscus sabdariffa L . – A phytochemical and pharmacological review. 2014;165:424–43.

11. Yeasmin S. Roselle : A Functional Food with High Nutritional and Medicinal Values Roselle : A Functional Food with High Nutritional and Medicinal Values. 2016;(August).

12. Al-snafi ALIE. Pharmacological and therapeutic importance of Hibiscus sabdariffa - A review. 2018;10(3):451–75.

13. Mohd-esa N, Hern FS, Ismail A, Yee CL. Antioxidant activity in different parts of roselle (Hibiscus sabdariffa L .) extracts and potential exploitation of the seeds. 2010;122:1055–60.

14. Ghodke S, Mane K. Processing of roselle (Hibiscus sabdariffa) calyces for value addition. Food Sci Res J. 2017;

15. Hassan HM. Determination of total phenolic content and antioxidant activity of Roselle (Hibiscus sabdariffa L .) Calyx ethanolic extract. 2014;(October).

16. Oloumi H, Shakeri S, Behzadi M. c r v i h o e f c r v i h o e f. 7(2):89–96.

17. Dabija A, Codin GG, Ropciuc S, Gˆ A. Assessment of the Antioxidant Activity and Quality Attributes of Yogurt Enhanced with Wild Herbs Extracts.

2018;2018.

18. Farida B. Physicochemical characteristics and phytochemical content of jam made from melon (Cucumis melo) Physicochemical characteristics and phytochemical content of jam made from melon (Cucumis melo). 2018;(February).

19. Alaga TO, Edema MO, Atayese AO, Bankole MO. Phytochemical and in vitro anti-bacterial properties of Hibiscus sabdariffa L (Roselle) juice. 2014;8(6):339–44.

20. Ijeomah A, Ugwuoma F, Abdullahi H. PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT. Niger J Agric Food Environ. 2012;8(1):10–6.

Figures

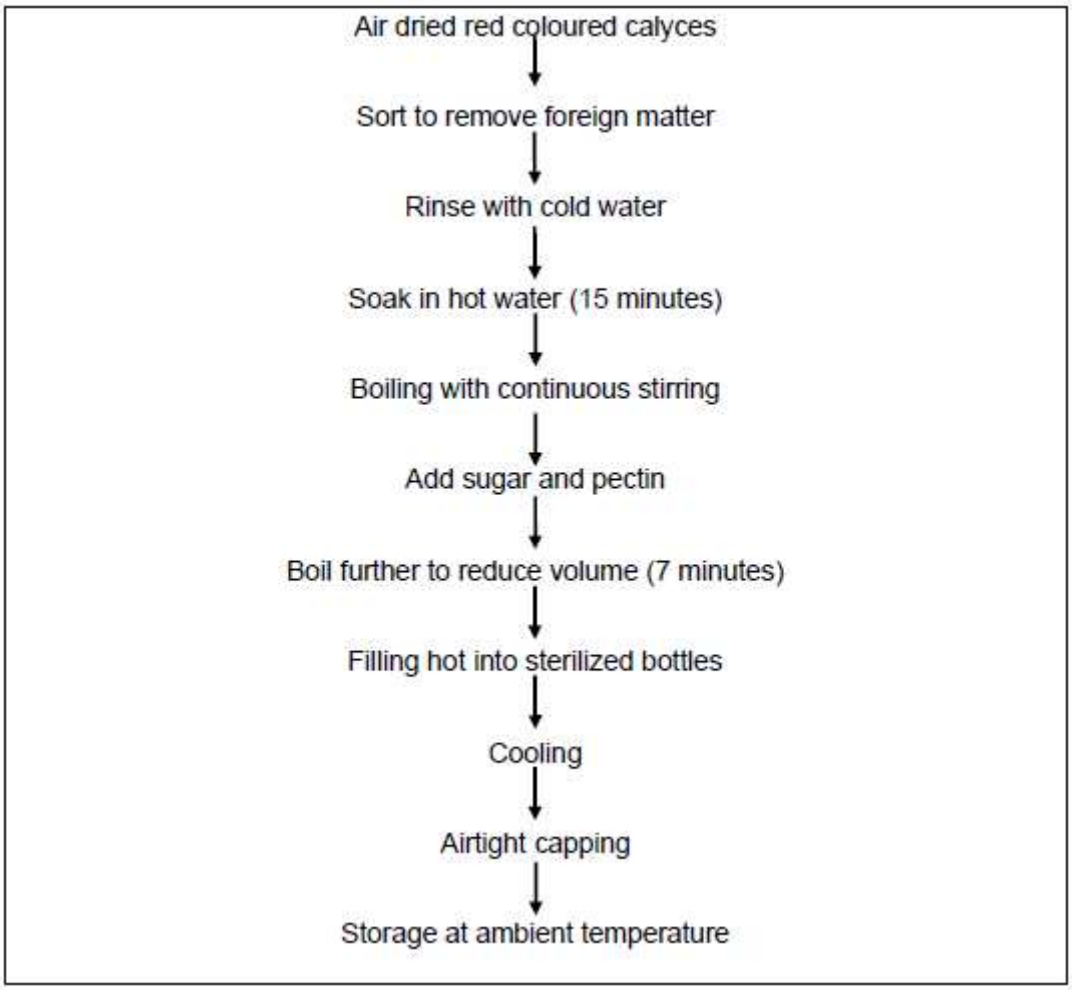

Figure 1

Preparation of Hibiscus jam