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Mineral Profiling and Antimicrobial Effects of the West Indian Cherry (*Malpighia emarginata* DC.) Fruit Extracts Against Selected Pathogenic Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bioactive compounds are found throughout the cherry fruit and these compounds give the fruits their antimicrobial properties. The objective of this study was to determine the mineral profile of *M. emarginata* for four maturity stages. The mineral potassium (K), phosphorus (P), and magnesium (Mg) content was evaluated by spectrophotometry. The highest amount of K was found in the half-green (HG) stage and the lowest in the full-ripe (FR). The highest concertation of P and Mg was found in the full-green (FG) stage and the lowest in the full-ripe. For P, there was an increase from the FG to the HG maturity stage, then the concentration declined until maturity, as it related to the

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concentration of Mg and K, there was a decline in the concentration as the fruit progressed maturity. Additionally, the antibacterial activity of the fruit extract was conducted on *E. coli* and *S. aureus* using the Kirby Bauer disc diffusion method at different concentrations of plant extracts. The ethanolic extracts (EE) (zone of inhibition 13.4 ± 1.9 to 21.8 ± 0.6**)** showed more antimicrobial activity than the aqueous extract (AE) with zones of inhibition diameters (mm) which ranged from 0 ± 0 to 18.2 ± 0.8. Both AE and EE were effective against *S. aureus* bacteria strain except the AE (crude) which had no zone of inhibition. All tested microorganisms were susceptible to the antibiotic ciprofloxacin. This study suggests that other types of solvents were used to investigate the antibacterial activities of the *M. emarginata* extract on a wider array of bacteria as the fruit demonstrated antimicrobial properties.

Keywords: Antimicrobial activity; bioactive compounds; maturity stage; mineral profile; zone of inhibition.

1. INTRODUCTION

A fruit undergoes physical changes such as a rapid increase in size, and loss of firmness and develops flavours and scent during ripening [1] and chemical changes, which are responsible for the development of flavours, colour and scent during the ripening stage [1]. Together, these physicochemical changes affect the increase or decrease of bioactive compounds (chlorophyll, carotenoid, anthocyanin, and vitamin C), which also affects the overall quality of the fruit. These events necessitate the timely processing of the *M. emarginata* to preserve its nutritional value. The accumulation of these compounds occurs rapidly as the fruit matures, which reduces the storage period and commercialization [1]. In the present paper, the accumulation of bioactive compounds, the factors that affect the physicochemical changes and health benefits of the fruit will be discussed. Mohammed [2] found bioactive compounds to have colour-changing effects. Chlorophyll is a pigment found in plants and is responsible for the green colour in both plants and fruits [3]. According to Mohammed [2] the chlorophylls in fruits decrease and eventually disappear as the synthesis of the non-green pigments (carotenoids and/or anthocyanin) occurs during ripening. This is to say that as the cherry transitions from green to red there must be a deduction of the chlorophyll which is responsible for the green colour so that the other chemical activities take place which is necessary for the red colour (anthocyanin and carotenoid) to come into play [2]. On the other hand, this fruit is high in anthocyanin, and it gives tart cherries their dark red shade colour changes while Carotenoid provides the colour from yellow to red [3]. Biosynthesis of anthocyanin is triggered as the chlorophyll and carotenoid degrades. Therefore, as the fruit approaches maturity, chlorophyll level and carotenoid levels decrease [3].

As a fruit ripens, its colour changes from green to red, and the ascorbic acid (vitamin C) declines. By the time the peel colour turned red, this result demonstrated that there were significant variances for each of the six development phases. The total sugars reduced as the fruit ripened [1,4]. looked at the *A. trifoliata* and found the following patterns: from the fully green to the half green stage, the sugar and starch content exhibited an upward trend, but in the three-quarter green stage or fully ripened stage, they saw a rapid decline. There were contradictions in the level of anthocyanin and ascorbic acid, where it was found that both compounds increased in *A. trifoliata* as the flesh darkened. Apart from vitamins and antioxidants, the West Indian cherry also contains the minerals K, Mg and P. Mg has a vital role to play in the growth of higher plants, it is important for plant food production (photosynthesis) and nucleic acid and protein synthesis, However, if Mg is deficient, it reduces the rate of photosynthetic activity and results in the yellowing of leaves [5]. In contrast, K plays a key role in various physiological and biochemical processes, including respiration, and photosynthesis [6]. According to Fu et al. [7]. Mg, P and K decreased as the fruit developed with the highest concentration noted in the young fruit stage (FG) and low concentration in the matured stage of the navel orange (*Citrus sinensis* L. Osbeck) [7]. These trends were noted by Rop et al. [8] who did a study on the Medlar (*Mespilus germanica* L.) fruit except the phosphorus, which remained stable.

Variation in bacterial cell wall structure and between different bacteria determines how susceptible it is to the bioactive compounds of the fruit extracts. Suriyaprom et al. [9] also noted that berries, which include cherry bioactive extracts, caused the release of lipopolysaccharide (LPS), increased cytoplasmic membrane permeability, and disintegration of the

outer membrane, which led to high antibacterial activity [9]. Cherries rich in bioactive compounds can inhibit the growth of pathogenic bacteria but it's not lethal to them [10]. These antimicrobial effects against pathogens have been demonstrated in several studies [11]. An antimicrobial analysis carried out on cornelian cherry (*Cornus mas* L.) showed that the extracts from this fruit had the strongest antimicrobial effects against *P. aeruginosa* and *E. faecalis,* Gram-positive and Gram-negative bacteria respectively [11]. Inhibitory effects of fresh fruits from *C. mas* against *S. aureus* and *P. aeruginosa* have also been observed in studies [11]. Another study tested the antibacterial efficacy of *cornelian* cherry fruits against four selected microorganisms, and it found that methicillin-susceptible *Staphylococcus aureus* bacteria (MSSA), *P. aeruginosa*, and *E. coli* greatly reduced the inhibition zone diameter, which was 8 mm for all strains [11].

Based on the results for antibacterial activity of EE, and AE extracted samples from the *Aesculus hippocastanum* L. against *E. coli* and *S. aureus* by disc diffusion method, demonstrated the degree of inhibition *S. aureus* of extracts increases as the concentration of *A. hippocastanum* extracts increases [12]. This means that *S. aureus* is highly susceptible to all extracts of *A. hippocastanum*. They also noted that the bacteria strains were susceptible to the antibiotics used [12].

2. MATERIALS AND METHODS

2.1 Study Site

In this study, the cherry fruits were collected at four different maturity stages (unripe, half-ripe, three-quarter ripe and fully ripe) during February. The cherries were obtained from the village of Buxton on the East Coast of Demerara. For the collection of samples, the fruits were harvested on the same day and separated per the peel's colours. The fully green fruits were selected, at the half-ripe stage, the yellow fruits were selected, at the three-quarter ripe stages, the yellow-red and for the final stage of maturity, fully red fruits were chosen (Fig. 1). Ten cherries for each colour change or 40 fruits were randomly selected for uniformity of colour development, irrespective of defects and size. The fruits were placed in covered containers to avoid mechanical damage.

This study utilized a randomized complete block experimental design to determine the concentrations of Mg, K and P in *M. emarginata* as the fruit progressed maturity and to explore the potential effects of the *M. emarginata* fruit extracts on *S. aureus* and *E. coli* bacteria.

For the analysis of magnesium, the PTPE digestion vessel, 10 ml concentrated HNO_3, pipette, fume hood, electronic scale, carousel, microwave (Mars 5) turntable, thermometer, oven, 2 ml of 30% H_2O_2, Whatman # 40 paper ashless paper, 50 ml volumetric flasks and the spectrophotometer at 420 nm. petri dish, ethanol, distilled water deionized water, 1g Na_2 SO_4 (1 Na_2 SO_4 Kjeldahl Catalyst Tablet), 0.5 ml 7% $CuSO_4$, $5H_2O$, 3 ml of conc. H_2 SO_4, hydrogen peroxide (H_20_2).

The tools used in this research were: test tubes, test tube racks, autoclaves, spirit lamps, cherry fruits, scale, micropipettes, incubators, stoves, and sterile petri dishes. The materials used in this study were MHA Media (Mueller-Hinton Agar), *M. emarginata* extract, bacterial culture of *S. aureus* and *E. coli*, discs containing the antibiotic ciprofloxacin, and empty test discs.

2.2 Extraction of the Bioactive Compounds

The collected fruit was washed and rinsed with distilled water samples and subjected to solar drying. After 24 hours, the fruit samples were transferred to the University of Guyana, Biology Laboratory for oven at 45 ℃ for 72 h. The fruit samples were turned daily to increase the surface area for better drying. To follow the moisture evolution during drying, the sample moisture was monitored by sample weighting every 24 hours after the first 48 hours. Extraction was performed on powders of dried cherry fruits. Each sample (50 g) was added to 96 %ethanol (300 ml) and 300 ml of distilled water with stirring for 96 h and filtered through double layers of gauze to attain a clear filtrate [13]. The filtrates were roto vaped at 45 ℃ for ethanol and 70 ℃ to concentrate the extracts. The ethanol evaporated faster than the water (100 ℃) since it has a lower boiling point. The extracts were stored in vials and stored in the dark at room temperature [14]. The crude was considered 100 % concentration of the extract for both ethanol and water. The concentrations of 75%, 50%, and 25% were produced by mixing with appropriate quantities of distilled water [15]. The discs were also soaked in ethanol, sterile water, and ciprofloxacin.

2.3 Preparation of the Agar

A conical flask containing 30.6 g of *Mueller-Hinton agar* (MH agar) was filled with 800 ml of

water. The agar dissolved after 30 minutes of gently swirling the mixture on a hot plate. When bubbles started to appear, the agar swirled at regular intervals, and the solution was then taken off the hot plate and sterilized in the autoclave for 15 minutes. To reduce the risk of contamination, the agar was poured in front of a spirit lamp in the microbiology laboratory [16]. After pouring, the plates were left to solidify at room temperature and then underwent a second 20-minute ultra-violet (UV) sterilization to avoid contamination. The plates were then inverted and then placed in the refrigerator overnight [17,18].

2.4 Bacterial Species

This experiment involved the utilization of two different microbial strains, based on therapeutic significance, the bacteria chosen from the University of Guyana's Department of Health Sciences were *E. coli* and *S. aureus.*

2.5 Antibiotic Sensitivity Testing

By using the disc diffusion method, the test microorganisms' susceptibility to the antibiotic ciprofloxacin was also evaluated. On the surface of sterile Muller-Hamilton agar plates, test cultures were transferred aseptically using sterile cotton swabs. The antibiotic disc was aseptically placed over the seeded agar plates using flamed forceps that had been dipped in ethanol. Measurements were made of the inhibition zone diameters after the plates had been incubated at 37 °C for 24 hours. The zones of inhibition were measured in mm [19,20].

2.6 Testing for Magnesium (Mg) and Potassium (K)

A dry sample weighing about 0.5 g was placed in a PTPE digestion tank. A digesting vessel was filled with 10 ml of concentrated HNO_3, swirled, and left to stand in a fume hood for 15 minutes. The containers were covered, secured, and weighed on a scale. They were thereafter seated on the microwave (Mars 5) turntable and uniformly dispersed around the carousel. The digester was set to run at 170°C for 15 minutes and then maintained for another 15 minutes. Using a modified software, biochar samples were digested as follows: samples were heated to 200°C for 20–25 minutes, then processed at that temperature for 10 minutes. After cooling the vessels, 2 ml of 30% H_2O_2 was added to each vessel. By adding 1 ml of 30% H_2O_2 and repeating the heating process for 12 minutes, samples were further digested. The containers were allowed to cool in the device for five minutes after the microwave procedure was finished before being removed [21,7].

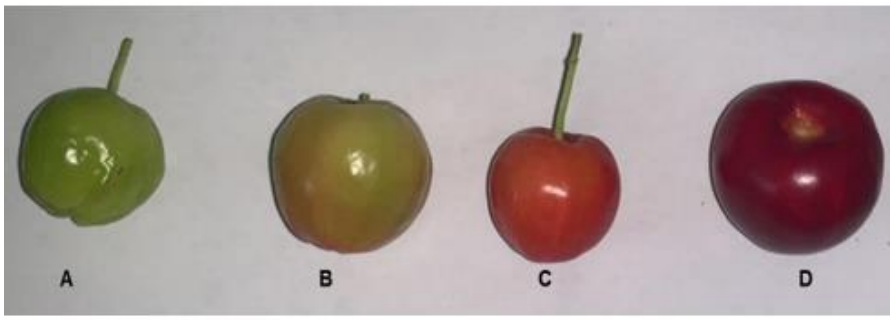

Fig. 1. A-Fully Green (FG), B. Half green (HG), C. Tree quarter green (TQR) and D. Fully ripe (FR)

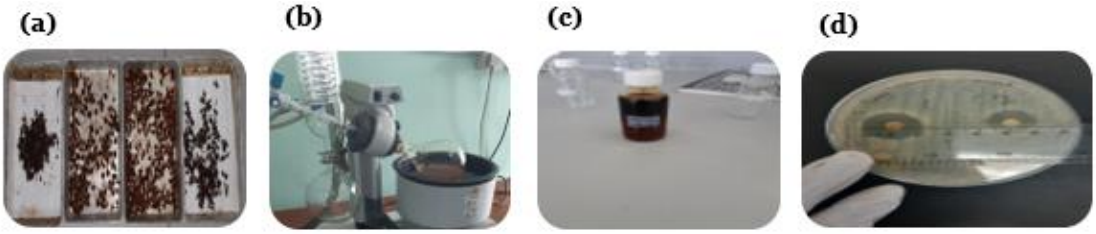

Fig. 2. The protocol for extraction and preparation of samples (a) Fruit subjected to oven drying at 45℃ for 72 hrs. (b) Aqueous and ethanolic extracts rotavaped (c) Crude extracts, and (d) Growth of inhibition zones (Ethanolic cherry extracts at 75% concentration

Additionally, each vessel was weighed with an approximation of 0.1 g and the samples were chilled to room temperature. In a fume hood, the samples were securely sealed with lids and vented. Some 50 ml volumetric flasks were used to filter the mixture with What-man #40 ash-less paper. A flame atomic absorption spectrophotometer (Agilent 240 AA series) was used to further analyze the materials [22]. By calibrating the device with known standards within the linear range of the absorption wavelength, the calibration plot of concentration against absorbance was created for each element. The sample's absorbance was diluted if it went beyond that of the appropriate calibration range [7,23].

2.7 Testing for Phosphorus

The ground cherry fruit sample was dried in an oven preheated at 65-70℃ for no more than four hours, and then stored in a desiccator containing self-indicating silica gel. In a tared weighing boat, the sample's weight of 0.1 g was measured. The desiccator received the sample that was still present. The weighing boat was placed almost horizontally in a clean dry boiling tube containing two glass beads. The handle of the boat was hooked with the hooking tool. The tube was slowly moved into the vertical position with one hand while turning over the boat to the tube. The boat was gently tapped face down in the tube a few times, the tool was used to facilitate the complete transferal of the sample [7,24]. The same tube was filled with 1 g of Na_2SO_4 (1 Na_2SO_4 Kjeldahl Catalyst Tablet), 0.5 ml of 7% CuSO4, and 3 ml of conc. H_2 SO_4. These reagents were used to create a blank sample. Each boiling tube received two glass beads [7,23].

2.8 Quality Control

Steps 1 and 2 were applied while the step with the blank was omitted, to one previously analyzed IPE (eternal) reference sample and one or more internal reference (or check) samples selected from the previously analyzed batch of samples. One internal reference had been selected for every 20 samples. The blank, samples and reference tubes were placed in the block digester. The Kjeldhal digested sample blank, sample, and reference solutions were divided into three separate aliquots and pipetted into 50 ml volumetric flasks, each of which contained roughly 35 ml of distilled deionized water. Each flask received 1 ml of 10% NaOH, 1 ml of Na_2OSiO_2, and 2 ml of Nessler's reagent. It was stopped and carefully mixed after being diluted to 50 ml with deionized distilled water [7,24,13,23,22].

Working 10 mg/L N standard aliquots of 0, 3, 4, and 5 ml were transferred to separate 50 ml volumetric flasks. Diluted to the appropriate amount, a deionized distilled water stopper was carefully combined. the UV-VIS spectrophotometer by the manufacturer's handbook or the relevant CAEMS SOP. The solutions contained 0, 0.6, 0.8, and 1.0 mg/L N. At 420 nm, the absorbance of the reference, standards, sample blank, and sample solutions was measured [7,23].

2.9 Statistical Analysis

The results were expressed as mean ± standard error after each assessment was conducted in triplicates. All statistical analyses were conducted using *R version 4.1.2* (R Core Team, 2020). The Shapiro-Wilk test was done to determine the normality of the data.

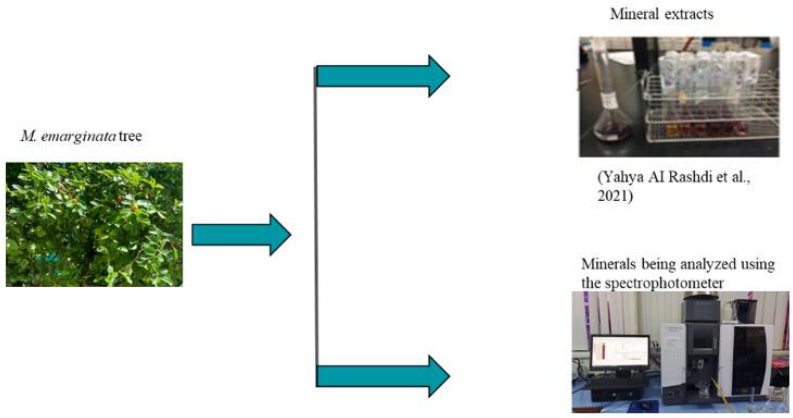

Fig. 3. The method used in carrying out the Mineral analysis Al-Yahya'ei et al., [25]

3. RESULTS

The analysis of nitric acid extracts of *M. emarginata* revealed the total percent (%) concentration of potassium was 0.12 ± 0.04, the total percent of Magnesium was 0.17 ± 0.006 and the total phosphorus concentration was 0.24 ± 0.003 (Table 1).

For the concentration of phosphorus in *M. emarginata* as it approaches maturity, it was observed that the highest concentration was recorded in the fully green (FG) stage with a concentration of 0.25 ± 0.002 while the lowest concentration was recorded in the Fully ripe (FR) stage with a concentration of 0.23 ± 0. The concentration was significantly higher than (p-value- 0.05).

For the concentration of phosphorus in M. emarginata as it approaches maturity, it was observed that the highest concentration was recorded in the half-green (HG) stage with a concentration of 2.18 ± 0.007 while the lowest concentration was recorded in the Fully ripe (FR) stage with a concentration of 1.82 ± 0.002. The concentration was significantly higher than (p-value- 0.05).

For the concentration of phosphorus in M. emarginata as it approaches maturity, it was observed that the highest concentration was recorded in the half-green (HG) stage with a concentration of 0.19 ±1.70 E-17 while the lowest concentration was recorded in the Fully ripe (FR) stage with a concentration of 0.14 ± 0.002. The concentration was significantly lower than (p-value- 0.05) (Table 2).

Table 1. K, Mg and P levels (%) of *M. emarginata* extracts at different maturity stage

Maturity stage	Concentration (%)		
	K	Mg	P
FG	2.12 ± 0.0047	0.19 ± 1.70 E-17	0.25 ± 0.0027
HG	2.18 ± 0.0072	0.17 ± 0.0027	0.24 ± 0.0027
TQG	2.15 ± 0.0027	0.17 ± 0	0.24 ± 0
FR	1.82 ± 0.0027	0.14 ± 0.0027	0.23 ± 0

Table 2. A pairwise post-hoc Dunn test with Bonferroni corrections showed statistical significance in magnesium concentrations for Fully Green - Fully Ripe (*p* = 0.008239492)

Comparison	Z	P-unadj	P. adj
FG – FR	3.200	0.00137	0.008239
FG-HG	1.422	0.155	0.930
FR-HG	-1.778	0.0754	0.452
FG-TQR	1.778	0.0754	0.452
FR-TQG	-1.422	-1.422	0.926
HG-TQR	0.356	0.722	1.000

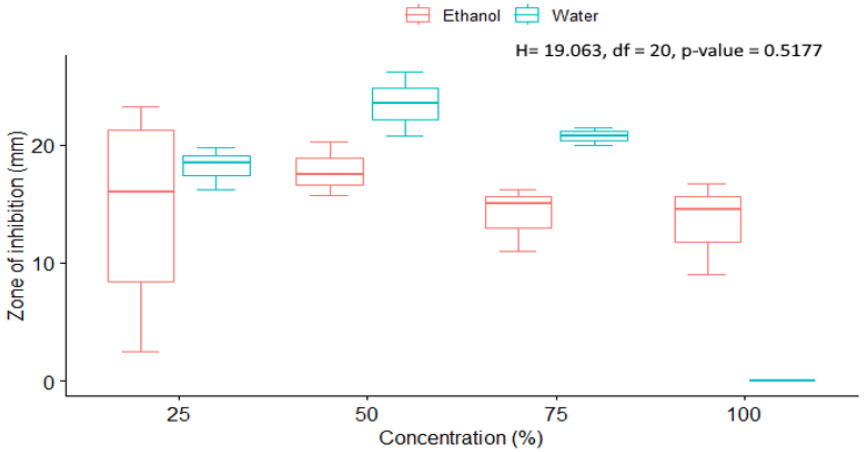

Fig. 4. P-value, H and df Zone of inhibition (mm) of each treatment group at different concentrations

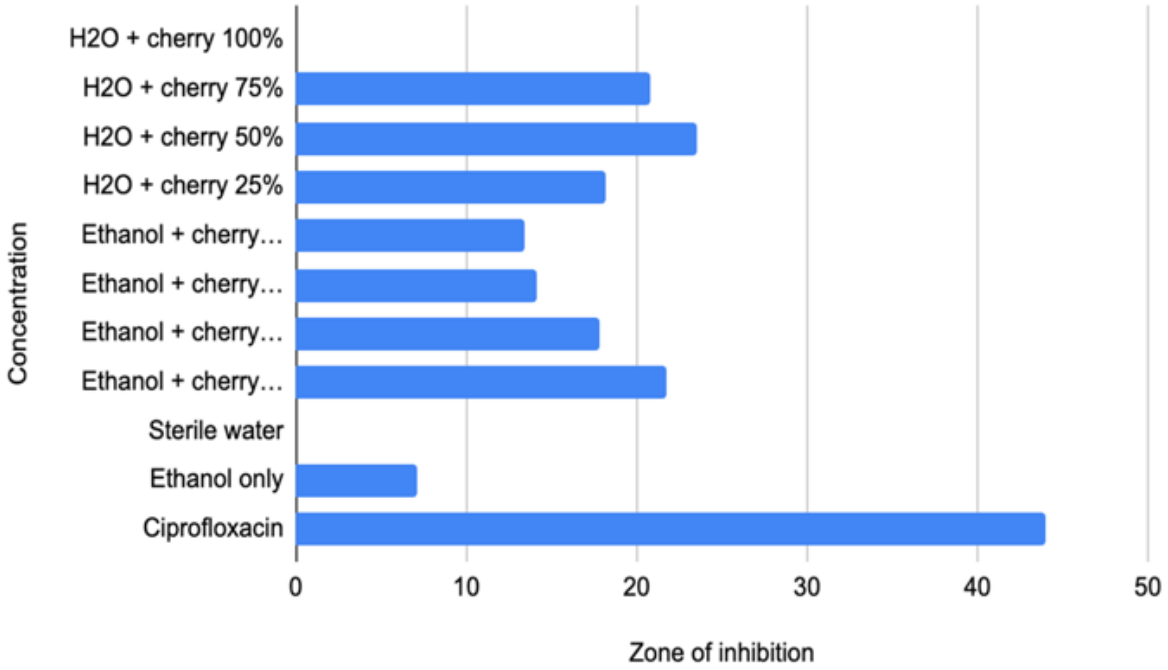

Fig. 5. The *S. aureus* inhibitory diameters of the ethanolic (EE) and aqueous (AE) fruit extract of *M. emarginata* against *S. aureus*

3.1 Antimicrobial Activity

Given the widespread knowledge of *E. coli* and *S. aureus* antibiotic resistance, these findings were of interest. The antibacterial activity against *E. coli* and *S. aureus* bacterial strains was assessed using the disc diffusion method using the extracts of the fruit *M. emarginata* at four different concentrations. In the present study, the antibiotic ciprofloxacin was utilized as a control at a concentration of 1 ml. Both the aqueous and the ethanolic fruit extracts from *M. emarginata* showed no antibacterial action against *E. coli*. The aqueous extract showed 50% of its highest efficacy against *S. aureus*.

The zones of inhibition (mm) of each treatment group against *S. aureus* at varied concentrations of 100%, 75%, 50%, and 25% (H = 19.063, df = 20, p-value = 0.5177). Results show the mean and standard deviation of three replicate measurements (Fig. 4 and Fig. 5). The Kruskal-Wallis H Test yielded a p-value of 0.5177, indicating that there were no statistically significant differences between the *M. emarginata* extracts and the *S. aureus* inhibition zones.

4. DISCUSSION

The West Indian cherry fruit is recognized as a fruit that protects health in considerable quantities due to its mineral richness and presence of a wide variety of bioactive substances [26]. Eating cherry fruit daily may provide numerous biological activities that are good for health and may assist in preventing chronic diseases [26]. While the fruit is growing and developing, these biochemical and mineral components are significantly altered. Additionally, the presence of these chemicals may be affected by micro-environmental conditions [7]. The present investigation was carried out to gain a thorough understanding of trace element alterations during the different maturity stages of fruit growth and the antimicrobial properties of the bioactive compounds.

The mineral content of the cherry showed a decreasing trend from fully green to fully ripe except for phosphorus which had fluctuation from fully green to fully ripe. Moreover, the contents of potassium (K) at the different stages of maturity varied from 2.12 ± 0.005 E- 17 to 1.82 ± 0.003. The concentration of K decreased in *M. emarginata* as fruits approached maturity, with the highest concentration being at the half-green (HG) stage and the lowest being at the fully ripened (FR) stage. Wu et al. [6] suggested that potassium is the most abundant mineral utilized in fruit maturity.

The contents of Mg at the different stages of fruit ripening in *M. emarginata* varied between 0.19 ± 1.6 E-17 to 0.14 ± 0.003 %. The content of Mg also showed a decreasing trend as it progressed ripening, with the highest and lowest concentrations being 0.19 ± 1.6 E-17 % and 0.14 ± 0.003 % respectively. These results coincide with other studies that noted a decrease in concentrations of both K and Mg as the fruit progressed to maturity [8]. However, the concentration of the phosphorus did not coincide with the literature. According to Rop et al. [8]. P concentrations were to remain stable but based on our results, Phosphorus content with regards to the maturity stage also had a decreasing trend. It varied from 0.25 ± 0.003 % to 0.23 ± 0.00 % as the fruit approached maturity, with the fully green stage being highest with percent concentration being at the fully green stage (0.25 ± 0.003 %) and the lowest % at the fully ripe stage (0.23 ± 0 %). The high levels of micro-elements found in the fruit are related to the fruit quality [7]. As a result, the overall quality of fruit will decline depending on whether these components are present in excess or deficit. For instance, excess N and K or P deficiency decreases the quality of fruit, whereas excess N or P can improve fruit quality [7].

The entire cherry fruit was investigated to evaluate the antimicrobial activity against the Gram-positive bacteria (*S. aureus)* and the Gram-negative bacteria (*E. coli*) using the disc diffusion method. Aqueous extracts were generally less active than ethanolic extracts (EE). Even though the yields of zones of inhibition with water were higher than those with ethanol, this is because ethanol removes more bioactive compounds than water. Khar'kov et al. [12] offered evidence for this conclusion. Flavonoids, polyphenols, tannins, and alkaloids are examples of chemicals having antimicrobial action that are typically soluble in ethanol but insoluble in water, according to several scientists [12].

The antimicrobial activity of *M. emarginata* extract exerted antimicrobial activity against *S. aureus* bacteria only. This is due to the Gram-negative bacteria having a thick layer of peptidoglycan making it difficult for the fruit extracts to penetrate the bacterial wall [12]. Aqueous extract at 50 %, (with the diameter of the inhibition zone of 23.5 ± 1.3 mm), showed a significantly stronger antimicrobial potential towards the bacteria strain when compared to the ethanolic extracts at 50%. These results were compared to previously reported studies and the results were by Aurori et al. [11] who demonstrated a strong inhibition effect of *Cornelian cherry* fruit extracts against *E. faecelis,* a Gram-positive bacterium. The lowest inhibition effect was recorded towards aqueous extracts at 100% (inhibition zone of 13.4 ± 1.9 mm).

Antibiotics are drugs that can inhibit the proliferation of bacteria [27]. These come in two varieties: those made naturally and those made artificially (synthetic antibiotics). Overuse of synthetic antibiotics can seriously harm important organs [27]. The synthetic drug used in this study was ciprofloxacin. The positive control experiment showed that Ciprofloxacin had the widest zone of inhibition against *E. coli* showing a 50.00 ± 14.47 mm zone of inhibition. This indicates that Ciprofloxacin is the best antibiotic for treating infection caused by *E. coli*. Ciprofloxacin also works against *S. aureus* (with an inhibition zone of 44 ± 0). From the results, ciprofloxacin is indeed a broad-spectrum antibiotic against many bacterial infections.

5. CONCLUSION

The results of this investigation showed that *M. emarginata*, a multifunctional traditional medicinal plant with a wide variety of vitamins, is also a rich source of bioactive chemicals. The *M. emarginata* fruit demonstrated a high concentration of phosphorus in the fully green stage and a high concentration of magnesium and potassium in its half-green stage. For potassium, it is recommended that the HG stage of the fruit is harvested as there is a huge loss during ripening and for phosphorus and magnesium, the FG stage is recommended to be eaten. The fruit extracts demonstrated significant antimicrobial activity against the *S. aureus* bacteria, except the aqueous crude extract (100 %), which had no antimicrobial activity (no zones of inhibition). The fruit extract has the potential to be used as a natural alternative preventative to control food poisoning diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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