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Organic Acid Content and Antimicrobial Properties of *Eleiodoxa conferta* Extracts at Different Maturity Stages

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Abstract

Eleiodoxa conferta water extracts at different maturity stages were shown to contain three types of organic acid which are oxalic, ascorbic and malic acids by HPLC analysis. The content of oxalic acid concentration was the highest at young stage (1.33 gml^{-1}) followed by mature stage (1.26 gml^{-1}) and ripe stage at 1.23 gml^{-1}. Malic acid content decreased during ripening from 1.38 gml^{-1} to 1.07 gml^{-1}. The concentration of ascorbic acid remained constants during the fruit ripening. When antimicrobial activity of the extract was tested against several bacteria it was observed that the activity decreased as the fruit ripen. Highest diameter of inhibition zone was recorded against *Escherichia coli* by the young fruit extract at 15.3 mm. MIC of 0.063 gml^{-1} exhibit by the young fruit extract was helpful in controlling the growth of Gram negative bacteria, *E. coli*. However, 0.063 gml^{-1} concentration of extract from mature fruit is shown to regulate the growth of Gram positive bacteria, *Staphylococus aureus*.

1. Introduction

Eleiodoxa conferta commonly called kelubi or asam paya belongs to the family Arecaceae is an underutilized fruit that has not been reported about their antimicrobial activity and organic acids content. Found in peat swamp and fresh water swamps area. It is clustering palm which forms dense thickets [1]. The leaves are up to 3.5 metres long, arranged along the rachis and toothed along the margins. Fruits skin has scaly appearance and very sour taste, reddish colour when ripe and has one or occasionally two seeds which can eat fresh or pickles for sale in local markets.

Organic acids are great significance in plants. As intermediates in the metabolic processes of the fruits including respiration and photosynthesis, these acids are contributed in growth, maturation and senescence. [2] studied the organic acid in tropical fruit longkong (*Aglaia dookoo Griff*.) at ripe stage and showed that citric acid and malic acid were present at the level of 0.22 % w/w and 0.15% w/w. [3] reported citric acid as the major organic acid in fully ripened strawberry and mulberry genotype fruit. [4] reported that malic acid is the dominant acid in various types of olive fruit such as Memecik, Domat and Akhisar Uslu varieties in ripe stage. This result is contrast to the recent studied by [2] reported that organic acids in foreign fruit such as strawberry, sweet cherry, and mulberry increased as maturity progressed. [5] identified three organic acids in orange juice and wines: citric, ascorbic and malic acids. The major organic acid was citric acid in orange juice (12.66 g L^{-1}) and wine (6.03 g L^{-1}).

In recent years, researchers have found that organic acids in some fruits can inhibit growth of some bacteria and fungi. [6] discovered that malic acid could

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inhibit the growth of *Listeria monocytogenes*, *Salmonella gaminara*, and *Escherichia coli*. [7] also found that organic acids such as malic, citric, lactic, and tartaric acid had antibacterial activity with specific pH conditions.

In view of these opinions and observations, the present investigation was taken up to screen this underutilized fruit to investigate its organic acids content and antimicrobial activity at different maturity stages.

2. Materials and Methods

2.1. Organic acid content analysis

Fruit of *Eleiodoxa conferta* was collected from local fruit farm in Jeli, Kelantan. The fruit was identified by Dr Shamsul Mohamad, lecturer in Universiti Malaysia Kelantan. Fruits with good condition at each maturity stages were selected. The ripening stage of the fruits was distinguished through physical observation. To obtain fruits extract, water extraction method was applied. Fruit was cut into two halves, and 50 g fruit was extracted by addition of 100 ml of water using electric blender. To obtain clear juice, the fruits extract was filtered through a muslin cloth or a stainless steel filter with small porosity. The filtered juices were immediately stored in freezer at 0°C for further analysis and also to avoid any colour changes. To start the analysis, the fruits extracts were clarified by centrifugation at 5000 rpm for 10 minutes and filtered through a 0.45 µm filter membrane before injection [8]. Two ml of extract was transferred into HPLC vial. HPLC analysis was carried out using Shimadzu HPLC equipped with a column oven C18 used for separation of organic acids. The mobile phase employed was ammonium di-hydrogen phosphate buffer (NH4H2PO4, 0.5% w/v). The flow rate of the mobile phase was maintained at 0.5 mlmin-1, the injection volume was 1 µL and the temperature of column oven was set at 40°C [9]. The detection wavelength was 254 nm. Organic acids were identified and quantified by comparison of their retention time and peak area to the standard.

2.2. Antimicrobial properties

Five species of bacteria were used in this study consists of two Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*), three Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella spp*.) were obtained from Director of Veterinary Research Institute, Ipoh, Malaysia. The test bacteria was sub-cultured into nutrient agar [10] and maintained at 4°C respectively. All the bacteria were sub-cultured on nutrient agar for inoculum with a loop. Single colony of each subculture bacteria was inoculated in 10 ml LB broth and incubated at 37°C for 24 hours on incubator shaker at 150 rpm. These stock cultures were kept at 4°C for antimicrobial assays [11]. The disc diffusion test was performed using a modified Kirby-Bauer method [12]. 100 µl of the microbial suspension was added and swabbed onto the surface of Nutrient Agar (NA). The plates were allowed to dry at room temperature within 15 minutes. Next, 10 µl of extract were immersed into a sterile 6 mm paper discs and allowed to dry for 1 hour in a laminar flow hood. Dry discs were aseptically placed on NA. The plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring inhibition zone diameters. The experiment was done in triplicate. The minimum inhibitory concentrations (MICs) of all fruit extracts against 5 microbial strains were determined using a broth dilution method. The fruit extract was diluted using serial two fold dilution to obtain 7 concentrations (0.5 mgml-1 to 0.008 mgml-1) [13]. Then 1 ml of the microbial suspension of *Staphylococcus aureus* and *Escherichia coli* (2×10^6 $cfuml^{-1}$) were added to each test tube and mixed thoroughly [14]. The control test tube containing the medium only was used to confirm the sterility of the medium. Then the culture tubes were incubated at 37°C for 24 hours. Afterwards, incubation tubes were observed for changes in turbidity as an indicator of growth. The lowest concentration that did not permit any visible growth was considered as MIC.

2.3. Statistical analysis

Experimental data were analysed using analysis of variance (ANOVA) and significant differences among means at $p < 0.05$ were determined by Tukey-test using MINITAB statistical software (Version 16).

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3. Results and Discussion

3.1. The composition and content of organic acids at different maturity stages of extracts

As shown in the Fig. 1, there were significant differences in the contents of organic acid among different maturity stages of extracts except for ascorbic acid. The main organic acid in Eleiodoxa conferta was oxalic acid. The content of oxalic acid concentration was the highest at young stage [1.33 gml-1] followed by mature stage (1.26 gml-1) and ripe stage of 1.23 gml-1. Malic acid content decreased during ripening from 1.38 gml-1 to 1.07 gml-1. The concentration of ascorbic acid remained constants during the fruit ripening.

3.2. Screening for antimicrobial activity at different maturity stages of fruit extracts

Young stage of extracts exhibited good activity against all the bacteria species as shown in Table 1. Highest diameter of inhibition zone was recorded against Escherichia coli by the young furit extact at 15.3 mm. Water extract of *Eleiodoxa conferta* exhibited good activity (diameter zone of inhibition > 8 mm) at the order of Salmonella spp > *Pseudomonas aeruginosa* for Gram negative bacteria and *Bacillus cereus* > *Staphylococcus aureus* for Gram positive bacteria.

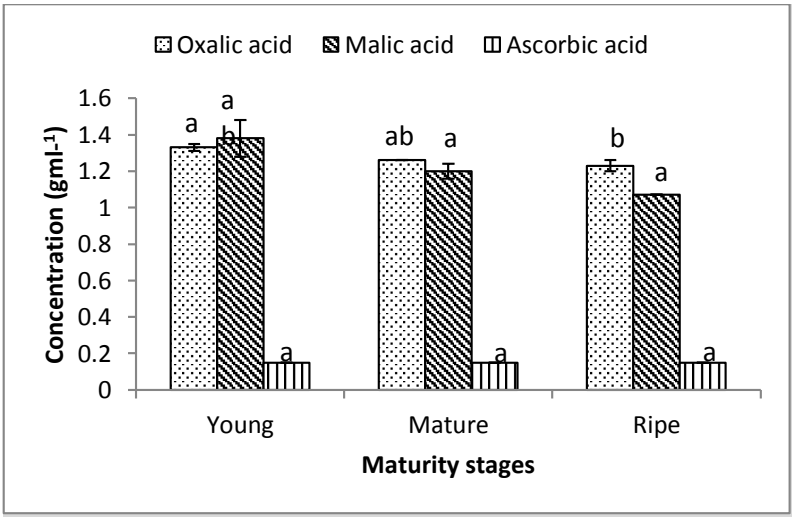

Figure 1: Distribution of organic acids at different maturity stages of *Eleiodoxa conferta* extracts at three different maturity stages. Different letters (a-c) in each stage of fruits indicate significant differences at $p < 0.05$. Each point represented the mean and error bars were ± SD of the mean.

Table 2: Zone of inhibition of *Eleiodoxa conferta* extracts at different maturity stages tested against Gram positive and Gram Negative bacteria using disc diffusion method

Stage of fruit development	Zone of inhibition [mm]				
	Gram +ve bacteria		Gram -ve bacteria		
	SA	BC	EC	PA	SS
Young	10.7	13.3	15.3	9.3	10.7
Mature	10.7	11.3	12.7	8.7	10.3
Ripe	12.3	12	10.7	8.7	8.3

SA – *Staphylococcus aureus,* BC – *Bacillus cereus,* EC – *Escherichia coli,* PA – *Pseudomonas aeruginosa,* SS – *Salmonella spp*.

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The minimum inhibitory concentration (MIC) was tested to observe the concentration required to inhibit the growth of bacterial as shown in Table 2. MIC of 0.063 gml-1 exhibit by the young fruit extract was helpful in controlling the growth of Gram negative bacteria, E. coli. However, 0.063 gml-1 concentration of extract from mature fruit is shown to regulate the growth of Gram positive bacteria, S. aureus. Overall, Eleiodoxa conferta proved to possess 50% inhibition against both bacterial strains.

Table 2: Minimum inhibitory concentration (MIC) of *Eleiodoxa conferta* extracts at different maturity stages against *E. coli* and *S. aureus*

Bacterial strains	MIC at each stages of extracts [gml^{-1}]		
	Young	Mature	Ripe
E. coli	0.063	0.125	0.125
S. aureus	0.125	0.063	0.125

Note: Concentration range of extract: 0.5 – 0.008 gml^{-1}

4. Conclusion

There were three kinds of organic acids present in the extracts of *Eleiodoxa conferta* which are oxalic, malic and ascorbic. The main organic acid was oxalic acid. In this study, we found that young stage of *Eleiodoxa conferta* extracts which has the highest concentration of organic acid contents also has greater antimicrobial activity. The antimicrobial activity was higher at the early fruits stages against Gram negative bacteria of E. coli and the minimum inhibitory concentrations against E. coli and S. aureus was 0.063 gml-1 at young and mature fruits stages. Therefore, it could be inferred that the amount of organic acid influences the antimicrobial activity of *Eleiodoxa conferta*. Acid penetrate the bacteria cell wall and disrupt the physiology of bacteria. [15] reported that the content of oxalic acid in other fruits species of *Averrhoa* can become a potent source of antioxidant and antimicrobial against S. aureus. The literature is also mention the possibility of organic acid altering the microbial potential and may function as acid resistant on certain bacteria species [16]. Thus, *Eleiodoxa conferta* fruits especially at young and mature stages have a great potential as antimicrobial agents.

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