THE PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF SPICED AND UNSPICED BEVERAGES FROM SORGHUM STEM SHEATH (*Sorghum bicolor*) AND BRAZILIAN PLUME LEAF

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ABSTRACT
This study was carried out to investigate the ability of nonalcoholic, spiced and unspiced beverages produced from sorghum stem sheath and Brazilian plume leaf to serve as functional drink. The beverage blends were produced from Sorghum stem sheath and Brazilian plume leaf in the proportion of 100:0, 0:100 and 50:50 with or without spices respectively. The beverages were subjected to assays to examine their antioxidant, antimicrobial, phytochemical, alpha amylase and alpha glucosidase inhibitory properties. The antioxidant properties of the beverages were in the range of 69 %-80 % for 1,1-diphenyl-2 picryl hydroxyl free radical (DPPH), 23.00 mg/ml-58.00 mg/ml for ferric reducing antioxidant power (FRAP),0.664 mg/100ml-12.84 mg/100ml for vitamin C and 238.0 mg/ml-288.0 mg/ml for anthocyanin content. The phytochemical content of the resultant beverages include flavonoid (6.12 mg/g-15.62 mg/g), alkaloid (1.52 mg/g -3.36 mg/g), tannin (0.77 mg/g-1.19 mg/g), oxalate (9.20 mg/g-19.71mg/g), saponin (2.52mg/g-8.78mg/g), anthraquinone (7.02 mg/g-11.82 mg/g), phenol (3.31 mg/g-8.21 mg/g) and terpenoid (29.14 mg/g-48.73 mg/g). The beverage blends were found to demonstrate very mild antimicrobial activities. The results showed that beverages exhibited good inhibitory potential against -amylase and -glucosidase enzymes which ranged from 60%-78% and 64%-77% respectively. The overall evaluation showed that spiced sorghum stem sheath-*Justicia carnea* beverages may be preferred to the unspiced beverage and could be considered as a functional drink.

Keywords:

1 | Introduction

Several researches have revealed growing recognition of the important roles played by foods and beverages in disease prevention, management and treatment with good attention given to consumption of sugar-sweetened beverages and increased risk of diet-related health problems (Malik *et al.*, 2010; Borokini *et al.*, 2019). Diet are excellent vehicles for delivering adequate nutrients and bioactive compounds into the body. beverages sub sector has been observed over 10% growth within the last decade and a predicted 48% of the total food market by 2027(Kotler & Keller, 2016). This innovative growth (of the beverages subsector) is linked to growing urbanisation, middle-class population expansion, fast-growing health concerns, perceived health promotion, and disease risk reduction potentials (Li *et al.*, 2016)

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This may be as a result of the ability of bioactive compounds from legumes, cereals, grains, fruits and vegetables to lower lipid and cholesterol levels, increase bone mineral density and antioxidant status and demonstrate anti-cancer properties (Eskin & Tamir, 2006). In addition, scientific investigations uphold the idea that diet may fulfill nutritional needs and render beneficial roles in the management of some noncommunicable diseases (Otles & Cangind, 2012). Hence, increase in the demand for "healthy" foods and beverages in many parts of the world in recent years (Ozen *et al*., 2012).

A functional food is considered to be similar to a conventional food and a part of a normal diet consumed consistently in good proportion but with proven health benefits that decreases the risk of specific chronic diseases or beneficially attends to specific purpose that surpasses its basic functions (Doyon & Labrecqu, 2008).

*Sorghum bicolor*is an essential staple food crop in Africa. Its stem sheath and leaf locally called 'poroporo baba' within many localities in Nigeria are generally recognized as medicinal herbs used as blood tonic in traditional treatment of anaemia and other blood related diseases especially in the South-Western part of Nigeria (Adetuyi, 2004).Sorghum stem sheath is cheap, locally available and also used as colour additive in cooking meals, for example, in the preparation of "Waakye", a local rice dish in Ghana and can be taken as beverages when steeped or boiled in water (Adetuyi, 2004).

Several species of *Justicia* are used in traditional medicine for the treatment of anemia, inflammatory fever, diarhorrea, liver diseases and arthritis, respiratory and gastro-intestinal disorder (Onyeabo *et al*., 2017). Recently, it has been reported that the species possess cardio-protective, antibacterial, antioxidant properties and generally rich in vitamins and minerals (Uroko *et al*., 2017; Ukpabi-Ugo *et al*., 2019). The leaves of *Justicia carnea* is usually prepared with edible vegetables to make soup, boiled separately in water to make tea or prepared by cooking with other medicinal plants for therapeutic purposes.

Spices are esoteric food adjuncts that are used to enhance the sensory quality of foods (Ndife, 2016). They have been used as flavourings since ancient times and as medicine and food preservatives in recent decades (Nabavi *et al.,* 2016). They impart characteristic flavor, aroma or pungency and color to foods, stimulate appetite as well as modify the texture of foods.Dietary spices in their minute quantities have colossal effect on human health by their antioxidant, chemo preventive, antimicrobial, anti-mutagenic, anti-inflammatory, immune modulatory effects on cells (Okwu *et al*., 2006; Ndife, 2016). Previous studies also revealed that, spice possesses antimicrobial properties (Ahn *et al*. 2007; Ifesan *et al*., 2009; Zaborowska *et al*., 2012).

Utilization of locally available raw materials inbeverage formulations have been developed with attainment and these include; Zobo and Kunnu Zaki (Bolade *et al.,* 2009), baobabfruit pulp (Ifesan *et al*., 2012), instant beverage infusion from blends of moringa leaf, zobo calyx and lemon grass (Ifesanand Olorunsola,2018), spiced and unspiced beverage from Kiaat bark (Abiodun *et al*., 2022) and beverage from blends of germinated pumpkin seed, ginger and zobo calyx (Faturoti *et al*., 2023). To further investigate the use ofnatural resources for healthy drinks, the study examined the potential of nonalcoholic, spiced and unspiced beverage from sorghum stem sheath and *Justicia carnea*leaf to serve as functional drink.

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2.0 | Materials and Methods

Collection and preparation of samples
Sorghum stem sheath and five (5) spices namely :*Xylopia aethiopica, Syzygium aromaticum, Monodora myristica, Piper guineense*and*Zingiberaceae*were purchased at Oba market, Owo, Ondo State, while *Justicia carnea* leaves were plucked from neighborhood gardens in Akure, Ondo State Nigeria.

2.1 | Preparation of mixed spices

Monodora myristica was cracked to separate the nuts from the kernel and was sun dried. *Piper guineense* was shredded, washed and dried in the sun, while *Xylopia aethiopica*, *Syzygium aromaticumand Zingiber officinale* where sorted, cleaned from extraneous materials and further sun dried. Five grams of each spice was weighed, blended together to obtain mixed spice powder for kunu beverage then stored in an air-tight container (Dada *et al*., 2013).

2.2 | Preparation of Sorghum stem sheath and *Justicia carnea* beverages

Ten grams of sorghum stem sheath and*Justicia carnea*leaveswere separately weighed, washed and boiled in 100 ml of water for 5 min. in the proportion of 100:0, 0:100 and 50:50 with or without the addition of 5 g of mixed spices respectively. The beverages were filtered using muslin cloth, and filled into sterile plastic bottles. The samples were divided into two portions, one was pasteurized at 60 ^{0}C for 30 min and the other set was unpasteurized. The beverages were cooled and stored at room temperature (27 ± 2 ^{0} C) Ifesan and Olorunsola, 2018).

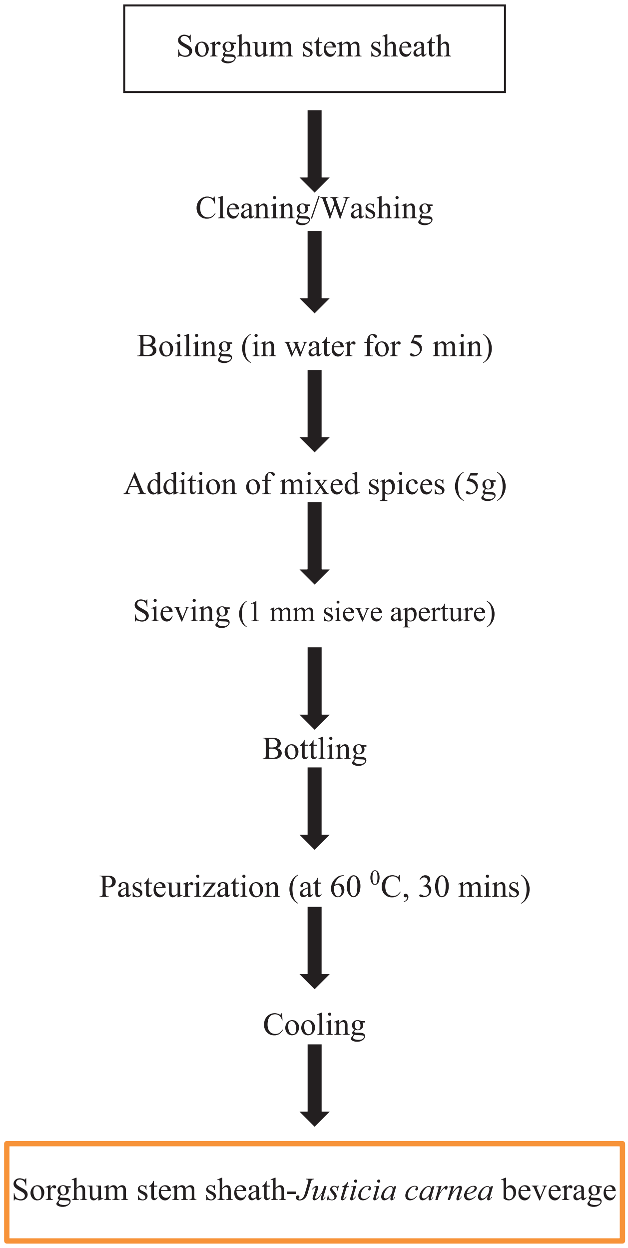

Figure 1: Flow chart for the production of Sorghum stem sheath-*Justicia carnea* beverage

Source: Tiburski, *et al*. (2011); Adetuyi, *et al*. (2007) modified.

2.3 | Determination of antioxidant properties of Sorghum stem sheath and *Justicia carnea* beverages

Determination of Vitamin C and DPPH

The vitamin C content of the aqueous extract was determined using the method of Benderitter *et al*. (1998). About 75 µl DNPH solution (2 g

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dinitrophenyl hydrazine, 230 mg thiourea and 270 mg copper sulphate ($CuSO_4$. $5H_2O$) in 100 ml of 5 ml/L H_2SO_4) were added to 500 µl extract mixture (300 µl of an appropriate dilution of the extract with 100 µl 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 h at 37 ºC, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm in a UV spectrophotometer (JENWAY 6305, Barloworld Scientific Ltd., Dunmow, Essex, UK). The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard. The free radical scavenging ability using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was determined as described by Singh *et al.* (2002). Different concentrations of the aqueous extract were taken in different test tubes and the volume was made up to 1 ml with distilled water. 4 ml of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature. A control was prepared as above without the sample and distilled water was used for base line correction. Changes in absorbance of samples were measured at 517 nm in a spectrophotometer (JENWAY 6305). Free radical scavenging ability was expressed as percentage inhibition and was calculated using the following formula: Free radical scavenging ability (%) = [(absorbance of control – absorbance of sample) / absorbance of control] ×100 % DPPH = (Absorbance of reference - Absorbance of sample) / Absorbance of reference x 100

2.4 | Determination of ferric reducing antioxidant property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce ferric chloride ($FeCl_3$) solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min; thereafter 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 2 000 x g for 10 min; 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer (JENWAY 6305) and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

2.5 | Determination of Antimicrobial properties of the beverages

The antimicrobial activities of beverage samples were carried out against selected food borne pathogenic bacteria (*Escherichia coli, Salmonella spp., Shigella spp., and Staphylococcus aureus*) and fungi (*Candida albicans* and *Aspergillus flavus*), obtained from Department of Microbiology, Federal University of Technology, Akure. The agar well diffusion method was used following Clinical and Laboratory Standard Institute (CLSI, 2006) and zone of inhibition was read in millimeter.

2.5 | Determination of phytochemical properties of sorghum stem sheath and *Justicia carnea* beverages

Phytate was determined according to the method of Wheeler and Ferrel, (1971). Oxalate was determined by the method of Day and Underwood (1986).Tannin quantification was carried out according to the method described by Phan *et al.* (2001). The spectrophotometric method of Brunner (1984) was used for saponin determination. The method of Harbone (1973) was used for alkaloids determination. Terpenoids and anthraquinone were determined using the method of Soladoye & Chukwuma (2012). The total

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phenol content of the beverages were determined by the method of Singleton *et al*.(1999). The total flavonoid content of the beverages were determined using a colorimeter assay developed by Bao (2005).

2.6 | Determination of Alpha-Amylase and Alpha-Glucosidase Inhibitory Activities of Sorghum stem sheath and *Justicia carnea* beverages

The alpha-amylase inhibition assay was carried out using the chromogenic non-pre-incubation method adapted from Sigma-Aldrich as described by Bernfeld, (1955), while the alpha-glucosidase inhibitory activity of the beverages was determined according to the method described by Kim *et al*.(2008), using alpha-glucosidase from *Saccharomyces cerevisiae*.

3.0 | Results and Discussion

3.1 | Antioxidant properties of Sorghum stem sheath and *Justicia carnea* beverages

Free radical scavenging (DPPH) activity of sorghum stem sheath and *Justicia carnea* beverages. The result displayed in Figures 1 showed that all the samples exhibited appreciable DPPH scavenging activities which ranged between 62%-80%, and it was observed that the addition of mixed spices enhanced significant differences. Spices have been reported to have superior antioxidant capacity than many plants materials (Wood & Pittler, 2000). This observation also agrees with the report by Adedeji *et al*., (2019) in which the addition of ginger extract at varying concentration increased the DPPH activity of sorghum stem sheath drink from 0.18%-1.91%. The percentage DPPH scavenging activity (79%) recorded for unspiced *Justicia carnea* beverage in this study is higher than 45%-65% reported by Asogwa *et al*. (2020) and Falode *et al*. (2022) for raw and boiled *Justicia carnea* extractIt was reported that sorghum stem sheath extract is rich in natural antioxidants that can scavenge free radicals to combat degenerative diseases, thus enhancing the antioxidants status of the body (Oboh *et al*., 2010).

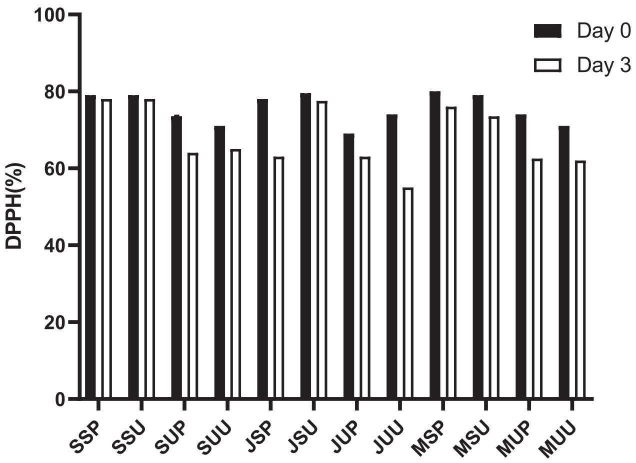

Figure 1 | Free radical scavenging (DPPH) content of Sorghum stem sheath and Justicia carnea beverages at Day 0 and Day 3

Means of triplicate readings ± SD, bars with the same alphabet are not significantly different ($p<0.05$).

SSP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SUP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized;

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MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized

3.2 | Ferric Reducing Antioxidant Potential of Sorghum stem sheath and *Justicia carnea* Beverages

Result in Figures 2 showed that the beverage samples demonstrated moderate reducing power in the range of 29.50 mg/GAE/100g -58.00 mg/GAE/100g The FRAP potential (45.50 mg/GAE/100g) of unspiced *Justicia carnea* beverage was found be higher than unspiced sorghum stem sheath beverage (24.00 mg/GAE/100g). This is in agreement with the reports of Asogwa *et al*., (2020) and Udedi *et al*., (2020) that *Justicia carnea* extracts possess high reducing power, which is an indication that the FRAP activity of *Justicia carnea* is stronger than that of sorghum stem sheath. The effect of storage time from day 0 to day 3 as investigated revealed that there was no significant effect on the antioxidants activities of the beverage samples, which implies that they were fairly stable during storage. The results however showed that the beverage blends from sorghum sheath stem and *Justicia carnea* did not show pronounced synergetic effect.Means of triplicate readings ± SD, bars with the same alphabet are not significantly different ($p<0.05$).

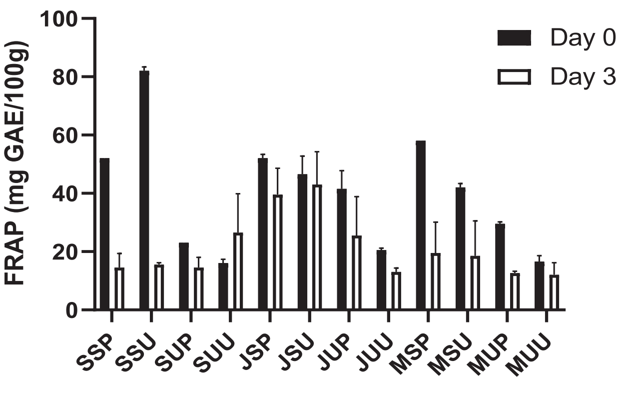

Figure 2 | FRAP Scavenging activity of Sorghum stem sheath and Justicia carnea beverages at Day 0 and Day 3

SSP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SUP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized; MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized.

3.3 | Vitamin C content of beverages from Sorghum stem sheath and *Justicia carnea* Beverages

The vitamin C contents of sorghum stem sheath and *Justicia carnea* beverages are shown on Table 1. The vitamin C content of beverages ranged from 0.66 mg/100ml-12.66 mg/100ml. It was observed that the addition of mixed spices significantly increased the vitamin C content of the beverage samples. Ali *et al*., (2005) reported that, the vitamin C content of beverages can be improved by blending with spices. Also, this report agrees with Adedeji *et al*., (2013), who reported a significant increase in the Vitamin C content of sorghum stem sheath spiced with ginger. The vitamin C content (0.660 mg/100ml) of unspiced sorghum stem sheath beverage, though relatively lower than that of *Justicia carnea* beverage (10.72 mg/100ml) negates some previous report that

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sorghum stem sheath lack vitamin C (Adetuji *et al*., 2007, Adedeji *et al*., 2021). However, Adedeji *et al.*, (2013) and Borokini *et al.* (2019) recorded 415 mg/100ml and 20% Vitamin C content respectively for sorghum sheath drink. This work revealed that *Justicia carnea* possess appreciable quantity of Vitamin C in which its addition increased the vitamin C content of sorghum stem sheath beverage from 0.8 mg/100ml to 11.36 mg/100ml. Adedeji *et al*., (2021) obtained similar report for vitamin C content of sorghum stem sheath drink enriched with moringa leaf (0.00-4.43 mg/100ml). Vitamin C is a powerful water soluble antioxidant that boost immune system (Rai & Amand, 2008). The range of 30 mg to 80mg per day for infants, children and adult is recommended (Bamishaye, 2011). Observation revealed a significant decrease in the Vitamin C content of the pasteurized samples. Vitamin C is said to decrease with exposure to heat, light and oxygen (Bamishaye *et al*., 2011). The effect of storage on vitamin C content of the beverages on the third day resulted in a significant loss. This observation is similar to the study of Egbere *et al*. (2007) who reported a significant reduction in zobo samples from 515 mg/100ml to 438mg/100ml during storage within the period of 4 days

Table 1 | Vitamin C composition of Sorghum stem sheath and *Justicia carnea* beverages (mg/100ml)

SAMPLES	Day 0	Day 3
SSP	9.29 ± 0.00^{e}	8.66±0.20c
SSU	10.50 ± 0.00^{cd}	8.63±0.70c
SUP	0.66 ± 0.10^{f}	0.52±0.10d
SUU	0.08 ± 0.20^{c}	0.45±0.00d
JSP	11.44 ± 0.00^{b}	9.81±0.80b
JSU	12.64 ± 0.10^{b}	11.53±0.10a
JUP	10.72 ± 0.10^{c}	9.49±0.30c
JCU	10.56 ± 0.50^{d}	9.13±0.10c
MSP	12.66 ± 0.00^{a}	11.07±0.00a
MSU	12.84 ± 0.10^{a}	11.77±0.10a
MUP	10.04 ± 0.20^{d}	8.67±0.70c
MUU	11.36 ± 0.50^{b}	8.40±0.10c

Means of triplicate readings ± SD, Values with the same superscripts along the same column are not significantly different ($p<0.05$).

SSP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SUP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized; MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized

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3.4 | Anthocyanin content of Sorghum stem sheath and *Justicia carnea* beverages

The anthocyanin content of sorghum stem sheath and *Justicia carnea* beverages are displayed in Figures 3 which ranged between 240.01 mg/ml to 289.03 mg/ml. Result showed that all the samples are rich in anthocyanin, however there was a significant increase in the anthocyanin content of the spiced samples. This is in agreement with the report of Shahidi and Naczk (2004) who reported that anthoayanin are significant type of phenolic flavoniods, which determines the colour of vegetables, fruits and spices. This implies that, the combination of sorghum stem sheath and *Justicia carnea* with the addition of mixed spice enhanced the quality of the beverages by improving the anthocyanin content. Anthocyanin, apart from being used as natural colourants, scientific studies such as animal models, culture studies and human clinical trials have revealed the beneficial health benefit in terms of antioxidants and antimicrobial activities (Khoo *et al*., 2017). The results of storage study revealed insignificant decrease in the anthocyanin content of the sample from day 0 to day 3. This is similar to the report of Gond *et al*, (2023) who observed that, the anthocyanin content of Roselle tea did not change much between 0 and 30 days.

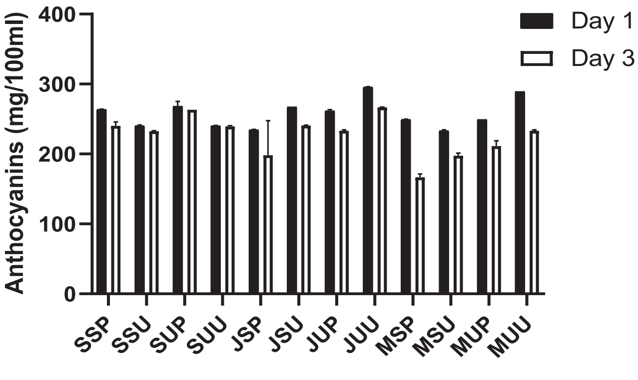

Figure 2 | Anthocyanin content of Sorghum stem sheath and *Justicia carnea* Beverages at Day 0 and Day 3

Means of triplicate readings ± SD, bars with the same alphabet are not significantly different ($p<0.05$).

SSP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SUP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized; MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized

3.5 | Antimicrobial properties of Sorghum stem sheath and *Justicia carnea* beverages

Antimicrobial activities of sorghum stem sheath and *Justicia carnea* against selected food borne pathogenic bacteria (*Escherichia coli, Salmonella spp., Shigella spp., and S. aureus*) and fungi (*Candida albicans and Aspergillus flavus*) showed that the beverages possessed very mild antimicrobial activities as shown in Table 2. It was observed that all the samples displayed inhibitory effects against *E. coli*, while samples SS 1 and JC 3 had the highest zone of inhibition (7.0 mm). The result obtained in this study for SS3 (5.0 mm) against *B. cereus* is similar to the value 4.0 mm reported for sorghum stem extract (Adetuyi *et al*., 2007). It was observed that the inhibitory activities of beverage samples reduced during storage.

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Table 1 | Antimicrobial activities of Sorghum stem sheath and *Justicia carnea* Beverages (mm) for Day 0

Samples	*E. coli*	*S.typhi*	*Shigella.spp.*	*S. aureus*	*B.cerueus*	*C. albicans*	*A.flavus*
SSP	7	3	-	5	4	3	-
SSU	3	1	2	2	-	5	-
SUP	2	-	-	1	5	4	-
SUU	2	-	-	3	2	-	-
JSP	6	1	2	1	5	5	-
JSU	4	-	-	1	2	4	-
JUP	7	-	-	-	4	4	-
JCU	3	-	-	-	3	-	-
MSP	5	2	1	-	4	5	-
MSU	3	-	-	-	3	2	-
MUP	4	-	1	4	3	2	-
MUU	3	-	1	2	-	-	-

Key:
SSP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SUP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized; MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized

3.6 | Phytochemical composition of Sorghum stem sheath and *Justicia carnea* beverages

The phytochemical content of sorghum sheath and *Justicia carnea* beverages are shown in Tables 3a and b. The result of phytochemical in this study showed that both sorghum stem sheath and *Justicia carnea* beverages have appreciable amount of phytochemicals. Studies have shown that sorghum stem sheath and *Justicia carnea* leaves are rich in phytochemcials (Anthonia *et al*., 2019; Adedeji *et al*., 2021; Ajuru *et al*., 2021,). Flavoniod (8.25 mg/ml-9.62 mg/ml) and alkaloid (2.22 mg/ml-2.65 mg/ml) for unspiced sorghum stem sheath beverage were found to be significantly higher than (5.51 mg/ml-6.12 mg/ml) and (1.52 mg/ml -1.67 mg/ml) obtained from unspiced *Justicia carnea* beverages. However, phytate, oxalate and saponin are significantly higher in unspiced *Justicia carnea* than in unspiced sorghum stem sheath beverage. It was observed that addition of mixed spices significantly increased the level of phytochemicals

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in the beverage samples. This is related to the claim of Okechukwu-Ezike and Oly-Alawuba, (2020) that spices are rich in phytochemicals. Flavoniods are the largest group of plant phenols that produce much of flavour and colour to fruits and vegetables, also serves as protection to biological system (Amadi *et al*., 2006).The result of the storage at room temperature from day 0 to day 3 showed a significant decrease in the phytochemcial content of the beverage samples. This is similar to the study of Ner *et al*., (2020) who reported a significant difference in phytochemical content of water melon juice stored for 9 days at room temperature.

Table 3a | Phytochemical composition of Sorghum (g/100g)stem sheath and *Justicia carnea* beverages for Days 0

SAMPLES	FLAVONIOD((mgQE/100 g)	ALKALOID	TANIN	PHYTATE	OXALATE	SAPONIN	ANTRAQUINONE	PHENOL (mgGAE/100 g))	TERPENOID
SSP	11.96 ± 1.3^{c}	3.36 ± 0.0^{c}	0.94 ± 0.0^{c}	12.29 ± 0.0^{f}	$4.69\pm0.2_{f}$	18.69 ± 0.4^{d}	11.82±1.6a	8.21 ± 0.4^{a}	46.34 ± 0.1^{bc}
SSU	12.38 ± 0.0^{bc}	2.59 ± 0.0^{d}	0.86 ± 0.0^{c}	13.82 ± 0.0^{e}	4.68 ± 0.0^{f}	18.00 ± 0.0^{d}	11.14 ± 0.9^{ab}	7.22 ± 0.5^{ab}	43.06 ± 1.4^{d}
SUP	9.62 ± 1.1^{de}	2.65 ± 0.0^{d}	0.80 ± 0.0^{de}	9.20 ± 0.1^{h}	2.68 ± 0.0^{g}	16.54 ± 0.4^{h}	8.55 ± 1.8^{cd}	4.00 ± 0.3^{fg}	39.05 ± 0.1^{e}
SUU	8.52 ± 1.1^{de}	2.22 ± 0.0^{de}	0.73 ± 0.0^{f}	9.63 ± 0.3^{g}	2.52 ± 0.0^{g}	16.24 ± 0.0^{h}	7.60 ± 0.3^{cd}	3.30 ± 0.7^{g}	39.02 ± 0.0^{e}
JSP	11.62 ± 0.5^{c}	1.52 ± 0.1^{g}	0.97 ± 0.0^{b}	15.76 ± 0.1^{d}	5.69 ± 0.0^{d}	20.41 ± 0.0^{d}	10.02 ± 0.6^{ab}	5.30 ± 0.1^{de}	46.07 ± 0.3^{bc}
JSU	12.45 ± 0.1^{bc}	1.93 ± 0.0^{g}	0.95 ± 0.0^{b}	15.75 ± 0.3^{d}	5.54 ± 0.0^{e}	20.21 ± 0.1^{d}	9.26 ± 0.5^{cd}	4.48 ± 0.5^{f}	44.60 ± 0.0^{cd}
JUP	5.51 ± 0.4^{g}	1.67 ± 0.1^{g}	0.84 ± 0.0^{cd}	13.59 ± 0.0^{e}	4.58 ± 0.1^{f}	18.70 ± 0.0^{e}	7.75 ± 0.6^{cd}	4.13 ± 0.4^{f}	38.43 ± 0.0^{e}
JCU	6.12 ± 0.6^{f}	1.51 ± 0.3^{g}	0.84 ± 0.0^{cd}	13.96 ± 0.0^{e}	4.67 ± 0.2^{f}	17.38 ± 0.3^{f}	7.02 ± 0.5^{cd}	3.46 ± 0.6^{g}	37.44 ± 0.3^{e}
MSP	15.62 ± 0.3^{a}	7.41 ± 0.0^{a}	0.14 ± 0.0^{a}	19.65 ± 0.1^{a}	8.78 ± 0.0^{a}	23.58 ± 0.8^{a}	10.33 ± 0.0^{ab}	6.06 ± 0.1^{cd}	48.73 ± 1.8^{a}
MSU	13.60 ± 0.1^{b}	5.20 ± 0.7^{b}	1.18 ± 0.0^{a}	19.71 ± 0.0^{a}	8.39 ± 0.0^{b}	23.67 ± 0.0^{a}	10.38 ± 0.1^{ab}	6.59 ± 0.2^{c}	47.67 ± 1.0^{ab}
MUP	10.95 ± 0.5^{c}	2.48 ± 0.1^{de}	0.77 ± 0.0^{ef}	18.66 ± 0.0^{c}	6.67 ± 0.0^{c}	21.32 ± 0.2^{b}	8.57 ± 0.4^{cd}	3.78 ± 0.0^{fg}	30.76 ± 1.0^{f}
MUU	8.10 ± 0.1^{g}	2.27 ± 0.1^{ef}	0.88 ± 0.0^{d}	17.69 ± 0.1^{b}	6.93 ± 0.0^{d}	21.25 ± 0.1^{b}	7.62±0.2cd	3.95±0.0fg	29.14 ± 0.8^{f}

Means of triplicate readings ± SD, Values with the same superscripts along the same column are not significantly different ($p<0.05$).

SSP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SUP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized; MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized

3.7 | Alpha Amylase and - Glucosidase inhibition of Sorghum stem sheath and *Justicia carnea* Beverage

The result of - amylase and -glucosidase inhibitory activities of the beverage samples are shown on Table 4. All the samples demonstrated strong activity against -amylase. However spiced beverages demonstrated stronger inhibitory activity. This is line with the report of Xiayan *et al*. (2017) that spices possesses bioactive compounds with potential for beneficial effects which have been successfully used for the management of diabetes mellitus. From the result, unspiced *Justicia carnea* beverage displayed 62.09%-63.88% -amylase inhibition. This is related to the findings of Ani *et al*., (2020) and Olugbenga *et al*., (2022) the range of 61%-79% -amylase for aqueous *Justicia carnea* at varying concentration. Studies have also revealed the *in-vitro* inhibitory

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Table 3b | Phytochemical composition (mg/100ml) of Sorghum stem sheath and *Justicia carnea* Beverages for Day 3

SAMPLES	FLAVONOID	ALKALOID	TANIN	PHYTATE	OXALATE	SAPONIN	ANTRAQUINONE	PHENOL	TERPENOID
SSP	10.43±0.5^{d}	1.58±0.1^{ab}	0.14±0.0^{g}	11.31±0.1^{e}	3.42±0.0^{d}	13.38±0.2^{d}	5.57±0.0^{ab}	5.72±0.1^{a}	17.70±0.0^{de}
SSU	11.52±0.4c	0.82±0.0^{e}	0.82±0.0^{a}	12.54±0.4^{d}	3.62±0.1^{d}	11.39±0.3^{fg}	7.18±0.1^{ab}	5.75±0.3^{a}	16.41±0.0^{de}
SUP	8.28±0.5^{e}	0.39±0.0^{e}	0.31±0.0^{c}	8.15±0.1^{f}	1.12±0.1^{e}	10.82±0.2^{gh}	4.51±1.6^{ab}	1.93±0.1^{d}	21.16±0.2^{ab}
SUU	7.93±1.0^{f}	1.28±0.0^{bc}	0.40±0.0^{b}	8.18±0.0^{f}	1.53±0.2^{e}	10.30±0.3^{h}	4.18±0.1^{cd}	1.65±0.1^{d}	19.56±0.0^{ab}
JSP	10.03±1.1^{d}	1.34±0.0^{bc}	0.29±0.0^{cd}	14.54±0.3^{c}	4.63±0.0^{c}	15.08±0.5^{c}	4.22±0.0^{cd}	3.17±0.1^{c}	19.46±0.2^{ab}
JSU	11.44±0.3^{c}	1.16±0.0^{c}	0.34±0.0^{bc}	14.47±0.3^{c}	4.80±0.0^{c}	15.53±0.2^{d}	7.42±0.4^{a}	2.96±0.0^{c}	20.28±0.2^{ab}
JUP	4.58±0.3^{g}	0.28±0.1^{ef}	0.22±0.0^{de}	12.53±0.4^{d}	3.63±0.3^{d}	11.97±0.3^{ef}	3.37±0.0^{d}	1.29±0.0^{f}	20.62±0.0^{ab}
JCU	6.59±0.8^{f}	0.23±1.0^{f}	0.33±0.0^{bc}	12.78±0.0^{d}	3.79±0.1^{d}	12.84±1.2^{de}	4.41±0.2^{cd}	1.51±0.2^{f}	20.78±0.2^{ab}
MSP	14.23±0.0^{a}	2.59±0.0^{a}	0.16±0.0^{fg}	18.11±0.5^{a}	7.67±0.0^{a}	18.71±0.5^{a}	5.43±0.1^{ab}	3.17±0.0^{c}	21.44±1.2^{ab}
MSU	12.33±0.2^{b}	2.71±0.0^{a}	0.23±0.0^{cd}	18.19±0.7^{a}	7.43±0.3^{a}	16.32±0.0^{b}	6.92±0.3^{ab}	3.83±0.0^{c}	22.70±1.2^{a}
MUP	9.17±0.0^{de}	1.48±0.0^{bc}	0.19±0.0^{ef}	16.52±0.1^{b}	5.69±0.2^{b}	15.29±0.0^{c}	5.15±0.5^{ab}	1.88±0.1^{d}	17.47±0.2^{de}
MUU	8.16±0.0^{g}	0.61±0.0^{de}	0.29±0.0^{cd}	17.65±0.0^{a}	4.51±0.4^{c}	16.78±0.0^{b}	4.07±0.0^{cd}	1.75±0.1^{d}	15.16±0.2^{e}

Means of triplicate readings ± SD, Values with the same superscripts along the same column are not significantly different ($p<0.05$).

SSP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SUP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized; MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized

potential of various leaf extract of *Justicia carnea* on diabetic-induced rats (Ani *et al*., 2020; Ukpabi-Ugo *et al*., 2020).

Alpha glucosidase inhibition of the beverages as observed from the table followed the same trend recorded for - amylase, with percentage of inhibition for unspiced sample ranged between 64.82% – 67.92%. This is also similar to the previous finding who reported 64.842% -69.92% inhibition for - glucosidase from aqueous *Justicia carnea* (Olugbenga *et al*., 2020). These reports are in agreement with the findings of Bhendari *et al*. (2008) that natural plants are good inhibitors of - amylase and - glucosidase and can be used in treatment of diabetes in traditional medicine. There is little or no information about - amylase and - glucosidase inhibition of sorghum sheath. However, study revealed strong evidence of reduced plasma and insulin level after

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consumption of food prepared with sorghum stem sheath in both animal model and human (Taylor & Emmambux, 2010). The effect of storage for 3 days did not have any significant effect on the antidiabetic activities of the samples, this could be as a result nature of the beverages and the condition of storage which favour the diet and decrease the inhibition of the beverage under the storage condition Although Ping *et al*., (2019) reported that extended storage time decrease antidiabetic property of white tea, which may be as a results of decrease in the polyphenol contents.

Table 4 | Effect of Sorghum stem sheath and *Justicia carnea* Beverages on α –Amylase and α –Glucosidase enzymes

	Day 0 (%)		Day 3 (%)	
SAMPLES	α –Amylase	α –Glucosidase	α –Amylase	α –Glucosidase
SSP	78.64±0.1[a]	74.07±0.1[ab]	50.84±0.3[a]	45.89±0.0[b]
SSU	77.21±2.0[a]	70.40±0.2[bc]	47.12±0.6[bc]	45.15±0.2[bc]
SUP	61.95±0.5[ef]	65.46±0.1[de]	44.14±0.1[cd]	43.88±0.4[bc]
SUU	60.59±1.3[f]	64.47±0.1[e]	41.56±1.0[ef]	42.33±2.5[d]
JSP	65.56±0.0[cd]	72.55±0.0[bc]	47.59±1.0[b]	58.17±1.0[a]
JSU	64.86±0.5[cd]	69.93±0.9[cd]	43.56±2.0[de]	56.14±1.6[a]
JUP	63.86±0.4[de]	67.92±0.0[cd]	40.76±0.0[ef]	44.11±0.1[bc]
JCU	62.09±1.0[ef]	64.82±0.2[e]	40.29±0.1[f]	42.88±0.1[cd]
MSP	66.47±1.0[bc]	77.34±0.0[a]	45.41±0.0[bc]	45.68±0.0[b]
MSU	67.14±0.2[b]	76.30±0.2[bc]	45.80±0.4[bc]	43.58±0.0[bc]
MUP	62.28±0.0[ef]	68.91±0.3[de]	47.57±2.7[b]	43.56±0.0[bc]
MUU	60.28±0.3[f]	69.44±0.1[de]	43.93±2.0[cd]	42.39±1.3[d]

Means of triplicate readings ± SD, Values with the same superscripts along the same column are not significantly different ($p<0.05$).

SUP=Sorghum sheath beverage spiced and pasteurized; SSU=Sorghum sheath beverage spiced and unpasteurized); SSP=Sorghum sheath beverage unspiced and pasteurized; SUU=Sorghum sheath beverage unspiced and unpasteurized; JSP=Justicia carnea beverage spiced and pasteurized; JSU= Justicia carnea beverage spiced and unpasteurized; JUP= Justicia carneabeverage unspiced and pasteurized; JCU= Justicia carnea beverage unspiced and unpasteurized; MSP=Sorghum sheath+ Justicia carnea beverage spiced and pasteurized; MSU= Sorghum sheath + Justicia carnea beverage spiced and unpasteurized; MUP= Sorghum sheath+ Justicia carnea beverage (unspiced and pasteurized; MUU= Sorghum sheath+ Justicia carnea beverage unspiced and unpasteurized

4.0 | Conclusion

From the study, beverages produced from sorghum stem sheath and *Justicia carnea* with or without mixed spices were found to have appreciable phytochemicals which may be responsible for the

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very high antioxidant properties displayed. However, the beverages exhibited very mild antimicrobial activities. Spiced sorghum stem sheath-*Justicia carnea* beverages demonstrated higher inhibition against alpha amylase and alpha glucosidase enzymes. It may be concluded that spiced beverages from sorghum stem sheath and *Justicia carnea* may be considered as a novel functional drink.

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