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Research Article

Antimicrobial Susceptibility of Spoilage Bacteria of Water Melon Juice to Ethanol Extract of Roselle (*Hibiscus sabdariffa*) Calyces and their Survival in the Extract Fortified Water Melon Juice

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Abstract

Aim: The aim of this research is to evaluate the antimicrobial potential of ethanol and aqueous (hot and cold) extracts of *Hibiscus sabdariffa* calyces and the survival of spoilage bacteria in water melon juice fortified with the ethanol extract.

Place of Study: Department of Microbiology University of Ibadan, Nigeria.

Materials and Methods: The isolation and identification of spoilage bacteria of water melon juice was carried out using culture dependent method and morphological and biochemical characterization respectively while extraction of phytochemicals was done with reference to standard procedures. The antimicrobial activities of the different solvent extracts were evaluated based on the agar well diffusion method while the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were carried out using the macro dilution broth method.

Results: The results obtained showed that *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Proteus vulgaris* were isolated as spoilage bacteria of water melon and phytochemical determination using different extracts showed that Ethanol extract contained 1.35 ± 0.5, 1.21 ± 0.3, 0.41 ± 0.1, 1.50 ± 0.2, 2.40 ± 0.0 mg/g of saponin, tannin, alkaloids, phenols and flavonoids respectively while the hot aqueous extract contained 0.2 ± 0.0, 0.79 ± 0.2, 0.27 ± 0.1 and 0.32 ± 0.1mg/g of saponin, tannin, alkaloids and phenol respectively and the cold aqueous extract contained 0.19 ± 0.1, 0.52 ± 0.2, 0.24 ± 0.1, and 0.11 ± 0.1 mg/g of saponin, tannin, alkaloids, phenol respectively while flavonoids was not detected in the hot and cold aqueous extract. The spoilage bacteria were inhibited by the different extracts showing inhibition zones of 15.3 ± 0.2, 14.3 ± 0.6, 10.7 ± 0.4, 11.0 ± 0.0, 10.7 ± 0.1 and 8.3 ± 0.5mm for *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Proteus vulgaris* at a concentration of 200 mg/ml respectively of Ethanol extract while the hot aqueous extract demonstrated inhibition zones of 10.7 ± 0.4, 11.0 ± 0.1, 9.0 ± 0.0, 6.7 ± 0.6, 7.3 ± 0.2 and 5.0 ± 0.0 mm for *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Proteus vulgaris* at a concentration of 200 mg/ml respectively and the cold aqueous extract showed inhibition zones of 6.7 ± 0.3, 6.0 ± 0.0, 4.0 ± 05, 3.7 ± 0.4, 4.3 ± 0.2 and 0.0 ± 0.0 mm for *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Proteus vulgaris* at a concentration of 200mg/ml respectively which were significantly different ($p < 0.05$). The MIC values for *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Proteus vulgaris* ranges from 50 ± 0.4 to100 ± 0.8 mg/ml using ethanol extracts while the MBC value for *Staphylococcus aureus, Bacillus cereus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris* using Ethanol extracts was 200 ± 0.9 mg/ml. The hot and cold aqueous did not have values for the MBC.

Conclusion: It could be concluded that the extract showed bio-preservative potential and could be applied in preservation or shelf life extension of water melon juice.

Keywords: *Hibiscus sabdariffa; Staphylococcus aureus; Bacillus cereus; Pseudomonas aeruginosa; Klebsiella pneumoniae; Escherichia coli; Proteus vulgaris*

Introduction

Microbial food spoilage has constituted an enormous threat to the food industry as it has consequences such as reduced textural characteristics, nutritional quality, sensory attributes and consumer's acceptability of finished food products amounting to huge economic loss. The over mentioned setbacks have necessitated the concept of microbial growth inhibition in foods which is significant for the current globalized food production. It is reported that approximately 25% of all food products harvested is wasted due to microbial deterioration [1]. The use of chemical additives in food preservation have been extensively reported to inhibit the survival and proliferation of microorganisms, however the problems associated with the usage of these chemicals on human health on a long term are of urgent concern to the food industry [2]. Recent research works are geared towards developing effective compounds for food preservation with apparently no adverse side effects on long term usage and this has led to the development of the concept of bio-preservation. The bio-preservatives are wide range of natural products obtained from plants, animals and microbial origin that possess ability to inhibit microbial growth and hence prolonging the shelf-life and increase overall quality of food [3]. These natural biological compounds possess antimicrobial properties which are capable of breaking down cellular membranes and disrupting microbial biosynthetic pathways [4,5]. Thee antimicrobial activity of different herbal extracts are well documented [6,7] and have been employed as preservative agents in food since ancient times, as folk medicine, and flavoring agents due to their antimicrobial activity against pathogens [8-11]. They are non toxic at levels consumed and are considered as GRAS (Generally Recognized as Safe) substance [12-14].

Hibiscus sabdariffa is an annual, tropical or subtropical shrub species belonging to the family Malvaceae and originated from West Africa. It is commonly known as roselle (English), l'Oiselle (French), Spanish (Jamaica), karkade (Arabic), and Krachiapdaeng (Thailand) [15]. There are two main types of the plant: *H. sabdariffa var. sabdariffa* and *H. sabdariffa var. altissima*. It is an annual plant that grows well in soils that are well drained and requires nighttime temperature higher than 21ºC (Stephens, 2012). The calyx vary in sizes with each variety, but ranges from ½ to 1 ½ inches in diameter with a deep penetrating tap root and highly medicinal. It is popularly known as 'roselle' and reported to contain high quantity of phytochemical substances such as flavonoids. glycosides, anthocyanins, phenolic acids, organic acids, saponins, alkaloids and polyphenolic compounds which are responsible for its medicinal and preservative properties [16-19]. In addition, extracts of *Hibiscus sabdariffa* calyces has been previously reported to confer health benefits which are dependent on the presence of antioxidants.

Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceae [20,21] and it is one of the most widely cultivated crops in the world with its global production of 89.9 million mega grams reported in 2002 [22,23]. China is the leading country in watermelon production followed by Turkey, United States, Iran and Republic of Korea [23]. There are over 1,200 varieties of watermelon grown all over the world and quite a number of these varieties are cultivated in Africa [24]. Nigeria was reported to produce the highest quantity of melon in 2011 [25]. Watermelon is used as a dessert fruit and a popular thirst quencher [26] with a huge economic importance [27]. They are sold in different forms, such as sliced, quarters, halves or chunks at sales point and nutritionally it is rich in some of the major antioxidants, vitamin C, and a good source of lycopene which is responsible for the red color, beta-carotene, potassium, vitamin A [28,29]. The components of water melon are important in maintaining healthy living and symptoms associated with the spoilage of water melon are off flavor formation, ropiness, turbidity, rot, discoloration, sliminess and putrefaction [30]. It possesses a high moisture content [31] which makes it susceptible to microbial spoilage. Generally, fruits are easily spoilt because they are metabolically active during the storage stage and this property accounts for their short shelf-life which is a very sensitive issue in the food industry as few days extension of shelf-life could represent a significant economic advantage [32].

This present research is intended to provide information on the antimicrobial activity of Ethanol extract of *Hibiscus sabdariffa* calyces, against spoilage bacteria of water melon juice and their survival in water melon juice fortified with Ethanol extract of *Hibiscus sabdariffa* calyces as parameters for evaluating its bio-preservative potential and shelf life extension of water melon juice.

Materials and Methods

Sample Collection

Samples of Water melon (*Citrullus lanatus*) and Roselle plant (*Hibiscus sabdariffa*) were procured from Bodija market in Ibadan, Oyo State, Nigeria and were authenticated by Professor E.A Ayodele a plant taxonomist in the Department of Botany University of Ibadan Nigeria. The dried calyces of the Roselle plant was transported in clean polyethylene bags to Pharmaceutical Chemistry laboratory, University of Ibadan, for the extraction and phytochemical analysis.

Extraction of water melon Juice

Water melon fruits were washed in saline water and sliced into many parts using a sterile knife. The edible red portion was cut into tiny cubes and transferred into electric blender for juice extraction. The juice obtained was filtered using a threefold muslin cloth and poured into a sterile container which was kept at room temperature for 72h to spoil. The spoilt water melon juice was serially diluted to dilution 10^{-7} using the method described by [33].

Isolation of bacteria

0ne ml from appropriate dilution was inoculated differently into sterile petri dishes and 20 ml of molten Nutrient agar, MacConkey agar and Mannitol salt agar were separately poured into the plates. Incubation was carried out at 37°C for 24 hours and plates examined for bacterial growth. Representative colonies were selected from the different plates and subcultured repeatedly The pure cultures obtained were transferred into nutrient agar slants in McCartney bottles and stored in the refrigerator for further work [34].

Identification of bacterial isolates

The pure isolates were identified on the basis of their colonial morphology and biochemical characteristics such as Gram stain, spore stain, catalase, oxidase, methyl red test, voges-proskauer tests, H_2S production, indole production, citrate utilisation, urease production, sugar fermentation and motility test and with reference to Bergey's manual of systematic bacteriology [35,36].

Preparation of *Hibiscus sabdariffa c*alyces extract

The calyces were thoroughly washed and dried properly in an oven at 60°C for 24h. The dried calyx was milled into fine powder using a mechanical grinder and sieved and transferred to a glass sealed container which was kept in the refrigerator prior to extraction.

Cold extraction

One hundred gm of the dried calyces powder was soaked in 500 ml of cold distilled water in sterile desiccators for 72h and filtered using sterile filter paper (Whatman No. 1) into a conical flask. The extract obtained was filtered with a muslin cloth and centrifuged at 10, 000 rpm for 5 minutes. The resulting supernatant was stored in the freezer at 4°C.

Hot extraction

One hundred gm of the dried calyces powder was soaked in 500ml of distilled boiled water in a sterile glass jar for 72 hours with continuous shaking and filtered using double layers of muslin cloth. Centrifugation was carried out at 10,000 rpm for 10 minutes. The supernatant was concentrated with the aid of a rotary evaporator and air dried. The extract yield was weighed and stored in the freezer at 4°C.

Ethanol extraction

One hundred and fifty gm of dried calyces powder was soaked in one and half liters of 70% ethanol with stirring for 5 days at room temperature and filtered through a sterile filter paper (Whatman No 1). The filtrate was centrifuged at 10,000 rpm for 10 minutes, evaporated and dried at 40°C under reduced pressure using a rotary evaporator. The extract yield were weighed and stored in the freezer at 4°C [37].

Determination of phytochemicals

Determination of total phenols

The total phenol content of the extract was determined using the Spectrophotometric method. 0ne ml of Folin-Ciocalteu's reagent was added to one milliliter of sample (250 µg/ml) and mixed thoroughly. The mixture was added to 4 ml of sodium carbonate (75 g/L) with 10 ml of distilled water and shacked thoroughly and allowed to stand for 2 hours at room temperature. Centrifugation was carried out at 2,000 rpm for 5 minutes and the absorbance of the supernatant was read at 505 nm.

Determination of alkaloid

Five gm of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4h. This was filtered and the filtrate was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium

hydroxide was added in drops to the extract until precipitation was completed and the solution was allowed to settle. The precipitate was washed with dilute ammonium hydroxide, filtered, dried and weighed (alkaloid) [38].

Determination of tannin

Fifty ml of distilled water was added to 500 mg of the sample in 100 ml plastic bottle and shaken for 1h in a mechanical shaker. The filtrate obtained in a 50 ml volumetric flask was made up to the mark. Five ml of the filtrate was pipette into a test tube and mixed with a solution containing 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008M potassium ferrocyanide and the absorbance was measured at 120 nm after10 minutes [39].

Determination of saponin

Ten g of the powered sample was weighed into 50 ml 20% ethanol in a conical flask and placed in a water bath at 55°C for 4 hours. The filtered residue was washed with 20% ethanol twice. The extracts were reduced to about 20 ml in the oven and 20 ml of diethyl ether was added and shaken vigorously. The ether layer was discarded and 30 ml of n-butanol was added. The mixture was washed with 10 ml of 5% sodium chloride and dried in the oven. The final content (Saponin) was weighed [40].

Determination of flavonoids

Ten g of the powdered sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The solution was filtered through the Whatman filter paper No 42 (125 mm) and the filtrate transferred into a crucible and evaporated into dryness over a water bath and weighed [41].

Assay for antimicrobial activity of different calyx extracts

The antibacterial activities of Ethanol, cold and hot aqueous extracts of *Hibiscus sabdariffa* calyces were investigated for their antimicrobial activity against spoilage bacteria of water melon juice using the agar well diffusion method as described Daoud [42]. A 24h old pure cultures of *Escherichia coli, Proteus vulgaris, Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* was differently inoculated into 5 ml of normal saline and the density of the inoculums was adjusted to 0.5 McFarland turbidity standard, resulting in a suspension of 1×10^7 cfu/ml of each of the test organism [43]. Mueller-hinton agar was poured aseptically into the plates which were seeded with the different standardized test organisms (*Escherichia coli, Proteus vulgaris, Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*). Wells were bored aseptically in the agar using 6mm cork borer and filled with 0.1 ml of concentration of the Ethanol extract. DMSO (5%) was used as negative control while ciprofloxacin (1 mg/ml) was used as positive control. The plates were kept at room temperature for 30 minutes to allow diffusion of the extracts into the agar and incubated at 37°C for 24 hours. Diameter of the zones of inhibition formed around each well was measured in millimeter [44,45]. The same was repeated for the other solvents extract of the *Hibiscus sabdariffa* calyces using different concentrations.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of the extracts were determined using macro broth dilution method [46]. One ml of nutrient broth with 1 ml of different concentration (200, 100, 50, 25 mg/ml) of the ethanol extracts of the *Hibiscus sabdariffa* calyxes was dispensed into different test tubes and 0.1 ml of the standardized test organisms (1×10^7 cfu/ml) was added to each of the test tubes containing the mixture and incubated aerobically at 37°C for 24h. A test tube containing the nutrient broth, normal saline and the test organisms was used as control. The tubes were examined for turbidity which indicates microbial growth. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extracts that showed no visible growth (no turbidity) when compared with the control tubes. The same was repeated for the other solvents extract of the *Hibiscus sabdariffa* calyces.

The minimum bactericidal concentration (MBC) was determined by streaking from each of the MIC (non turbid) test tube on fresh solid medium and then incubated at 37°C for 24 hours. The lowest concentration of the extract at which the bacteria were killed was regarded as MBC. Each experiment was done in duplicates.

Survival of spoilage bacteria in water melon juice supplemented with different concentration of Ethanol extract of *Hibiscus sabdariffa* calyces

The survival of the spoilage bacteria in water melon juice fortified with different concentrations for example (200, 100, 50, 25 mg/ml) of ethanol extract of *Hibiscus sabdariffa c*alyces was determined according to the method of [47]. Twenty ml of different concentrations of ethanol extract of HSC were dispensed differently in McCartney bottles containing 20 ml of pasteurized fresh water melon juice and one ml (1×10^7 cfu/ml) of the standardized test organisms (*Escherichia coli, Proteus vulgaris, Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*) was inoculated differently into the mixture. Twenty ml of pasteurized water melon juice with the standardized organisms was used as control. The bacterial growth was monitored for 150 mins at 30 minutes interval by serially diluting 1 ml from the twenty ml of pasteurized water melon juice containing spoilage bacteria and ethanol extract of HSC and 1 ml of appropriate dilution was plated on nutrient agar and incubated at 37°C for 18h.

Statistical analysis

All the assays were carried out in triplicates and the experimental results were expressed as mean ± standard deviation. A statistical comparison of means of different treatments was carried out using one way analysis of variance and treatment means were separated using the Duncan Multiple Range Test. Significance level was set $p < 0.05$. The data analysis was done using SPSS version 13.0 for window

Results

Table 1 shows the morphological and biochemical characteristics of spoilage bacteria of water melon juice. Twenty five bacterial isolates were obtained from spoilt water melon juice and base on the combined results of the morphological and biochemical characteristics and with reference to Bergeys manual of systematic bacteriology the isolates were identified as *Bacillus cereus, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aeruginosa.*

Isolate Code	**MA6**	**NA5**	**NA2**	**MA4**	**MA9**	**MA12**
Gram Reaction	+	+	-	-	-	-
Shape	Cocci	Rods	Rods	Rods	Rods	Rods
Spore	-	+	-	-	-	-
Catalase	+	+	+	+	+	+
Oxidase	-	-	+	-	-	+
H_2S	-	-	-	-	-	+
Indole	-	-	-	-	+	+
Motility	-	+	+	-	+	+
Citrate	+	+	+	+	-	+
Urease	+	-	-	+	-	+
Methyl Red	+	-	-	-	+	+
Voges Proskauer	+	+	-	+	-	-
Glucose	+	+	-	+	+	+
Fructose	+	+	-	-	-	-
Mannitol	+	-	+	+	-	-
Lactose	+	-	-	+	+	-
Maltose	+	+	+	+	+	-
Sucrose	+	+	-	+	+	-
Probable Organism	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Escherichia coli*	*Proteus vulgaris*

Table 1: *Morphological and biochemical characteristics of spoilage bacteria of water melon juice.*

(+): Positive; (-): Negative.

Determination of phytochemicals

The result of the quantitative determination of the phytochemical constituents of the different solvent extracts of *Hibiscus sabdariffa* calyces is presented in table 2. The quantities of Saponin, tannin, alkaloids, phenol and flavonoids present in ethanol extracts were 1.35 ± 0.5, 1.21 ± 0.3, 0.41 ± 0.1, 1.50 ± 0.2 and 2.40 ± 0.0mg/g respectively while the hot aqueous extract contained 0.20 ± 0.0, 0.79 ± 0.2, 0.27 ± 0.1 and 0.32 ± 0.1mg/g of saponin, tannin, alkaloids and phenol respectively and the cold aqueous extract contained 0.19 ± 0.1, 0.52 ± 0.2, 0.24 ± 0.1 and 0.11 ± 0.1mg/g of saponin, tannin, alkaloids and phenol respectively which were significantly different. Flavonoids were not detected in both hot and cold aqueous extracts

Samples	Saponin mg/g	Tannin mg/g	Alkaloids mg/g	Phenol mg/g	Flavonoid mg/g
Ethanol	1.35 ± 0.5^{b}	1.21 ± 0.3^{c}	0.41 ± 0.1^{b}	1.50 ± 0.2^{c}	2.40 ± 0.0^{b}
Aqueous (Hot)	0.20 ± 0.0^{a}	0.79 ± 0.2^{b}	0.27 ± 0.1^{a}	0.32 ± 0.1^{b}	0.00 ± 0^{a}
Aqueous (Cold)	0.19 ± 0.1^{a}	0.52 ± 0.2 ^{a}	0.24 ± 0.1^{a}	0.11 ± 0.1^{a}	0.00 ± 0^{a}

Table 2: *Quantitative phytochemicals of different extracts of Hibiscus sabdariffa calyx.*
Means within a column followed by the same letter are not significant by Duncan's Multiple Range Test at 5% level of significance.

Table 3 shows the antibacterial activities of the different extract of *Hibiscus sabdariffa* calyces against spoilage bacteria of water melon. It was observed that Ethanol extract demonstrated the highest antimicrobial activity and inhibited *Staphylococcus aureus, Bacillus cereus*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli and Proteus vulgaris* showing zones of inhibition of 15.3 ± 0.2, 14.3 ± 0.6, 10.7 ± 0.4, 11.0 ± 0.0, 10.7 ± 0.1 and 8.3 ± 0.5 mm at concentration of 200 mg/ml respectively while at 200 mg/ml the hot aqueous extract inhibited *Staphylococcus aureus, Bacillus cereus*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli and Proteus vulgaris* showing zones of inhibition of 10.7 ± 0.4, 11.0 ± 0.1, 9.0 ± 0.0, 6.7 ± 0.6, 7.3 ± 0.2 and 5.0 ± 0.0 mm respectively and inhibition zones of 6.7 ± 0.3, 6.0 ± 0.0, 4.0 ± 0.5, 3.7 ± 0.4, 4.3 ± 0.2 and 0.0 ± 0.0 mm were seen against *Staphylococcus aureus, Bacillus cereus*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli and Proteus vulgaris* respectively at 200 mg/ml by the cold aqueous extract. The same trend was exhibited by the other concentrations of the extract used.

Solvent Extract		Conc.mg/ml	Zones of Inhibition (mm)				
Concentration		*S. aureus*	*B. cereus*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*	*P. vulgaris*
Ethanol	200	15.3 ± 0.2^{d}	14.3 ± 0.6^{d}	10.7 ± 0.4^{d}	11.0 ± 0.0^{d}	10.7 ± 0.1^{c}	8.3 ± 0.5^{b}
	100	11.0 ± 0.0^{c}	10.7 ± 0.3^{c}	9.3 ± 01^{b}	9.7 ± 0.6^{c}	8.3 ± 0.2^{c}	6.3 ± 0.6^{b}
	50	6.3. ± 0.6^{b}	5.3 ± 0.4^{b}	4..0 ± 0.0^{a}	4.3 ± 0.2^{b}	3.7 ± 0.6^{a}	3 ± .0 ± 0.0^{a}
	25	3.3 ± 0.3^{a}	2.0 ± 0.0^{a}	2.0 ± 0.4^{a}	3.0 ± 0.5^{a}	3.0 ± 0.0^{a}	2.7 ± 0.6^{a}
Ciprofloxacin		21.3 ± 0.6^{e}	19.3 ± 0.1^{e}	17.7 ± 0.5^{e}	15.7 ± 0.2^{e}	15.3 ± 0^{e}	16.5 ± 0.8^{e}
Hot Aqueous	200	10.7 ± 0.4^{d}	11.0 ± 0.1^{b}	9.0 ± 0.0^{b}	6.7 ± 0.6^{b}	7.3 ± 0.2^{b}	5.0 ± 0.0^{b}
	100	8.7 ± 0.6^{b}	7.3 ± 0.7^{c}	6.1 ± 0.3^{b}	3.7 ± 0.5^{b}	4.1 ± 0.0^{b}	3.5 ± 0.3^{b}
	50	4.0 ± 0.0^{a}	3.9 ± 0.3^{b}	2.0 ± 0.0^{a}	1.0 ± 0.0^{a}	1.3 ± 0.0^{a}	0.8 ± 0.0^{a}
	25	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Cold Aqueous	200	6.7 ± 0.3^{c}	6.0 ± 0.0^{b}	4.0 ± 0.5^{a}	3.7 ± 0.4^{b}	4.3 ± 0.2^{b}	0.0 ± 0.0^{a}
	100	5.0 ± 0.6^{b}	.4 ± 0.1^{a}	3.2 ± 0.3^{b}	2.3.0 ± 0.0^{a}	3.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	50	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0a
	25	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.00 ± 0.0^{a}

Table 3: *Zones of inhibition (mm) of different extracts against bacteria isolates.*
Means within a column for each attribute followed by the same letter are not significant by Duncans Multiple Range Test at 5% level of significance.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of different solvent extracts of *Hibiscus sabdariffa* calyces against spoilage bacteria of water melon juice is shown in table 4. The minimum inhibitory concentrations recorded by the ethanol extract for *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris* fell within the range of 50 to 100 mg/ml while their corresponding MBCs values fell within the range of 100 to 200mg/ml. The MIC values for *Staphylococcus aureus, Bacillus cereus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* fell within the range 0 to 200 mg/ml in the hot aqueous extract while their MBC values were not detected. However, MIC values for the spoilage bacteria were not detected when the cold aqueous extract was tested.

Bacteria isolates			Solvents			
	Ethanol		Aqueous (hot)		Aqueous (cold)	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	50 ± 00^{a}	100 ± 0.0^{b}	200 ± 0.0^{c}	-	-	-
Bacillus cereus	50 ± 0.0^{a}	100 ± 0.0^{b}	200 ± 0.0^{c}	-	-	-
Pseudomonas aeruginosa	100 ± 0.0^{b}	200 ± 0.0^{c}	-	-	-	-
Klebsiella pneumoniae	100 ± 0.0^{b}	200 ± 0.0^{c}	200 ± 0.0^{c}	-	-	-
Escherichia coli	100 ± 0.0^{b}	200 ± 0.0^{c}	200 ± 0.0^{c}	-	-	-
Proteus vulgaris	100 ± 0.0^{b}	200 ± 0.0c	-	-	-	-

Table 4: *Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of different extracts of Hibiscus sabdariffa calyces against spoilage bacteria of water melon juice.*

Means within a column followed by the same letter are not significant different by Duncan's Multiple Range Test at 5% level of significance.

Survival of spoilage bacteria in water melon juice fortified with different concentration of ethanol extract of *Hibiscus sabdariffa* calyces

The result of survival rate of spoilage bacteria in water melon juice supplemented with different concentration of Ethanol extract of *Hibiscus sabdariffa* calyces were shown in Figures 1-6 respectively. The results showed that the microbial load of the spoilage bacteria decreased as time increased with concentration of the HSB extract.

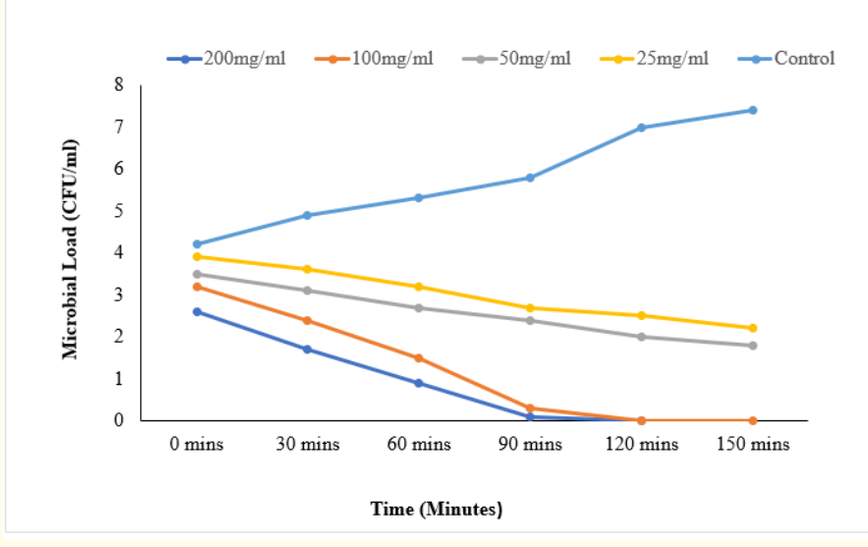

Figure 1: *Survival of Staphylococcus aureus in water melon juice supplemented with different concentrations of ethanol extract of Hibiscus sabdariffa calyces.*

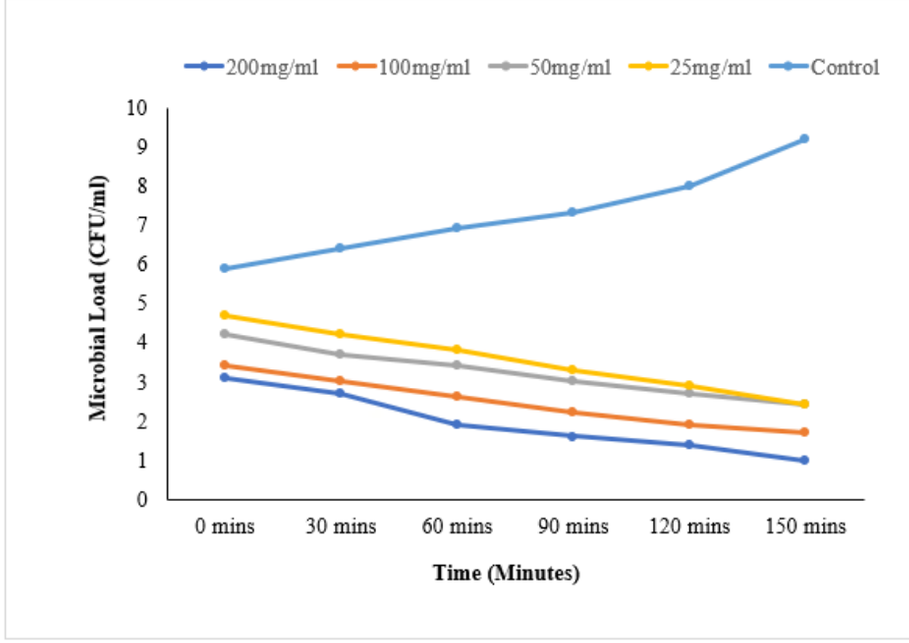

Figure 2: *Survival of Klebsiella pneumonia in water melon juice supplemented with different concentrations of Ethanol extract of Hibiscus sabdariffa calyces.*

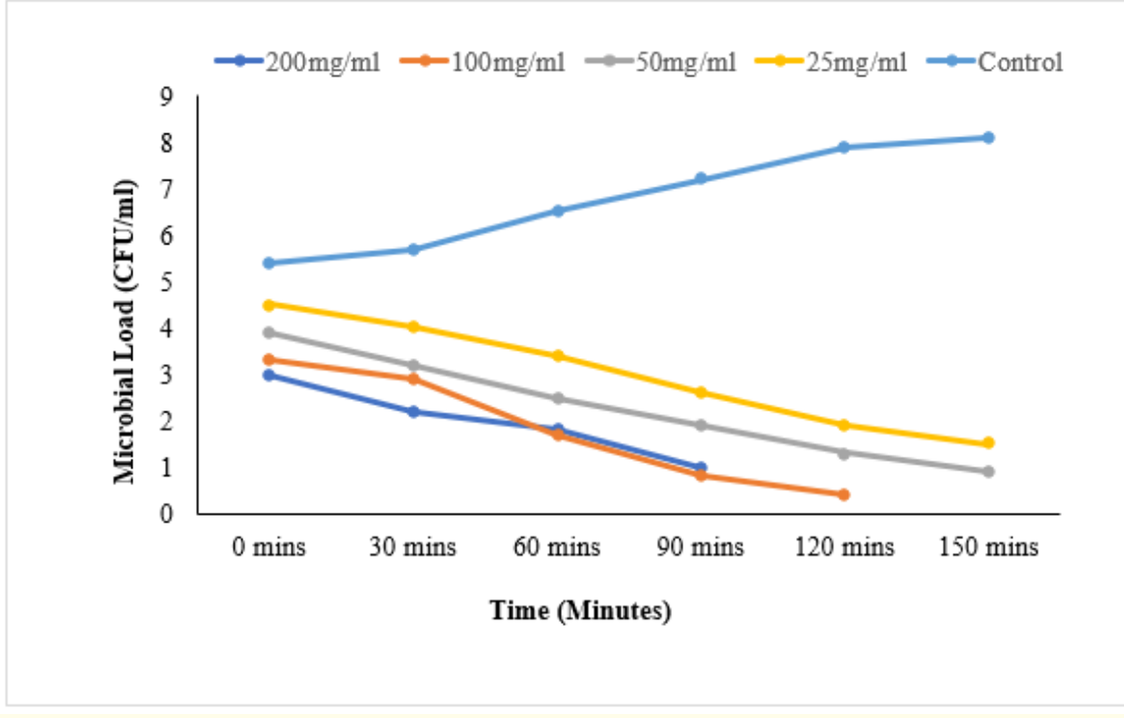

Figure 3: *Survival of Bacillus cereus in water melon juice supplemented with different concentrations of Ethanol extract of Hibiscus sabdariffa calyces.*

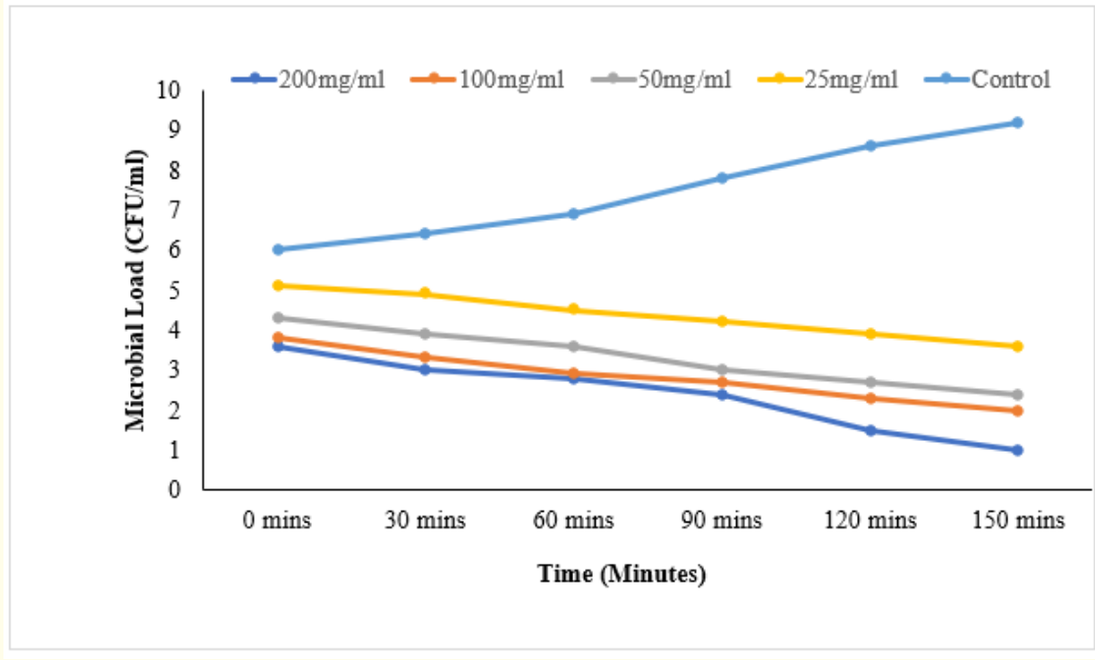

Figure 4: *Survival of Pseudomonas aeruginosa in water melon juice supplemented with different concentrations of Ethanol extract of Hibiscus sabdariffa calyces.*

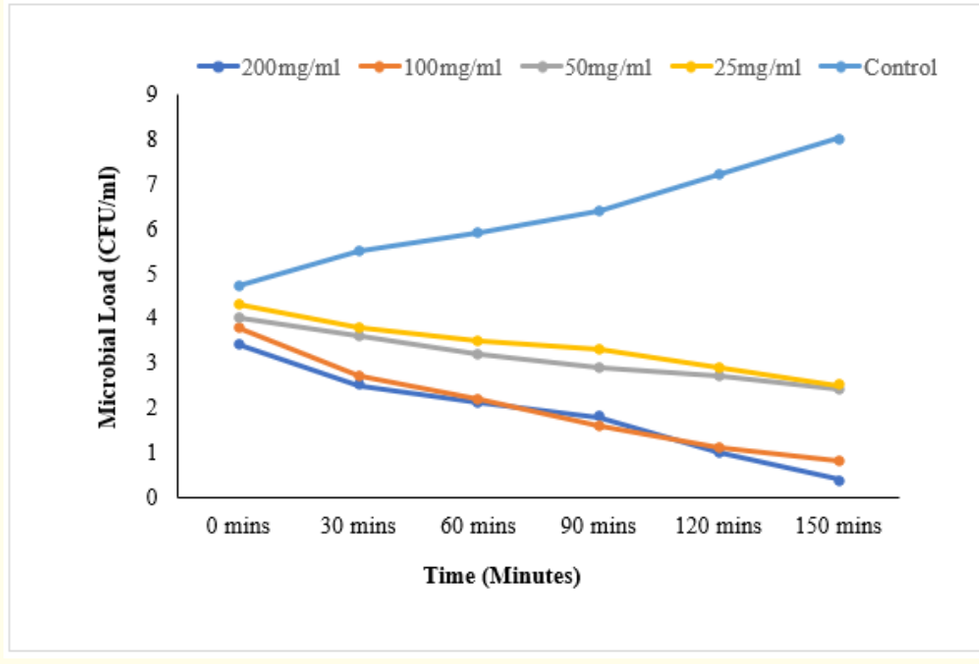

Figure 5: *Survival of Escherichia coli in water melon juice supplemented with different concentrations of Ethanol extract of Hibiscus sabdariffa calyces.*

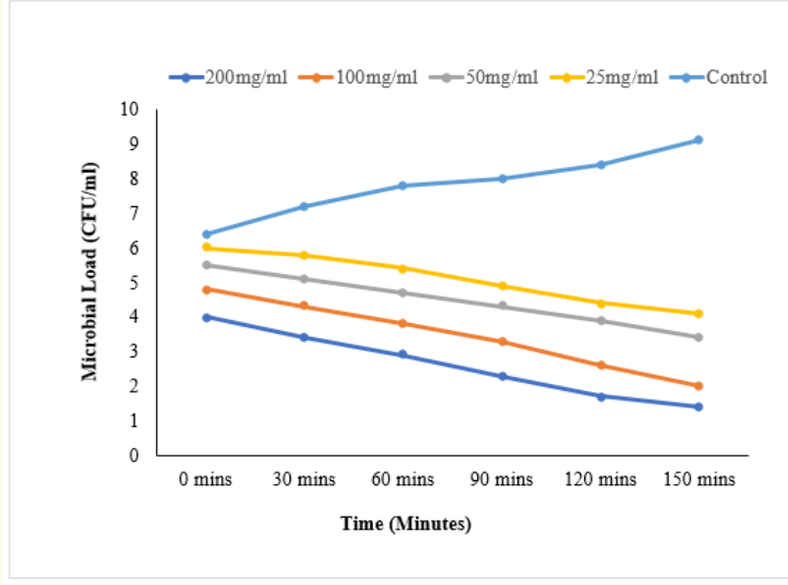

Figure 6: *Survival of Proteus vulgaris in water melon juice supplemented with different concentrations of Ethanol extract of Hibiscus sabdariffa calyces.*

Figure 1 shows survival of *Staphylococcus aureus* in water melon juice supplemented with different concentrations of Ethanol extract of *Hibiscus sabdariffa* calyces. At 200 mg/ml, the microbial loads were 2.6 x 10^2 ± 0.3, 1.7 x 10^2 ± 0.1, 0.9 x 10^1 ± 0.5, 0.1 x 10^1 ± 0.0, 0.0 ± 0.0 and 0.0 ± 0.0 CFU/ml at 0 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes respectively while at 100 mg/ml, the microbial load were 3.2 x 10^2 ± 0.2, 2.4 x 10^2 ± 0.5, 1.5 x 10^1 ± 0.3, 0.3 x 10^1 ± 0.2, 0.0 ± 0.0, 0.0 ± 0.0 CFU/ml at 0 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes respectively. At 50 mg/ml, the microbial load were 3.5 x 10^2 ± 0.1, 3.1 x 10^2 ± 0.4, 2.7 x 10^2 ± 0.6, 2.4 x 10^2 ± 0.2, 2.0 x 10^2 ± 0.5 and 1.8 x 10^2 ± 0.3 CFU/ml at 0 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes. At 25 mg/ml, the microbial load were 3.9 x 10^2 ± 0.3, 3.6 x 10^2 ± 0.1, 3.2 x 10^2 ± 0.5, 2.7 x 10^2 ± 0.1, 2.5 x 10^2 ± 0.4 and 2.2 x 10^2 ± 0.3 CFU/ml at 0 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes respectively. At control, the microbial load were 4.2 x 10^2 ± 0.3, 4.9 x 10^2 ± 0.5, 5.3 x 10^2 ± 0.6, 5.8 x 10^2 ± 0.8, 7.0 x 10^2 ± 1.0 and 7.4 x 10^2 ± 0.5 CFU/ml at 0 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes respectively. The same survival trend was seen in the other food spoilage bacteria when exposed to the different concentration of the extract with time.

Discussion and Conclusion

This study provides empirical information on the isolation and identification of spoilage bacteria of water melon juice and the evaluation of the antimicrobial potential of Ethanol, cold and hot water solvent extracts of *Hibiscus sabdariffa* calyces against spoilage bacteria of water melon and the survival of spoilage bacteria in water melon juice fortified with different concentration of Ethanol extract .The susceptibility of water melon juice to microbial spoilage is due to the presence of high water activity and other components of juice such as minerals and sugar which are necessary for the growth of spoilage bacteria and their ubiquitous nature [48]. In addition, these spoilage bacteria are able to synthesize enzymes which are capable of hydrolysing the sugar and other components present in water melon juice for their growth and metabolism [49,50].

The presence of phytochemicals in the different extracts of *Hibiscus sabdariffa* calyces had been earlier reported by Nkumah [51]. However, there are documented reports on the synthesis of phytochemicals by plants which accounts for their occurrence. The highest quantity of phytochemicals seen in the ethanol extract may be due to its outstanding extraction capacity and in addition, alcohol are polar solvents solubilsing most of the active ingredients present in *H. sabdariffa* calyces thereby enhancing substantial quantities [52] while lower yield of phytochemicals obtained using the other extraction solvents may be attributed due to their low solubility capacities. According to Walsh., *et al*. [53] different solvents have diverse solubility capacities for different phytochemicals. Equally the best antimicrobial activity

demonstrated by the Ethanol extract could also be linked to presence of relatively higher quantities of phytochemicals. This reported finding is in agreement with the earlier submission of Walsh., *et al.* [53] that confirmed that ethanol extract demonstrated a higher antimicrobial activity than aqueous extracts. The antimicrobial ability of the extract may be due to the presence of phytochemicals which act by inhibition of electron transport, protein translocation, phosphorylation steps, and other enzyme-dependent reactions, followed by an increase in plasma membrane permeability and finally ion leakage from the bacterial cells [53]. The therapeutic use of plants especially as antimicrobials has been reported by many scientists [55-57]. Previous reports on the antimicrobial activity of *H. sabdariffa* revealed that it is inhibitory against gram positive and gram negative bacteria which confirms its broad spectrum activity [56] but inhibition is higher against gram positive bacteria than gram negative bacteria as seen in this present study. Susceptibility difference between Gram positive and Gram negative bacteria may be due to the structural differences in their cell membranes [58,59]. This observation is in consonance with previous reports of [57]. In addition, Nair and Chanda [60] and Borrás-Linares., *et al*. [61] reported that higher inhibition zones were seen against gram positive bacteria when compared to the gram negative bacteria from a study conducted on antimicrobial activity of Ethanol extract from 25 varieties of Mexican *Hibiscus sabdariffa* calyces. The potency of Ethanol extracts of *H. sabdariffa* calyces against these bacteria as seen in this study gives scientific basis for their uses in folk medicine as applied to treatment of bacterial infection [62].

The low MIC and higher MBC values recorded by extracts of *H. Sabdariffa* calyces indicated its high potency and bacteriostatic tendency at lower concentrations and bactericidal tendency at higher concentration [63]. It could be suggested that it can be used to inhibit spoilage bacteria and be applied in food preservation. However the use of plant extracts in food preservation has earlier been reported [64,65].

The selection of the Ethanol extract for further was due to the higher inhibition zone observed against spoilage bacteria of water melon. The survival rate is dependent on time and concentration of the extract. This observation was previously reported by [43,47,66]. In conclusion, from the empirical results obtained in this study specifically in the outstanding inhibitory performance of the Ethanol extract of *Hibiscus sabdariffa* calyces, it can be suggested that the extract could be used as potential bio preservative agent of water melon juice.

Bibliography

1. Bondi M. "Natural Preservatives to Improve Food Quality and Safety". Journal of Food Quality (2017).
2. Hamid SAN., *et al*. "Food: Its preservatives Additives and applications". *International Journal of Chemical and Biochemical Sciences* (2012).
3. Seetaramaiah K., *et al*. "Preservatives in Food Products - Review". *International Journal of Pharmaceutical and Biological Archive* 2.2 (2011): 583-599.
4. Burt S. "Essential oils: their antibacterial properties and potential applications in foods-A review" *International journal of food microbiology Elsevier* 94.3 (2004): 223-253.
5. Ponce A., *et al*. "Shelf life of leafy vegetables treated with natural essential oils". *Journal of Food Science* 69.2 (2004): 550-556.
6. Bonjar S. "Evaluation of antibacterial properties of some medicinal plants used in Iran". *Journal of Ethnopharmacology* 94 (2004): 301-305.
7. De Boer H., *et al*. "Antifungal and antibacterial activity of some herbal remedies from Tanzania". *Journal of Ethnopharmacology* 96.3 (2005): 461-469.
8. Cutler HG. "Natural flavor compounds as potential antimicrobials insecticides and medicina". *L AgroFOOD Industry Hi-tech* 6 (1995): 19-23.
9. Wood C., *et al*. "Herbals cancer prevention and health". *The Journal of Nutrition* 131 (2001): 3034-303.
10. Erasto, P., *et al*. "Antioxidant favonoids from the root wood of Bohisanthus speciosus". *Phytochemistry* 65 (2004): 875-880.

11. Tepe B., *et al*. "*In vitro* antimicrobial and antioxidant activity of the essential oils and various extracts of Thymus eigil M Zohary et P.H Davis". *Journal of Agricultural and Food Chemistry* 52 (2004): 1132-1137.

12. Pandit VA and Shelf LA. "Sensitivity of Listeria monocytogenes to rosemary (Rosimarinus oficilalis L.). *Food Microbiology* 11 (1994): 57-63.

13. Stephens JM. "Horticultural Sciences Department Cooperative Extension Service". Institute of Food and Agricultural Sciences, University of Florida, Gainesville FL 32611 (2012).

14. Souza E., *et al*. "Antimicrobial effectiveness of spices an approach for use in food conservation systems". *Brazilian Archives of Biology and Technology* 48 (2005): 1-13.

15. Maganha EG., *et al*. "Pharmacological evidence for the extracts and secondary metabolites from plants of the genus Hibiscus". *Food Chemistry* 118 (2010): 1-10.

16. Dokosi OB. Herbs of Ghana. Ghana Universities Press. (1998): 141-157.

17. Tsai J., *et al*. "Anthocyanin and antioxidant capacity in roselle (Hibiscus sabdariffa L.) extract". *JCIFST* 35 (2002): 351-356

18. Ali BH., *et al*. "Phytochemical, Pharmacological and Toxicological Aspects of Hibiscus sabdariffa L.: A Review". *Phytotherapy Research* 375 (2005): 369-375.

19. Olaleye T. "Cytotoxicity and antibacterial activity of mEthanol extract of Hibiscus sabdariffa". J. Med. Plants Res. 1.1 (2007): 9-13.

20. Edwards AJ., *et al*. "Consumption of watermelon juice increases plasma concentrations of lycopene and β-carotene in humans". *The Journal of Nutrition* 133 (2003): 1043-1050.

21. Roger GD. "Healthy Foods, New Lifestyle". 1st Edition. Safeliz S.L. Spain 236 (2006).

22. FAO. "World Agriculture: towards 2015/2030". Summary report, Rome (2003).

23. Huh YC., *et al*. "Morphological characterization of Korean and Turkish watermelon germplasm. 1 Cucurbitaceae". Proceedings of the 9th EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M. Ed.), INRA Avignon, France (2008): 21-24.

24. Zohary D and Hopf V. "Domestication of Plants in the Old World". 3rd Edition. Oxford University Press. (2000):193. 59

25. This Day Live. "How Export Farm Produce will Improve Nigeria's Foreign Earnings". Media Report 19 (2014).

26. Quek SY., *et al*. "The physicochemical properties of spray-dried watermelon powders". *Chemical Engineering Processing* 46 (2007): 386- 392.

27. Artes-Hernandez., *et al*. "Low UV-C illumination for keeping overall quality of fresh-cut watermelon". *Postharvest Biology and Technology* 55 (2010): 114-120.

28. Oms-Oliu G., *et al*. "Use of Weibull distribution for describing kinetics of antioxidant potential changes in fresh-cut watermelon". *Journal of Food Engineering* 95 (2009): 99-105.

29. United States Department of Agriculture (USDA). Nutrient Lab. Watermelon (2009).

30. Sapers GM., *et al*. "Antimicrobial treatments for minimally processed cantaloupe melon". *Journal of Food Science* 66 (2001): 345-349.

31. Erukainure OL., *et al*. "Improvement of the Biochemical Properties of Watermelon Rinds Subjected to Saccharomycescerevisae Solid Media Fermentation *Pakistan Journal of Nutrition* 9.8 (2010) : 806-809.

32. Anitha M. "Prevalence of disease causing microrganisms in decaying fruits with analysis of fungal and bacteria species". *International journal of research in health sciences* 2.2 (2014): 547-554.

33. Meynell G and Meynell E. "Theory and practice in experimental bacteriology". University Press Cambridge, UK, 347 (1965).

34. Willey JM., *et al*. "Isolation of pure culture". In: Prescott's Microbiology *Mc*Graw-Hill International Edition (8th Edition). McGraw-Hill press, New York. 4 (2011): 150-152.

35. Holt JG., *et al*. "Bergey's manual of determinative bacteriology 9th edition" (1994).

36. Cheesbrough M. District Laboratory Practice in Tropical Countries Part 2 Cambridge University Press Cambridge (2006): 47- 54.

37. Sukhdev SH., *et al.* Extraction Technologies for Medicinal and Aromatic Plants". United Nations Industrial Development Organization and the International Science and High Technology, Trieste, Italy 7 (2008).

38. Harborne JB. "Phytochemical methods". Chapman and Hall Ltd., London. 125 (1988).

39. Van-Burden TP and Robinson WC. "Formation of complexes between protein and Tannin acid". *Journal of Agricultural and Food Chemistry* 1 (1981): 77-82.

40. Obodoni BO and Ochuko PO. "Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria". *Global Journal of Pure Applied Science* 8.3 (2001): 203-208.

41. Boham BA and Kocipai - Abyazan R. Flavonoids and condensed tannins from leaves of Vaccinium vaticulatum and V. calycinium. *Pacific Science* 48 (1994): 458 - 463.

42. Daoud A. "Assessment of polyphenol composition Antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars". *Arabian Journal of Chemistry Elsevier* (2015).

43. Bhalodia NR and Shukla VJ. "Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant". *Journal of advanced pharmaceutical technology and research medknow Publications* 2.2 (2011): 104.

44. Okeke MJ., *et al*. "Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity". *Journal of Ethnopharmacology* 78 (2001): 119-127,

45. Uddin SJ., *et al*. "Neuropharmacological properties of Xylocarpus molucensis". *Fitoterapia* 78 (2007): 107-111.

46. Nweze EI., *et al*. "Antimicrobial activities of mEthanol extract of Tremaguineensis (Schumm and Thorn) and Morindalucidaused in Nigeria herbal medicine practice". *Journal of Biological Research and Biotechnology* 2.1 (2004): 36-39.

47. Oramadike CE and Ogunbanwo ST. "Antagonistic activity of Thymus vulgaris extract against Vibro species isolated from sea foods". *Journal of food science and technology* 54 (2017): 1199-1205.

48. Splittstoesser DF., *et al*. "Growth characteristics of aciduric spore forming bacilli isolated from fruit juices". *Journal of Food Protection* 57.12 (1994): 1080-1083.

49. Antony U and Chandra TS. "Microbial population and biochemical changes in fermenting finger millet (Eleusine coracana)". *World Journal of Microbiology and Biotechnology* 13 (1997): 533-537.

50. Ferrati AR., *et al*. "A comparison of ready-to-use systems for evaluating the microbiological quality of acidic fruit juices using non-pasteurized orange juice as an experimental model". *International Microbiology* 8 (2005): 49-53.

51. Nkumah O. "Phytochemical analysis and medicinal uses of Hibiscus sabdariffa". *International journal of herbal medicine* 2 (2015): 16-19.

52. Tschesche R. "Advances in the chemistry of antibiotics substances from higher plants: In proceeding of the 1st international Congress Murich". *Journal of Pharmacognosy and Phytochemistry* (1970): 274-289.

53. Walsh SE., *et al*. "Activity and mechanism of action of selected biocidal agents on Gram -positive and -negative bacteria". *Journal of Applied Microbiology* 94 (2003): 240-247.

54. Al-Hashmi AG. "Antioxidant and antibacterial activities of Hibiscus sabdariffa L. extracts". 6.21 (2012): 506-511

55. Sharaf A., *et al*. "Further study on the antibacterial effect of H sabdariffa". *Microbial Pathogenesis* 29.1 (1966): 120-125.

56. Cowan MM., "Plant products as antimicrobial agents". *Clinical Microbiology Reviews* 12.4 (1999): 564-582

57. González- Lamothe R., *et al*. "Plant Antimicrobial Agents and Their Effects on Plant and Human Pathogens". *International Journal of Molecular Sciences* 10.8 (2009): 3400-3419.

58. Lennette E., *et al*. "Manual of clinical microbiology". 4th edition Amer. Soc ***M***icrobio Washington (1995).

59. Baron EJ., *et al*. "Baily scotts diagnostic microbiology". 9th edition mosby year book, inc St Louis (1994).

60. Nair R and Kalariya T. "Antibacterial Activity of Some Selected Indian Medicinal Flora". *Medicina Experimentalis* 29 (2005): 41-47.

61. Borrás-Linares I. "Characterization of phenolic compounds Anthocyanidin Antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscussabdariffa*)". *Industrial Crops and Products* 69 (2015): 385-394.

62. Morton JF. "Roselle". In: Fruits of warm climate (Dowling CF, ed) *M*edia, Inc., Greensboro, NC (1987): 281-286

63. Rahman MS., *et al*. "Antibacterial activity of Argemone Mexicana L Against water borne microbes". *Research Journal of Medicinal Plants* 5.5 (2011): 621-626.

64. Nassar-Abbas S and Kadir-Halkman A. "Antimicrobial effect of water extract of Sumac (Hruscor coriaria L.) on the growth of some food borne bacteria including pathogens". *Journal of Food Microbiology* 97 (2004): 63-69

65. Hara-Kudo Y., *et al*. "Antibacterial activities of plants used in cooking for aroma and taste". *Journal of Food Protection* 67 (2004): 2820-2824.

66. Banso A., *et al*. "Antimicrobial properties of Vermonia amygdalina extract". *Journal of Applied Sciences and Environmental Management* 3 (1999): 9-11.

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