Original Research Article

Effect of Processing Methods on the Micronutrient Profile, Colour, and Anti-Nutritive Components of *JusticiaHeterocarpa* (*mwidu*)

ABSTRACT

JusticiaHeterocarpa (mwidu) is a popular indigenous leafy vegetable picked wild in rural regions of Morogoro, Tanzania, during the wet seasons. This research examined the impact of processing *on*micronutrients,Total phenols and anti-nutrient content. The fresh leaves (FL) underwent direct shade drying (UBLDDR), blanched shade drying (BLDR), blanched oven drying (BLDO), fermentation (FFL), and gas and microwave cooking (FLCO5, FLCO10, and FLMCO2). Vitamins, chlorophyll, minerals, phenolic compounds and anti-nutrients were analyzed. All laboratory experimentsadhered to procedures guidelines. The pH dropped more significantly to <3.5 in a 3% salt.3% sugar brine solution with 1.328± 0.006 mg/100 g of lactic acid compared to its counterpart. Blanched leaves dried in 5 days, but un-blanched leaves took 15 days.Fermented samples demonstrated a notable reduction in Total chlorophyll concentration (0.0964± 0.075 mg/g) compared to other processing techniques..The nutritional and anti-nutritional composition of *JusticiaHeterocarpa*showed significant change *(P*< 0.05) depending on processing methods. The results indicated a significant loss of vitamin C in the fermented and ten-minute cooked samples, at 74.57% and 61.64%, respectively. Beta-carotene levels in cooked samples FLMCO2 (107.4%, 11.29± 0.03 mg/100 g) and FLCO5 (86.26%, 10.51± 0.02 mg/100 g) above those in Fresh Leaves (3.67 mg/100 g) by more than two-fold. In comparison to alternative processing procedures, samples cooked for 10 minutes exhibited significant mineral leaching, whereas un-blanched direct shade drying preserved the highest mineral concentration. Fermented samples (532.83± 14.91 GAE/100g) exhibited a 64.19% increase in Total Phenolic Compounds compared to Fresh leaves (190.83± 14.91 GAE/100g). Nonetheless, tannins increased by 68.1% (254.44 ± 7.45 GAE/100g) in the fermented samples. Samples exposed to extended cooking (17.8± 3.17 mg/100 g) and fermentation (40.28± 3.34 mg/100 g) exhibited the lowest levels of phytates. The oxalate concentration was significantly decreased in the cooked samples. *JusticiaHeterocarpa* may serve as a sustainable food supply in areas of Tanzania experiencing nutritional deficits.

Keywords:Anti-nutrients,Indigenous Leafy Vegetables,*JusticiaHeterocarpa,*Micronutrientsand Processing

1.0. INTRODUCTION

Micronutrient deficiencies are prevalent in many underdeveloped nations, where populations often depend on staple cereals like maize and rice, resulting in minimal dietary diversification (1–3). Micronutrient deficiency or hidden hunger arises when the intake and absorption of essential vitamins and minerals, including vitamins A, C, zinc, and iron, are inadequate to support optimal health and development (4). Vitamin A deficiency accounts for approximately 1.7% of deaths among children under five in Africa (5) In Tanzania, the prevalence of anemia is 59% among children aged 6-59 months (6,7), 37.8% among pregnant women in the Mbeya region(8) and 23% among adolescents in boarding schools in Kilimanjaro (9). The prevalence of stunting and underweight in Morogoro is notably elevated as a result of nutritional deficiencies. A 24-hour dietary recall indicates that the prevalence rates of anemia, vitamin A deficiency, and zinc deficiency in children are 42.9%, 29.3%, and 24.9%, respectively (10). The World Health Organization recommends a daily intake of 400 grams of fruits and vegetables for adults to combat hidden hunger(11–13). Due to challenging economic conditions, numerous rural regions in Sub-Saharan Africa, including Tanzania, lack the financial capacity to afford such quantities (6,11,13).

Indigenous leafy vegetables (ILVs) represent a valuable resource for enhancing dietary diversity and ensuring food security at the household level in developing countries (14–17).Recent studies have identified ILVs as a significant source of nutrition(16–18), economic potential (19) and adaptability to climatic conditions (16,17,20).Indigenous leafy vegetables, while beneficial, experience considerable post-harvest losses during the rainy season (16,21) and are often scarce in the dry season due to their wild growth and reliance on natural rainfall(3). Furthermore, ILVs contain anti-nutrients that hinder the body's capacity to digest and absorb essential nutrients (7,22). Phytates and oxalates are capable of chelating metal ions, including iron, zinc, and calcium, resulting in the formation of insoluble complexes (23). Wide research has been conducted on the processing and preservation of indigenous leafy vegetables (ILVs)(1,7,11,24). Additionally, the effects of processing on the reduction of anti-nutrients in specific ILVs have also been examined (7,11,25). *Justiciaheterocarpa,* referred to as "mwidu," is a popular indigenous leafy vegetable among the residents of the Morogoro Region in Tanzania (26). This plant flourishes in rainy seasons and grows abundantly in fields and around family compounds (26). The fresh leaves exhibit a notable micronutrient profile(27). *Justiciaheterocarpa* is significantly impacted by drought during arid seasons. Furthermore, the restricted preservation methods impede its accessibility and availability in dry seasons. This study examined the impact of processing methods (blanching, drying, fermenting, and cooking) on the retention of nutrients and the reduction of anti-nutritional components in *JusticiaHeterocarpa.*

2.0. MATERIALS AND METHODS

2.1. Samples collection

JusticiaHeterocarpa leaves were collected from a farmer's plots at Kiroka ward, Morogoro Rural District, Tanzania. Tender leaves were plucked with a sharp knife from each plot during the early morning hours, packaged in polyethylene bags and immediately transported to Food Science and Agro-processing laboratory. The leaves were trimmed, sorted, washed, and placed in a perforated vessel for water drainage. The vegetables were thoroughly mixed to obtain a representative sample

2.2. Sample Preparation

Fresh leaves were washed, drained, and vacuum-packaged in polyethylene bags, then freeze-dried until analysis. In contrast, to obtain the un-blanched room-dried leaves, the leaves were washed, drained, and placed on drying racks at room temperature (25±2°C) until a crispy texture was achieved, which took 15 days. dried leaves were vacuum-packaged and freeze-dried before to analysis.

Blanched dried leaves were washed, drained, and secured in net cloth, subsequently immersed in boiling water at 100°C for one minute. They were immediately rinsed in ice water for one minute, followed by drying on racks at room temperature for five days. The blanched leaves were subsequently dried in an oven at 40°C for 12 hours. The moisture content was measured using a moisture meter (Denver, IR35M-0002300V1-GERMANY). After attaining a crispy texture, the leaves were vacuum packaged and then freeze-dried before analysis.

For fermented leaves, previously trimmed and sorted vegetables were washed with distilled water and placed in the perforated tray with a clean paper towel to enhance the removal of excess water. Fermentation was done in submerged brine (1:3 vegetables: water). Two distinct concentrations of salt to sugar ratios were employed (3%:3% and 4%:2%) as previously outlined by(25) and (12) with certain modifications. Fermenting vessels utilized were 800ml plastic containers with secure seals. Locally produced crock pots, consisting of polyethylene bags filled with water, were utilized to submerge the vegetables, preventing the leaves from floating to the surface and thereby mitigating mold growth during fermentation. The experimental setup was incubated at 25°C, which is the optimal temperature for the growth of fermenting bacteria. Samples were collected at 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, and 144 hours. The efficacy of fermentation was evaluated by monitoring and recording pH and lactic acid levels in triplicate. Subsequent to pH measurement, the samples were promptly freeze dried, waiting laboratory examination of lactic acid using spectrophotometer (JENWAY 6715 UV/Vis U.K)

Regarding cooked leaves, the cooking time for *J Heterocarpa* leaves varied from 3 to 20 minutes, according to pilot data collected from the study area. 500 grams of leaves were boiled in a pot with three hundred milliliters of water on a gas cooker for 5 and 10 minutes, respectively. Furthermore, 500 grams of the identical vegetables were subjected to microwave cooking for a duration of 2 minutes. Finally, the vegetables were cooled to room temperature and transferred, along with the cooking water, into plastic jars with lids for freeze-drying prior to laboratory analysis.

2.3. Chemical Analysis

2.3.1 Determination of nutritional composition

Beta-carotene

Total Beta–carotene was done as per (28) with slight modification. Frozen samples were thawed to room temperature (25 ± 2°C) and homogenized using a blender. A total of 2.000 g of sample was weighed in duplicate in 15 ml screw-capped glass tubes, followed by the addition of 10 ml of acetone (Sigma-Aldrich, USA). The samples were vortexed for a minute, then incubated in a water bath (SW23GB, JULABO) set at 85°C for 10 min. After that, 120 µl of 80% (w/v) potassium hydroxide (Sigma-Aldrich, USA) was added to the tubes, vortexed for 1 min, and incubated further at 85°C for 5 min. The tubes were transferred in ice to cool before the addition of 4 ml deionized water. The tubes were then vortexed for 1 min, and 5 ml of hexane (Sigma-Aldrich, HPLC grade, USA) was added to each tube and further vortexed for 1 min before centrifugation at 3000 rpm for 5 minutes. The upper phase of hexane was pipetted into a separate 25 ml labeled flask. Extraction from the pellet was repeated four times using 4×3×3×3 ml hexane, and the extract was combined in the 25 ml labeled flask. Leaves containing higher concentrations of beta carotene were further extracted by carotenoids. Sometimes, more extraction was necessary. 5ml deionized water was added into the extracts, mixed with vortex for 1 minute and centrifuged for 5 mins at 3000 rpm. The hexane layer in each was recovered into a clean test tube and evaporated the hexane under nitrogen in the N-Evaporator machine (Organomation, Model OA-8125) with its water bath set at 40°C to dry. Dried extracts in tubes were reconstituted by adding 10 ml of methanol (HPLC grade Sigma Aldrich) to the tubes, mixing homogeneously for 1 min by a vortex. The samples were transferred in 1 ml to HPLC vials. A volume of 30 µl was injected into the HPLC (SPD-20A SHIMADZU CORPORATION, Japan) with the type and conditions explained above. The method's limit of detection and limits of quantification (LOD and LOQ) were 3.1µg/ml and 5.2 µg/ml, whilst the percentage recovery was 103%, respectively.

Vitamin C (Ascorbic Acid)

The quantification of *JusticiaHeterocarpa* ascorbic acid was conducted utilizing the 2,4-dinitrophenylhydrazine (2,4-DNPH) colorimetric assay method as previously described by (29). Each sample was finely chopped, blended, and homogenized using an electric blender. A total of 50 mL of a 3% metaphosphoric acid and 8% acetic acid solution was mixed with 10 mL of the blended sample. The resulting mixture was then diluted to the desired concentration by adding the same metaphosphoric-acetic acid solution to a 100 mL volumetric flask. After filtration, the clear filtrate was collected for vitamin C determination. A few drops of bromine water were added to the filtered sample solution to oxidize the ascorbic acid to dehydroascorbic acid. Excess bromine was neutralized with 10% thiourea, yielding a clear solution. Standard ascorbic acid solutions were prepared by diluting the 500 ppm stock to final concentrations of 5, 10, 15, 20, and 25 ppm. For both the oxidized sample and standard solutions, 1 mL of 2,4-DNPH solution was added. These were incubated in a 37°C water bath for 3 hours. After incubation, the solutions were cooled in an ice bath for 30 minutes and then treated with 5 mL of 85% H_2SO_4, resulting in a colored solution. The absorbance was measured at 530 nm, and ascorbic acid content was quantified using a standard calibration curve.

Minerals

The mineral content (Fe, Zn, Ca, and Mg) was analyzed through microwave-assisted acid digestion and ICP-OES, as outlined by (30) with some modifications Approximately 0.5 g of the pulverized plant material sample was weighed into a digestion flask. A 10 mL mixture of concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) (typically in a 3:1 ratio) was added to the flask, which was then allowed to stand for 4 hours in the fume hood. The flask was placed on a microwave digestion system, and the mixture was heated in a hot block (Hot block 150 models: SC-154-240) at 95°C and boiled for 30 minutes until it reached 85°C and became apparent, which took 1-2 hours. After digestion, the flask was allowed to cool, and the solution was diluted to a known volume of 50 mL with distilled water in a volumetric flask following filtration. The machine was turned on and allowed to warm up. The digested samples in the test tubes were introduced into the Inductively Coupled Plasma Optical Emission Spectroscopy machine (ICP-OES-5900 Agilent) with the model number (Agilent 5900 SVDV ICP-OES), Serial number MY2215CP04, Software version 7.6.0.12121, and firmware version 5590). they were using an auto-sampler. The emission signals from the samples were monitored, and the instrument parameters (e.g., plasma conditions and nebulization rate) were optimized for the analysis. The instrument outputted the concentration of the minerals, which were displayed on the computer.

Determination of Total Phenolic Compounds

The total phenol for the *JusticiaHeterocarpa* sample was determined by using Folin-Ciocalteu method as described by (31) with some modifications. 80 mg of the sample was added to 2 mL of 50% methanol and extracted by maceration for 2 hours. The resulting mixture was centrifuged at 10,000 rpm for 10 minutes to separate the supernatant, and the volume was adjusted to 5 mL with 50% methanol in a volumetric flask. A 1 mL aliquot of the sample extract was transferred into a test tube, followed by the addition of 9 mL of distilled water and 1.5 mL of Folin-Ciocalteu's reagent. The mixture was incubated at room temperature for 5 minutes. Afterwards, 4 mL of 35% (w/w) Na_2CO_3 was added, and the volume was adjusted to 25 mL with distilled water. The mixture was agitated and left to stand for 30 minutes at room temperature. The absorbance of the sample was measured at 765 nm using a UV-Vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. Total phenolic content was expressed as mg Gallic acid equivalents per gram of sample (mg/g).

2.3.2. Determination of anti-nutrients composition

Tannins

The tannin content in each sample was determined using insoluble polyvinyl-polypyrrolidone (PVPP), as described by (32). Specifically, 1.0 mL of the extract dissolved in methanol (1%) was mixed with 100 mg of PVPP. The mixture was vortexed and then incubated for 15 minutes at 4°C. The sample was centrifuged for 10 minutes at 3,000 rpm to separate the precipitated tannins. The clear supernatant obtained after centrifugation contained non-tannin phenolic, which was subsequently analyzed using the Folin-Ciocalteu method to determine total phenolic content. The difference in absorbance values before and after the removal of tannins was regarded as the absorbance value for total tannins in the sample.

Phytates

The phytates content was assessed through the iron-precipitation colorimetric method utilizing thiocyanate detection, following the approach outlined by(22) with certain modifications. Each powdered sample, weighing approximately 0.2 g, was carefully measured into a 125 ml Erlenmeyer flask. To extract the phytic acid, 50 ml of 3% trichloroacetic acid (TCA) was added, and the mixture was swirled by hand for 45 minutes with occasional swirling during the first 30 minutes. After centrifugation, 10 ml of the supernatant was transferred to a 50 ml conical flask. Next, 4 ml of $FeCl_3$ solution was rapidly added to the aliquot, and the content was heated in a

boiling water bath for 45 minutes. After 30 minutes of heating, two drops of 3% sodium sulfate were added to the extract, and the heating process continued. The resulting supernatant was centrifuged for 15 minutes and carefully decanted. The precipitate was washed twice with 20 to 25 ml of 3% TCA by thoroughly dispensing, followed by heating in a boiling water bath for 10 minutes and centrifugation. This washing process was repeated with water. The precipitate was then dispersed in 27 ml of water and mixed with 3 ml of 1.5 N NaOH. The volume was adjusted to approximately 30 ml with water and heated in a boiling water bath for 30 minutes. The precipitate was filtered through moderately retentive filter paper, such as Whatman No. 2. The precipitate was washed with 70 ml of hot water, and the filtrate was discarded. The precipitate was then dissolved from the filter paper using 40 ml of 3.2 N HNO_3 into a 100 ml volumetric flask. The filter paper was thoroughly washed with multiple portions of water, and the washings were collected in the same flask without exceeding the 100 ml volume. The flask was allowed to cool to room temperature and diluted to volume with water. A 5 ml aliquot was transferred to a 100 ml volumetric flask and diluted to approximately 70 ml. Subsequently, 20 ml of 1.5 M KSCN was added, and the solution was diluted to volume. The resulting color was measured immediately within 1 minute at 480 nm using a spectrophotometer. A reagent blank was included for each set of samples to ensure accuracy. The standard stock solution was prepared by taking 1000 mg/L of phytate (phytic acid) from potassium phytate ($K_3C_6H_6O_{18}P_3$). Precisely, 2.064589 g of potassium phytate was weighed and dissolved in a 1000 mL volumetric flask to prepare a working solution of different concentrations. The standard solutions were then subjected to coloration, and the absorbance was measured at 480 nm. A standard plot of concentration against standard absorbance was created, and a linear regression equation was obtained. The concentration of phytates was calculated using the Beer-Lambert equation.

Oxalates

The oxalic acid content was quantified utilizing the UV-Vis spectrophotometric approach, employing iron Ferron reagent detection, as outlined by(33), with modifications. A 1.00 g sample of dried ground compost was extracted in a 100-ml conical flask with 10 ml of water and 10 ml of citric acid reagent. The flask was heated on a hot plate, allowing it to simmer for 30 minutes with a small glass funnel placed in the neck of the flask as a condenser. The mixture was filtered through No. 30 Whatman filter paper and thoroughly washed with cold water. The flask was drained, and the filter paper and the precipitate were partially dried at 100°C for one hour before both were returned to the flask. Based on the expected calcium oxalate concentration, 25 ml or 50 ml of 0.4 N hydrochloric acid was added using a pipette (25 ml if the expected concentration was below 1% and 50 ml between 1% and 3%). The solution was heated to near the boiling point and maintained at this temperature for 5 minutes with occasional swirling. The solution was then filtered through a small, dry filter paper. The initial filtrate was discarded, and 4 ml of the remaining filtrate was pipetted into a dry beaker. To the filtrate, 5 ml of iron ferron reagent was added. The solution was protected from direct sunlight, and the absorbance was measured at 525 nm using a UV-Vis spectrophotometer (JENWAY 6715 UV/Vis U.K) with the instrument zeroed against a reagent blank containing 4 ml of 0.4 N hydrochloric acid and 5 ml of iron Ferron reagent. A standard curve was constructed using solutions with calcium oxalate ($CaC_2O_4 \cdot H_2O$) concentrations ranging from 0 to 4 mg, dissolved in 4 ml of 0.4 N hydrochloric acid.

2.3.3 Determination of physico-chemical parameters

Chlorophyll Content

The chlorophyll content was assessed using acetone extraction and UV-Vis spectrophotometry, following the methodology outlined by(25), with modifications. Use a mortar and pestle to crush 1.0 g of the material into a fine pulp with 10 ml of 80% acetone (LOBA, India). The pulp was centrifuged at 4000 rpm at room temperature for 5 minutes, then the green supernatant was

transferred to a 50 ml volumetric flask for chlorophyll analysis. The centrifuge tube sediment was scraped and pulverized with the same mortar and pestle and a little 80% acetone to remove the remaining chlorophyll. The supernatant was mixed with the previous supernatant in the volumetric flask after 5 minutes of centrifugation at 4000 rpm at room temperature. Re-extraction was repeated until the residue was green-free. A 50 ml volumetric flask with 80% acetone received the supernatant. The extract was chilled for 10 minutes. With 80% acetone as the blank, a Spectrophotometer (JENWAY 6715 UV/Vis U.K) assessed the extract's absorbance at 663 and 645 nm. Chlorophyll was calculated using the empirical formula;

$$\text{Chlorophyll a, mg/g tissue} = 12.7\,(A663) - 2.69\,(A645) \times V / 100 \times W.$$

$$\text{Chlorophyll b, mg/g} = 22.9\,(A645) - 4.68(A663) \times V/100XW$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

A represents absorbance at designated wavelengths; V is the final volume of the chlorophyll extract; W indicates the fresh weight of the extracted tissue.

Lactic acid

Colorimetric Method for Lactic Acid Quantification Using Ferric Chloride was used as outlined by (34), incorporating specific modifications. Five grams of dried leaf sample were placed in a beaker, to which boiled distilled water was added at a ratio of approximately 1:20 to 1:50, and allowed to cool. Following heating, the extract was allowed to cool to room temperature. The extract was then filtered through filter paper (Whatman No. 1) to remove the solid leaf residue, the filtrate test solution (50 µL) containing lactic acid and 2 mL of a 0.1% iron (III) chloride solution in a cuvette and stirred was conducted at 25 ± 5°C. The reaction was allowed to proceed at room temperature (26.2°C) for 10–15 minutes to ensure the lactic acid fully reacted with the ferric ions, forming a yellow-coloured complex. The absorbance was measured at 390 nm. To create the calibration curve, the absorbance of the lactic acid (1.2 g) with a known concentration of 89% (density = 1.2 g/mL) was measured; a stock solution containing 89 g/L of lactic acid was prepared. From this stock solution, a series of lactic acid solutions with different concentrations was prepared by dilution. A plot of absorbance against concentration was generated. The absorbance of the samples was then measured, and the lactic acid concentration was calculated using the calibration curve based on the Beer-Lambert law.

2.4. Statistical Analysis

All data were subjected to Statistical Package for Social Sciences (SPSS) version 27. A one-way analysis of variance (ANOVA) test was performed following a post-hoc Turkey test, with significant differences determined at a 5% level ($P<0.05$). All results were expressed as mean ± SD of triplicate values except for beta carotene and minerals, where the values were duplicate.

3.0 RESULTS AND DISCUSSION

3.1 Effect of processing on Physicochemical properties of *JusticiaHeterocarpa*

3.1.1. Dynamic trend in pH and lactic acid during spontaneous fermentation

The results of dynamic trend in pH and lactic acid of *JusticiaHeterocarpa* leaves are presented in Table 1. Findings suggest significant trends at $P<0.05$ for pH and lactic acid during the spontaneous fermentation of *Justiciaheterocarpa* leaves exposed to two distinct doses of salt-sugar brine solution. The pH drops from neutral to acidic, measuring 3.89 and 3.52 for 4%salt-2%sugar and 3%salt-3%sugar, respectively, within 48 hours, with 3%salt-3%sugar brine concentration showed a more significant pH reduction than its counterpart. The variation could be due to the increased sugar content, which promoted bacterial growth by giving fermentation additional substrate. The quick pH decrease establishes acidic environment that suppresses spoiling organisms which cannot tolerate low pH and promotes beneficial bacteria (11). The study on the fermentation of pumpkin leaves in Kenya reported the spontaneous fermenting microbes present in pumpkin leaves when treated with 3%salt-3%sugar brine solution were *Leuconostoccitreum* at time zero, after 48 hours *Weissellacibaria* prevail the fermentation process , and*Lactiplantibacillusplantaram* finalize the fermentation process (3).The work conducted in Tanzania by (25), demonstrated successful spontaneous fermentation of *Solanum* species using a 4%salt-2%sugar brine solution, resulting in a pH reduction to 3.7 within 48 hours. Acidification of the fermented vegetables is very essential for preservation of the vegetable and extend its shelf life also achieving the desirable flavor (35).

Table 1: Lactic acid and pH dynamics in spontaneous fermentation (SF)

	Lactic Acid (mg/100ml)		P^H	
TIME	4%salt-2%sugar	3%salt-3%sugar	4%salt-2%sugar	3%salt-3%sugar
0 hrs.	0.099 ± 0.011^{a}	0.06 ± 0.011^{a}	6.68 ± 0.006^{a}	6.62 ± 0.021^{a}
12 hrs.	0.125 ± 0.006^{ab}	0.12 ± 0.011^{b}	5.23 ± 0.01^{ab}	5.66 ± 0.023^{a}
24 hrs.	$0.177 \pm 0.011^{b*}$	0.22 ± 0.006^{c}	4.45 ± 0.044^{ab}	4.35 ± 0.031^{ab}
36 hrs.	$0.26 \pm 0.023^{c*}$	0.35 ± 0.006^{d}	4.0 ± 0.02^{abc}	3.87 ± 0.02^{b}
48 hrs.	0.28 ± 0.042^{cd}	0.36 ± 0.011^{d}	3.89 ± 0.012^{bcd}	3.53 ± 0.023^{b}
60 hrs.	0.29 ± 0.006^{cd}	0.42 ± 0.040^{e}	3.82 ± 0.02^{cde}	3.4 ± 0.012^{c}
72 hrs.	0.33 ± 0.006^{de}	0.55 ± 0.022^{f}	3.78 ± 0.02^{de}	3.2 ± 0.015^{c}
84 hrs.	0.35 ± 0.011^{e}	0.65 ± 0.006^{g}	3.7 ± 0.0^{ef}	3.31 ± 0.006^{c}
96 hrs.	0.36 ± 0.017^{e}	0.75 ± 0.011^{h}	3.77 ± 0.026^{f}	3.40 ± 0.015^{d}
108 hrs.	0.42 ± 0.028^{e}	0.89 ± 0.006^{i}	3.68 ± 0.025^{g}	3.39 ± 0.012^{e}
120 hrs.	0.52 ± 0.011^{f}	0.96 ± 0.011^{j}	3.66 ± 0.057^{h}	3.27 ± 0.058^{f}
132 hrs.	0.69 ± 0.017^{g}	1.20 ± 0.011^{k}	3.72 ± 0.012^{i}	3.30 ± 0.006^{g}
144 hrs.	0.78 ± 0.028^{h}	1.328 ± 0.006l	3.64 ± 0.032^{j}	3.21 ± 0.023^{h}

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at $P<0.05$.

In comparison to a 4%salt-2%sugar brine solution, a 3%sugar-3%salt brine solution exhibited a considerable rise in lactic acid content over time during spontaneous fermentation, indicating enhanced efficacy in producing an acceptable quantity of lactic acid. Upon completion of fermentation, a 3%sugar-3%salt brine solution yielded 1.3288 mg/100ml of lactic acid, which was substantially greater (P<0.05) than the 4%salt-2%sugar brine solution, which produced 0.7877 mg/100ml (Table 1). The lactic acid produced acts as a preservative of the vegetables because spoilage and pathogenic microbes cannot tolerate high acid. However, it contributes to flavor and aroma development, improving fermented vegetables' sensory appeal (11). The study conducted by (25) on the fermentation of nightshade leaves in 4% and 2% salt sugar brine solution, utilizing the titration method, yielded results comparable to those of the current study.

3.1.2.Moisture Content (MC) of dried *JusticiaHeterocarpa*

The moisture content changes with time for un-branched leaves directly dried in the room (UNLDDR), blanched leaves dried in room (BLDR) and blanched leaves dried in oven (BLDO) during drying of *JusticiaHeterocarpa* are displayed in Table 2. All three methods displayed substantial moisture reduction of moisture content in which moisture was reduced from 87.83 to 12.38% for UBLDDR, 85.4 to 10.29 for BLDR, and 86.11 to 9.89%. Samples of blanched leaves dried at room (BLDR) temperature for 48 hours exhibited a significant higher moisture content (P<0.05) compared to those un-branched leaves dried directly at room temperature (UBLDDR). The study by(36), indicated a reduction in moisture content to 11.82% in black nightshades and amaranth when dried in shade. This finding is consistent with the current study, which observed that *J. Heterocarpa* achieved a moisture content of 12.38% under similar drying conditions. While oven drying proved to be the most effective drying method (9.89%, 12 hrs) in current study due to its efficiency, it is less feasible in resource-limited settings as it necessitates electricity and specialized equipment. Blanching facilitates moisture reduction by disrupting cell walls, thereby enhancing the rate of moisture escape. Achieving the desired moisture content in unbranched vegetables presents greater challenges. The process required 15 days to achieve a moisture content of 12.38%, rendering it less suitable for rapid processing, though viable under resource constraints. Other studies have highlighted the importance of blanching before drying, which leads to minimal nutrient leaching and preserves more nutrients (37).

Table 02. Moisture Content of Different Drying Methods

Time	Moisture%		
	UBLDDR	BLDR	BLDO
0 hours	87.83 ± 1.444g	84.33 ± 1.624e	86.11 ± 0.596
12 hours	73.35 ± 2.024f	43.86 ± 3.879d	9.89 ± 0.157
24 hours	51.00 ± 2.036e	28.07 ± 1.356c	
48 hours	19.08 ± 0.251d	15.41 ± 1.181b	
72 hours	16.58 ± 0.592cd	13.06 ± 0.648ab	
96 h0urs	15.05 ± 0.196abc	11.95 ± 0.205ab	
120 hours	15.15 ± 0.933abc	10.29 ± 0.121a	
144 hours	15.73 ± 0.529bc		
168 hours	14.21 ± 0.754abc		
Day 8	13.68 ± 0.7abc		
day 12	14.04 ± 0.072abc		
day 13	13.44 ± 0.516ab		
day 14	12.77 ± 0.252a		
day 15	12.38 ± 0.27		

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at $P<0.05$. Abbreviations: BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room

3.1.4 Effects of processing on chlorophyll content

Chlorophyll content of fresh and processed leaves of *JusticiaHeterocarpa* are presented in Table 03. The total chlorophyll content of the leaves subjected to different processing method varied and were significantly reduced to 0.0964 ± 0.075 mg/g in fermented samples and 6.7688 ± 0.448mg/g for the ten minutes cooked leave samples (Table 3). Total chlorophyll levels were highest in fresh leaves (FL), measuring 13.429 ± 0.522 mg/g. Leaves dried at room temperature (BLDR) and those microwaved for 2 minutes (FLMCO2) maintained total chlorophyll levels of approximately 12.92 and 12.15 mg/g, respectively, and did not show a significant difference ($p>0.05$) compared to fresh leaves. Leaves dried at room temperature retained a highest chlorophyll level than all other processing methods, which suggests that blanching before drying can control chlorophyll degradation. Fermented freshly leaves (FFL) samples exhibited significantly reduced chlorophyll levels of 0.0964 ± 0.075 mg/g (table 03), indicating substantial degradation of chlorophyll during acidic fermentation, resulting in the transformation into non-green compounds and observable color changes. Numerous studies indicate a significant decrease in chlorophyll levels in fermented ILVs. The study conducted by (25) on the impact of fermentation on chlorophyll reduction in nightshade species indicated a gradual decrease in chlorophyll a, b, and total chlorophyll levels. (35) reported that the accumulation of acids such as acetic and lactic is the primary factor contributing to the reduction of chlorophyll levels in fermented nightshades. Chlorophyll present in green leafy vegetables serves as a significant antioxidant, aiding in the mitigation of oxidative stress and the prevention of cellular damage. Due to its anti-inflammatory properties, it may reduce chronic inflammation and enhance overall health (38).

Table 3. Chlorophyll Content in raw and processed JusticiaHeterocarpa

Sample	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
FL	8.019 ± 0.104^{d}	5.414 ± 0.422d	13.429 ± 0.522^{f}
BLDR	7.927 ± 0.108^{d}	4.995 ± 0.795^{cd}	12.918 ± 0.836^{ef}
BLDO	6.403 ± 0.956^{c}	4.316 ± 0.117^{cd}	10.715 ± 0.942^{cd}
UBLDDR	7.35 ± 0.36624^{cd}	4.0759 ± 0.151^{c}	11.4228 ± 0.381^{cde}
FFL	0.1065 ± 0.126^{a}	-0.01 ± 0.0530^{a}	0.0964 ± 0.075^{a}
FLCO5	6.3389 ± 0.551^{c}	3.8569 ± 0.790^{bc}	10.193 ± 1.066^{c}
FLCO10	3.945 ± 0.5236^{b}	2.8258 ± 0.173^{b}	6.7688 ± 0.448^{b}
FLMCO2	7.7804 ± 0.294^{d}	4.3774 ± 0.235^{cd}	12.1545 ± 0.527^{def}

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at$P<0.05$. Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented fresh leaves; FLCO5:5 min cooked sample in gas cooker, FLCO10:10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

3.2 Effect of processing on *JusticiaHeterocapa* nutritional composition

Ascorbic Acid

Table 4 presents the beta carotene and ascorbic acid content of processed *Justiciaheterocarpa* leaves. Unprocessed fresh leaves (FL) exhibited a high concentration of ascorbic acid (23.21 mg/100g), which significantly decreased ($P<0.05$) following various processing methods.

Table 4. Beta carotene and Ascorbic acid of *JusticiaHeterocarpa* processed leaves (FW)

	Ascorbic acid (mg/100g)	% Reduction	Beta carotene (mg/100g)	% Reduction
FL	23.21 ± 0.742^{e}	-	3.67 ± 0.072^{c}	-
BLDR	18.62 ± 0.557^{d}	19.79	2.06 ± 0.038^{b}	43.82
BLDO	16.52 ± 0.185c^{d}	28.84	5.72 ± 0.156^{d}	-55.79
UBLDDR	19.41 ± 0.186^{d}	16.40	1.55 ± 0.013^{a}	57.78
FFL	5.88 ± 0.743^{a}	74.66	1.29 ± 0.053^{a}	64.69
FLCO5	14.02 ± 1.114^{c}	39.59	10.51 ± 0.002^{f}	-186.26
FLCO10	8.90 ± 1.299^{b}	61.65	9.14 ± 0.035^{e}	-148.77
FLMCO2	18.22 ± 0.371^{d}	21.49	11.29 ± 0.030^{g}	-207.42

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at $P<0.05$. % Reduction: the percentage ascorbic acid and beta carotene (%). Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented freshly leaves; FLCO5:5 min cooked sample in gas cooker, FLCO10:10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

The fermentation of flesh leaves and gas cooking for ten minutes resulted in a significant reduction of ascorbic acid content, approximately by 74.66% and 61.65%, respectively. Various processing methods resulted in a moderate decrease in ascorbic acid levels. Specifically, BLDO decreased ascorbic acid by 28.84%, FLCO5 by 39.59%, and FLMCO2 by 21.49%. In contrast, BLDR and UBLDDR led to reductions of only 19.79% and 16.40%, respectively. Despite the lack of significant difference ($p>0.05$), the loss of ascorbic acid in blanched dried in oven leave samples (BLDO) was greater (28.84%) compared to the blanched leaves dried at room temperature (BLDR; 19.79%). Blanching prior to drying enhances the retention of ascorbic acid by inactivating oxidative enzymes, such as ascorbic acid oxidase, responsible for its degradation.Oven drying is a rapid process; however, it results in a significant degradation of ascorbic acid when compared to room drying (BLDR). This degradation may be attributed to elevated thermal temperatures, which enhance the rates of oxidation. The current study achieved a reduction of approximately 75% in ascorbic acid through the fermentation process of *JusticiaHeterocarpa*. This reduction may be attributed to the solubility of ascorbic acid (vitamin C) in brine solution, as ascorbic acid is soluble in water(27). The findings align with those reported by (25) in their study conducted in Tanzania, which indicated a significant loss of vitamin C, ranging from 88.33% to 95%, during the fermentation of *solanum species*. Furthermore, the gas cooking of flesh leaves for ten minutes resulted in approximately a 61% reduction in ascorbic acid in this study, likely due to leaching

and thermal degradation. A prior study indicated that pasteurizing yellow and red tomatoes at 100°C for 10 minutes resulted in an up to 80% reduction in vitamin C, while cooking broccoli for 5 minutes was associated with a loss of approximately 66% of this vitamin (39).The findings of vitamin C content of J. Heterocarpa from the present study are less than those of daily recommended value for adult men and women (110mg/day and 95mg/day respectively) (39). Traditionally, *JusticiaHeterocarpa* is cooked as a composite dish mixed with legumes (cowpea, pigeon peas, beans) and okra (author's unpublished surveyed data). This combination can boost the level of vitamin C to meet the required amount in a day. *Justiciaheterocarpa* was reported to be similar with African nightshade (*Solanumnigrum complex*) in taste (25) and that incorporating *Solanum species* or *Justiciaheterocarpa* into meat, other vegetables, and legumes such as cowpea improves taste, appetite, and increase carotenoids and vitamin C (40).

Beta-carotene

Beta-carotene levels differed substantially across all processing processes at$P<0.05$, with the cooked samples FLCO5 and FLCO10 having the greatest quantities (10.5115 mg/100g and 11.2885 mg/100g, respectively) (Table 4). The FFL and UBLDD samples exhibited the lowest concentrations of beta carotene, with values of 1.30 mg/100g and 1.55 mg/100g, respectively, and were not statistically different from one another. The cooked samples shown an increase in beta-carotene content, with FLCO5 exhibiting an 86.26% increase, FLCO10 a 48.77% increase, and FLMCO2 a 107.42% increase. Additionally, the blanched oven-dried vegetables (BLDO) showed a 55% increase in beta-carotene content. The substantial increase in beta-carotene levels in cooked samples may be attributed to the relative heat stability of beta-carotene, as the plant matrix facilitates its release through cell wall breakdown, thereby enhancing its bioavailability (27). This study demonstrated a reduction of approximately 65% in beta-carotene via the fermentation process of *JusticiaHeterocarpa*. This reduction can be attributed to the sensitivity of beta-carotene to oxidation, which affects its stability over time.(41) reported that submerged fermentation of vegetables reduces approximately 20% of total beta-carotene content. However, the study by (25) highlighted an increase in beta-carotene during the fermentation of nightshade species. Findings from the present study showed that microwaving cooking resulted in more than a threefold increase in this vitamin. Several studies have reported similar outcomes, with(42) (2017) noting that microwaving of selected ILVs retained more beta-carotene compared to their fresh counterparts. The observed reduction of beta-carotene in room temperature dried samples (UBLDDR and BLDR) may be attributed to the oxidation of beta-carotene. Research by (42) similarly indicated a reduction in beta-carotene levels in certain vegetables dried at 65°C using a hot air dryer. The study by (43) reported an increase in beta-carotene content in African nightshade and cowpea leaves subjected to various sun drying treatments.

Minerals:

Macro minerals (calcium, magnesium) and trace minerals (iron, zinc) exhibited significant differences across all processing techniques at ($P<0.05$) Considerable concentrations of Fe (9.46-16.74 mg/100g), Zn (3.041-4.39 mg/100g), Ca (255.2-348 mg/100g), and Mg (71.779-159.9 mg/100g) were recorded (Table 5).

Table 5: Mineral Content of raw and processed *JusticiaHeterocarpa* in Fresh Weight

	Minerals (mg/100g)			
Sample	Iron	Zinc	Calcium	Magnesium
FL	16.74 ± 0.353^{d}	4.39± 0.1718^{d}	348.73 ± 0.57^{f}	159.91 ± 0.04^{g}
BLDR	13.40 ± 0.472^{c}	3.58 ± 0.0665^{b}	312.74 ± 0.9^{d}	123.86 ± 0.04^{b}
BLDO	13.84 ± 0.182^{c}	3.4 ± 0.087^{b}	338.30 ± 1.34^{e}	135.30 ± 0.02^{c}
UBLDDR	15.85 ± 0.088^{d}	3.96 ± 0.0411^{c}	314.17 ± 0.1^{d}	149.4 ± 0.66^{f}
FFL	14.25 ± 0.266^{c}	3.53 ± 0.0553^{b}	262.36 ± 0.28^{b}	71.78 ± 0.08^{a}
FLCO5	11.32 ± 0.242^{b}	3.28 ± 0.0661^{a}	286.46 ± 2.12^{c}	140.18 ± 0.023^{e}
FLCO10	9.46 ± 0.354^{a}	3.04 ± 0.0738^{a}	255.20 ± 1.57^{a}	139.09± 0.04^{d}
FLMCO2	13.46 ± 0.512^{c}	4.04 ± 0.0019^{c}	339.85 ± 1.51^{e}	138.92 ± 0.06^{d}

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at$P$<0.05. Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented freshly leaves; FLCO5:5 min cooked sample in gas cooker, FLCO10:10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

Iron: Unprocessed fresh leaves (FL) demonstrated a high iron concentration of 16.74 mg/100g, which significantly decreased (P<0.05) after certain processing methods of *JusticiaHeterocarpa*. The lowest iron content was observed in samples cooked for ten minutes (FLCO10, 9.46 mg/100g), indicating significant iron leaching during prolonged cooking. The iron content among the samples (BLDO, BLDR, FFL, and FLCO) exhibited no significant variation, indicating the stability of iron levels across certain processing methods. (11) reported a decrease in iron content in fermented African spider plant and African black nightshade, which aligns with the current study. Conversely, the study by (25) utilizing Energy-Dispersive X-ray Fluorescence equipment reported an increase in iron content in fermented *Solanum species*. This study corroborates findings indicating that iron is susceptible to leaching during cooking and blanching(44). Iron is essential in the human body for hemoglobin-mediated oxygen transport, energy synthesis, immune function, and muscle performance. Additionally, it contributes to DNA synthesis and cognitive development (45). The intake of 100 grams of *JusticiaHeterocarpa* provides the daily iron requirement for the body, supplying 18 mg for adult women and 6-12 mg for adult men (46).

Zinc: Unprocessed fresh leaves (FL) exhibited a zinc concentration of 4.39 mg/100g, which significantly decreased (P<0.05) following particular processing methods of *JusticiaHeterocarpa*, as indicated in Table 5. The study indicated no significant difference in zinc content between blanched dried and fermented samples, likely due to the relative stability of zinc across various processing methods. Furthermore, there is no significant difference between gas-cooked samples subjected to different durations (FLCO5 and FLCO10). A minimal reduction of zinc was observed in microwaved cooked and un-blanched room-dried samples, likely due to the absence of liquid, which reduces mineral loss. The zinc content of the fresh leaves in this study is comparable to that reported by Gowele*et al.* (2019) for the same vegetable (3.9 mg/100 g, FW). The research by Marynurce (2023) indicated a decrease in zinc

levels across various drying techniques of nightshade species, consistent with the current study. However, the study by(47) reported a contrasting finding, indicating that zinc content increased more than threefold in selected indigenous leafy vegetables (ILVs) dried using sun, oven, and shade methods. Furthermore, the research by (48) indicated a reduction in zinc content in blanched sun-dried selected indigenous leafy vegetables by as much as 50%. (49) reported a decrease in zinc content in the selected cooked indigenous vegetables. (25) reported an increase in zinc content in fermented nightshade species, which contradicts the findings of the current study. The zinc content in fresh leaves of *Justiciaheterocarpa* exceeds that found in soy (3.7 mg/100 g), indicating a significant mineral concentration in this vegetable (50). Zinc is an essential trace element involved in numerous physiological processes in the body. Its participation in over 300 enzymatic activities and metabolic processes underscores its essential role in maintaining overall health and wellbeing. (51). Excessive zinc consumption has been associated with health risks, including zinc-induced copper deficiency, symptoms of anemia, and neutropenia (51).

Calcium

Unprocessed fresh leaves (FL) demonstrated a calcium concentration of 348.73 mg/100g, which significantly decreased($P<0.05$) after specific processing methods of *JusticiaHeterocarpa*, as shown in Table 5. There was no significant variation ($p<0.05$) in calcium content was observed between shade-dried samples (BLDR and UBLDDR) and high thermal-treated samples (BLDO and FLMCO2). This stability can be attributed to calcium's resistance to changes during various processing methods, as it is not sensitive to heat, light, or oxygen. The research conducted by (47) revealed a significant increase in calcium content, exceeding threefold, in selected indigenous leafy vegetables (ILVs) dried through sun, oven, and shade methods. The study by (48) demonstrated a reduction in calcium content in blanched sun-dried selected indigenous leafy vegetables by up to 50%. (49) observed a reduction in calcium content in the chosen cooked indigenous vegetables, consistent with the findings of the current study. (11) reported a decrease in calcium content in fermented African black nightshade and African spider plant, which supports the findings of the current research. (25) reported an increase in calcium content in fermented nightshade species, which contradicts the findings of this study. Numerous studies indicate that the calcium content in ILVs is higher than that observed in the current study (52,53). Calcium is vital for bone and dental health, regulates blood coagulation, influences muscle and nerve function, and activates various enzymes, such as ATPases, crucial for energy production(53). Daily calcium intake should be between 1000 and 1300 mg(45).

Magnesium

Unprocessed fresh leaves (FL) exhibited a magnesium concentration of 159.9 mg/100g, which significantly decreased ($p<0.05$) following specific processing methods of JusticiaHeterocarpa, as indicated in Table 5. No significant variation was observed between samples cooked in a microwave and those cooked with gas for ten minutes (FLMCO2 and FLCO10), as magnesium remains stable and does not degrade or volatilize at standard cooking temperatures. Fermented samples exhibit a notably low magnesium content of 71.78 mg/100g, likely due to magnesium leaching into the brine solution. (49) reported a slight reduction in magnesium content in blanched and sautéed indigenous vegetables, which is consistent with the findings of the current study. Factors influencing magnesium bioavailability include dietary fat content, the presence of anti-nutrients, and the individual's age and sex (27). Magnesium facilitates energy production, the transport of calcium and potassium ions, nerve conduction, muscular contraction, vasomotor tone, and the maintenance of regular heart rhythm. It serves as a crucial cofactor in over 300 enzymatic systems (54). The daily recommended intake of magnesium is between 300 and 429 mg (54).

3.4 Effect of processing on Total Phenolic Compounds(TPC) and ant-nutrient composition of *JusticiaHeterocarpa.*

The anti-nutrient profiles of *Justiciaheterocarpa* exhibited significant variation under different processing methods, with(P<0.05) , as indicated in Table 6. The reduction of oxalates, tannins, and phytates following processing improves the bioavailability of minerals, thereby supporting its potential application in addressing micronutrient deficiency.

Table 06: TPC and Anti-Nutrient Profiles in raw and processed *JusticiaHeterocarpa*

	Ant-nutrient (FW)			
Sample	Total phenol(GAE/100g)	Tannins (GAE/100g)	Phytates (mg/100g)	Oxalates (mg/100g)
FL	190.83 ± 14.91^{c}	81.17 ± 3.56^{d}	103.56 ± 4.68^{d}	560.46 ± 19.16^{f}
BLDR	142.95 ± 5.92^{b}	59.51 ± 2.96^{c}	76.24 ± 7.83^{c}	351.39 ± 7.41^{d}
BLDO	130.42 ± 5.22^{b}	53.24 ± 2.61^{c}	73.82 ± 5.99^{c}	242.44 ± 4.50^{c}
UBLDDR	352.71 ± 12.01^{d}	164.39 ± 6.01^{e}	94.92 ± 1.80^{d}	417.16 ± 4.50^{e}
FFL	532.83 ± 14.91^{e}	254.44 ± 7.45^{f}	40.28 ± 3.34^{b}	225.76 ± 10.34^{bc}
FLCO5	117.19 ± 7.90^{b}	21.32 ± 2.61^{b}	50.66 ± 6.25^{b}	171.77 ± 50.34^{ab}
FLCO10	64.75 ± 7.90^{a}	4.22 ± 1.98^{a}	17.81 ± 3.17^{a}	151.16 ± 6.13^{a}
FLMCO2	149.11 ± 29.81^{b}	25.31 ± 7.45^{b}	75.9 ± 1.59^{c}	219.87 ± 1.7^{bc}

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at (P<0.05) Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented freshly leaves; FLCO5:5 min cooked sample in gas cooker, FLCO10:10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

Total phenolic Compounds (TPC)

The concentration of Total Phenolic Compounds (TPC) in unprocessed leaves of *JusticiaHeterocarpa*varied significantly across different processing methods as indicated in Table 06. No significant variation in TPC was observed in the BLDR, BLDO, and FLCO5 samples. The highest levels of TPC were observed in FFL, which exhibited an increase of 64.19% from the unprocessed leaves, and in UBLDDR, where the increase was 45.9%. The increase in Total Phenolic Compounds (TPC) in un-blanched leaves dried at room temperature may be attributed to the concentration of TPC during the drying process. The increase in TPC in the FFL sample may result from the liberation of bound phenols, enhancing their concentration and bioavailability (55). The low pH stabilizes phenolic compounds, which can be detected in significant quantities (11,35). Reduced phenol levels following blanching (BLDR) and extended cooking (FLC10) suggest heat sensitivity and potential loss of phenolic components due to oxidation (32)(Mohankumar*et al.,* 2018). Total phenolic compounds exhibit various health benefits, such as antioxidant activity, anti-inflammatory properties, and antimicrobial effects (56).

Tannins:

The concentration of total tannins in unprocessed leaves of *JusticiaHeterocarpa* exhibited significant variation across various processing methods as shown in Table 06. No significant variation was observed between the short time cooked samples (FLCO5 and FLMCO2), likely due to their comparable cooking times, which resulted in similar tannin binding to the food

matrix, as well as between the blanched dried samples (BLDR and BLDO). The fermented leaves (FFL) and UBLDDR exhibited the highest tannin concentrations, measuring 254.44 GAE/100g and 164.39 GAE/100g, respectively. An increase of 68.1% in the total tannins in the FFL sample compared to the fresh sample may result from spontaneous fermentation of *JusticiaHeterocarpa* leaves. During this process, microbial activity and enzymatic actions break down complex tannin compounds and degrade cell walls, thereby releasing bound tannins into a more measurable and soluble form. The ten-minute cooked samples exhibited the lowest tannin levels at 4.22 mg/100g, reflecting a reduction of 94.6% compared to the two- and five-minute cooking durations. This demonstrates the efficacy of extended cooking in diminishing tannin levels. (11) reported comparable findings, indicating that tannin content was significantly elevated in spontaneously fermented African black nightshade and African spider plants. In contrast to this study, the research conducted by (25) indicated a reduction in total tannins in fermented *nightshade species*. A study by(44) reported reduced tannin levels in false sesame and common bean leaves when exposed to varying blanching times and drying techniques, corroborating the findings of the present study. Tannins, due to their pronounced properties and capacity to bind with proteins, can influence nutrient absorption efficiency. Tannins can interact with proteins via covalent and hydrogen bonding, resulting in the formation of a complex protein precipitate that inhibits absorption (50).

Phytates

The levels of phytates in unprocessed leaves of *JusticiaHeterocarpa* demonstrated considerable significant variation at(P<0.05) among different processing method as indicated in Table 06. No significant difference was observed between the blanched dried and microwaved cooked samples (BLDR, BLDO, and FLMCO2), indicating a moderate reduction of phytates across all methods. Furthermore, the absence of a significant difference between the unprocessed fresh leaves and the un-blanched leaves dried at room temperature may be attributed to Shade drying entails limited processing, thereby retaining certain compounds similar to those found in fresh leaves. The lowest total tannin content was observed in the fermented and ten-minute cooked samples, measuring 40.28 mg/100g and 17.8077 mg/100g, respectively. In spontaneous fermentation, phytase enzymes generated by fermenting microorganisms hydrolyze phytic acid, the storage form of phosphorus in plants, into inositol and free phosphate, resulting in a significant reduction of phytate levels in FFL samples (57). (23) reported a significant reduction of phytates in fermented spinach. Furthermore, researches conducted by (11,25) indicated a decrease in phytates in fermented nightshade species, corroborating the results of the present study. Several studies indicate that blanching and boiling vegetables decrease the phytate content in ILVs (7,22,24), which is consistent with the findings of the current study. Phytates bind essential minerals, forming insoluble complexes that decrease their bioavailability (58). Phytic acid consumption (4-9 mg/100 g) has been shown to decrease iron absorption by 4 to 5 times (59).

Oxalates:

The concentration of oxalates in unprocessed leaves of *JusticiaHeterocarpa* exhibited significant variation*(P*<0.05) across different processing methods, as shown in Table 06. The concentrations of oxalates did not significantly vary between the cooked samples (FLMCO2, FLCO10 and FLCO5), indicating a substantial reduction of oxalates in these methods relative to other processing techniques. The minimum reduction of oxalates was observed in un-blanched samples dried directly in the room, consistent with the findings of(50), which indicated that direct drying and storage did not significantly alter the reduction of oxalic acid in ILVs. Numerous studies indicate that oxalates can be effectively reduced through boiling and blanching (7,22,50) corroborating the findings of the current research. The total oxalic acid content in fresh leaves of *JusticiaHeterocarpa* is lower than that found in other green leafy vegetables, including spinach

(329.6-2350 mg/100g FW) and rhubarb (1235 mg/100g FW) (33). Soybeans have been reported to contain lower levels of oxalates (124-497 mg/100 g FW) (33) ,compared to the fresh leaves of *JusticiaHeterocarpa.* This suggests that certain indigenous leafy vegetables (ILVs) may have higher concentrations of anti-nutrients than legumes, which are typically recognized for their anti-nutrient content. The lethal dose of oxalate in adults has been documented to range from 10 to 15 grams per body weight(7).

4.0 CONCLUSION AND RECOMMENDATION

The present study examined how processing affects *JusticiaHeterocarpa's* nutritional and anti-nutritional characteristics. Cooking for ten minutes and blanching before drying reduced anti-nutrients more than un-blanched samples. Beta-carotene, vitamin C, and minerals were moderately preserved in microwaved cooked and un-blanched shade-dried samples. Fermented leaves had highest Total Phenolic Compounds (TPC), which protect against non-communicable diseases. The findings are important for Tanzanian communities with dietary inadequacies. To promote *JusticiaHeterocarpa* as a functional food across various dietary patterns, further research is necessary to ascertain its phytochemical composition and antioxidant activity, as well as the role these compounds may play in the prevention of chronic diseases such as cancer and heart disease.

5.0 **Data availability**

The data produced and examined in this study can be obtained from the corresponding author upon a reasonable request**.**

Disclaimer (Artificial intelligence)
Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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