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Phytochemical Screening and Toxicity Activities of *Eleiodoxa conferta* Plant Extracts

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Abstract. Researchers are actively exploring the locally available fruits that may use for therapeutic remedies. *Eleiodoxa conferta* is an underutilized Malaysian fruit. This study was conducted to examine bioactive compounds and toxicity activities of different parts of *E. conferta* using 100% ethanol, 50% ethanol, and water for extraction. Results showed that Ethanol (50% v/v) was the best extraction solvent. The highest and lowest yield shown in the flesh extract and seed extract at 39.247% and 4.89%, respectively. The peel of *E. conferta* always showed higher Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) as compared to other parts of the plant. Phytochemical compounds such as flavonoids, phenol, tannin, to name a few, were abundantly present in most extractions. The toxicity screening revealed that the toxicity level was high in the seed part of *E. conferta* while low toxicity shown in the flesh of the fruit. Overall, this fruit is rich in bioactivities. Further studies can be done to uncover its true potential application, especially the application of those bioactive components as antimicrobial agents in preserving food.

1. Introduction

Plant is a wonderful living thing in Earth. Plant source food is a constituent of human diets. Besides add beauty, it also gives significant medicinal value to human. Many research has been done on plants and their potential in remediation has been scientifically proven [1-4]. Yet, there are many plants have yet to be discovered and these plants could have great significance effect in therapeutic remedies. *Eleiodoxa conferta*, or popularly called *kelubi* or *salak* is one of the cultivations where its potential is underutilized. It is a plant found in Southeast Asia. Studies had shown that the plant has an undiscovered potential [1]. It belongs to the family of Arecaceae which is abundantly found in peat swamp forest. Dense thickets are seen due to the clustering palm of the plant [5]. The fruits has scaly skin, and it turns reddish when mature [1]. *E. conferta* is a popular fruit among the local community. The local community uses the ripen fruits as a substitute for sour fruit for vegetable spices.

Recently, it was found that *E. conferta* has some advantageous properties. There are many studies done on the antioxidant activity as well as other bioactive properties of *E. conferta*. However, limited

information are available especially on the potential bioactive components and toxic activity of various parts of *E. conferta*. Since *E. conferta* has the prospective quality as an essential source for developing new drug molecules, this study was performed to screen and determine the selected bioactive contents and toxicity activities of different parts (peel, flesh, and seed) of the fruit using various extraction solvents (100% ethanol, 50% ethanol, and water).

2. Materials and Methods

2.1. Materials

The apparatus and equipment employed in this research were oven, beakers (100 mL, 250 mL, 500 mL), conical flask (1000 mL), measuring cylinder (10 mL, 50 mL), round bottom flask 29/32 (500 mL and 1000 mL), microcentrifuge tube (2 mL), glass rod, dropper, filter funnel, rubber gloves, hot plate, weighing balance, pipette (1000 mL), micropipette tubes, test tubes, and boiling tubes.

2.2. Methods

2.2.1. Sample Preparation. Ripped *E. conferta* fruits, harvested in Kelantan were de-branched and cleaned with tap water prior to segregating them into three biomass components (peel, flesh, and seed). They were cut into small pieces before dried them separately in oven at 50°C. They were then grounded into a fine powder and stored in a storage container with airtight lid for subsequent analysis.

2.2.2. Extraction. Around 200 g of different parts of *E. conferta* in powder form were extracted by solvents100% ethanol, 50% ethanol, and water according to Chowdhury et al.'s method [2]. The process was done by placing the fine powder with the solvent in a closed system for three days under room temperature (25°C) with frequent agitation with spatula until the soluble matter had dissolved. The macerates were then filtered twice with muslin, and the marc was pressed. Later, the filtrate was subjected to a rotary evaporator to obtain the crude extracts at 45°C (ethanol solvent) and water at 100°C (water), respectively. The crude extract, collected in the round bottom flask was then transferred into a beaker. The crude extracts were kept for bioactivity assay.

2.2.3. Determination of Total Phenolic Content (TPC). Method of Folin-Ciocalteu [2] was applied for the assay of TPC. A total of 200 µL of extract solution (1 mg/mL) and 500 µL of Folin-Ciocalteu reagent were added, and the contents were mixed thoroughly. After 3 minutes, 2 mL of 20% Na2CO3 was added, and then the mixture was allowed to stand for 2 hours at room temperature in the dark. The absorbance was measured at 765 nm using spectrophotometer. The gallic acid calibration standard was prepared based on [4] method with slight modification. The gallic acid solution was then analysed with the Folin-Ciocalteu method. A calibration curve was plotted for calculating the TPC of the samples.

2.2.4. Determination of Total Flavonoid Content (TFC). Aluminium chloride colorimetric method was used to determine the flavonoids into the defined extracts of *E. conferta* fruit. The first step was about 1.5 mL methanol, and 0.1 mL aluminium chloride (10%) were added into a test tube containing 0.5 mL of extracts (1 mg/mL), followed by 0.1 mL of potassium acetate solution (1M), and 2.8 mL of distilled water and then mixed well. After incubation at room temperature (25°C) for 30 minutes, the absorbance was measured at 415 nm using a Spectro (ThermoFisher Scientific, model 4001/4) spectrophotometer. Quercetin from Sigma Aldrich (6.25 to 100 µg/mL) was used to make the standard calibration curve. A standard calibration plot was constructed to determine the concentration of flavonoids in the extract. The standard test was performed in triplicate, and the concentration of flavonoid in the extracts, expressing in mg quercetin Equivalent/g of extract was calculated from the calibration plot [3].

2.2.5. Phytochemical Screening. Phytochemical screening involving Dragendoff test, Sodium Hydroxide test, Lead acetate test Foam test, Salkowski test, Ferric Chloride Test were performed to detect the presence of different phytochemicals on the different parts of *E. conferta* fruit extracts.

2.2.6. Toxicity Screening. Brine Shrimp Lethality Assay (BSLA) was used to test the toxicity at triplicate. A concentration of 500 and 1000 µg/mL extracts of *E. conferta* in sample vials A, B, and C, which contained brine shrimp nauplii with 500 µg/mL, respectively. After 24 hours, dead and alive nauplii in the tube were counted and recorded. Distilled water introduced as control sample together with nauplii was also prepared to ensure the mortality effect of plant extracts. The percentage of mortality (%) of the nauplii in different concentration were calculated [4]. The percentage of mortality (%) was calculated using Equation 1.

$$\text{Percentage of mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Total nauplii}} \times 100\ \% \quad (1)$$

2.2.7. Statistical Analysis. All analyses were expressed as mean ± standard deviation of triplicate measurements, subjected to two-way ANOVA and Tukey's test by Minitab version 18 at 0.05 confidence level.

3. Result and Discussion

3.1. Extraction Yields

E. conferta flesh extracted by 50% ethanolic extract exhibited the highest yield among others; while the seeds extracted by water shown least efficient as compared to others (Table 1). In this study, different parts of *E. conferta* extracts were acquired using ethanol (50% and 100%) and water. From Table 1, the highest extraction yield was found in the flesh sample ranged from 16.367 ± 0.21% for 100% ethanol extract to 39.247 ± 0.01% for 50% ethanol extract whereas the lowest extraction yield was found in the seed sample ranged from 4.89% ± 0.01% for water extract to 26.967 ± 0.06% for 50% ethanolic extracts. Note that the 50% ethanolic solvent gives the highest yield while water gives the lowest extraction yield.

Table 1. Extraction yield (%) of different parts of *E. conferta* using different extraction solvents.

Samples	Extraction yield (%)
Peel ethanolic extract	18.303 ± 0.01^{E}
Peel 50% ethanolic extract	34.767 ± 0.06^{B}
Peel water extract	9.5700 ± 0.01^{H}
Flesh ethanolic extract	23.467 ± 0.06^{D}
Flesh 50% ethanolic extract	39.247 ± 0.01^{A}
Flesh water extract	16.367 ± 0.21^{F}
Seed ethanolic extract	11.487 ± 0.01^{G}
Seed 50% ethanolic extract	26.967 ± 0.06^{C}
Seed water extract	4.890 ± 0.01^{I}

Data were represented by mean ± standard deviation (n=3). Means that did not share a same letter were significantly different at $p < 0.05$ using Tukey's test.

There are influences of different parts of fruit residues on extraction yield and observed that residue type has more influential over the solvent system on extraction yield [6]. Extraction yield, however, does not sorely rely on the sample but also the solvent used for the extraction. To recover polyphenols from plant residues, polar solvents are widely employed. The polarity of the solvent for the different parts of the fruit affects the efficiency of the extraction and the activity of the obtained extracts [7]. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Nevertheless, samples extracted by 50% ethanol has the highest extraction yield. This is because extraction yield will increase if the water concentration in the organic solvent is enhanced [8].

Water is rather polar with its polarity index of 9.0. Inclusion of water can surely inflate the solubility of hydroxylated and methoxylated compounds. This in turn can result in improvement in total yield of extraction. Hence, the combined use of water and organic solvent may facilitate the extraction of chemicals that are soluble in water or organic solvent. An extraction solvent is that which can obtain extracts with high yield but with minimal changes to the functional properties of the extracts required. Several studies have reported variations in the biological activities of extracts prepared using different extraction techniques [9].

3.2. Total Phenolic Content (TPC)

TPC was measured using the Folin-Ciocalteu method. The TPC values of the extracts obtained from the calibration curve of gallic acid standard (Table 2). The highest TPC was taken in the ethanolic peel extract (80.653 mg/mL) whereas the lowest TPC value was taken in the water extract of seed (17.99 mg/mL). Among the different extraction solvents system, 50% ethanol extracts contained the highest TPC, followed by order of ethanol > water. The peel sample had the highest TPC while seed sample showed a low content of TPC. The TPC value decreased ($p<0.05$) in the range of peel > flesh > seed.

Table 2. TPC in different parts of *E. conferta* fruit by using different extraction solvents.

	Total Phenolic Content (GAE mg/g)		
	Ethanol	**50% ethanol**	**Water**
Peel	69.787 ± 0.006^{A}	80.653 ± 0.075^{D}	46.757 ± 0.029^{G}
Flesh	56.570 ± 0.069^{B}	78.780 ± 0.069^{E}	34.447 ± 0.075^{H}
Seed	47.820 ± 0.069^{C}	68.320 ± 0.069^{F}	17.990 ± 0.029^{I}

Data were presented by mean ± standard deviation (n=3). Means that did not share a same letter were significantly different at $p < 0.05$ using Tukey's test. Data were expressed as mg of gallic acid equivalent (GAE) per 10 mg of the sample.

The recovery and yield of phenolics in an extract are influenced by the type and polarity of extracting solvents and physical characteristic of the samples [10]. The different parts of the fruits may have varying physical characteristics mainly the sensory attributes due to different chemical composition. For instance, the variation in the content of reducing agents such as ascorbic acid, minerals and carotenoids in the samples.

It is found that the ethanol has been extensively used to extract bioactive compounds from various plants and plant-based foods like fruits, vegetables and so on but considering water extract, some constituents such as phenolic compounds have less solubility in water. The content of non-phenol compounds, for example, carbohydrates and terpene are more detected in water extracts than in other extracts.

The difference in TPC content may also be caused by the possible complex formation of some phenolic compounds that are more soluble in ethanol. These phenolic compounds may possess more phenol groups or have higher molecular weights than the phenolics in the water extracts. Apparently, the result in this study showed that 50% aqueous solvent gave a better extraction of TPC compared to those of pure solvent [11]. This is because it has a high extraction efficiency due to the difference in polarities of which affects the solubility of chemical constituents in the sample.

3.3. Total Flavonoid Content (TFC)

TFC values of the extracts (Table 3) were measured using quercetin standard method. The highest amount of flavonoid content was found in the peel followed by the flesh, and seed. It implied that the peel extract has more potentially effective compounds. Similar to phenolic content, 50% ethanolic peel extract had higher flavonoid content compared to that of the other parts of the fruits (91.00 QE/10g) while the water extract of seed sample indicated the lowest TFC value (7.95 QE/10g). It was observed that the effect of solvents on TFC is similar to that on TPC. The highest TFC was obtained in the 50% ethanol extract, followed by the 100% ethanol and water extract.

Table 3. TFC in different parts of *E. conferta* fruit by using different extraction solvents.

	Total Flavonoid Content (QE mg/g)		
	Ethanol	**50% ethanol**	**Water**
Peel	69.140 ± 0.520^{A}	91.000 ± 0.300^{C}	47.667 ± 1.518^{E}
Flesh	69.067 ± 0.306^{B}	88.017 ± 0.306^{C}	30.123 ± 0.300^{G}
Seed	36.857 ± 0.029^{D}	62.227 ± 0.306^{F}	7.950 ± 0.277^{H}

Data were represented by mean ± standard deviation (n=3). Means that did not share a same letter were significantly different at $p < 0.05$ using Tukey's test. Data are expressed as mg of quercetin equivalent (QE) per 10 mg of the sample.

From the results, it can be proved that there are possible pharmacological effects present in different parts of the *E. conferta*, but the intensity of the reaction would be varying due to different concentration of TFC. Flavonoids are effective antioxidants in preventing the risks of developing cancer, cardiovascular disease, etc. Polyphenolic nature contained in the flavonoids make them an effective free radical scavenger, in which injurious free radicals like super oxide and hydroxyl radicals can easily be scavenged [12]. Thus, there is advantageous use of different parts of the *E. conferta* fruit.

It is found that flavonoids are a widely distributed group of plant phenolic compounds which are occurring virtually in all parts of the fruit. Flavonoids possess many biochemical properties that contribute to the human and animal diet [13]. Synthesizing flavonoids by humans and animals is impossible. Thus, flavonoids found in animals are of plant origin rather than being biosynthesized in situ. These bioactive compounds in food are generally responsible for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes. The estimation of the dietary intake of flavonoids is difficult due to wide varieties of available flavonoids, the extensive distribution in various plants, and also the diverse consumption in humans [14].

3.4. Phytochemical Screening

Qualitative phytochemical screening was done to detect the presence of different phytochemicals on the different parts of *E. conferta* fruit extracts and the results were tabulated in Table 4.

Table 4. Phytochemical screening of different parts of *E. conferta* fruit using various solvents.

	Types of Samples								
	PE	P50E	PW	FE	F50E	FW	SE	S50E	SW
Acidic compounds	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	−	+	+	−	+	−	−
Betacyanin	+	+	+	+	+	+	+	−	−
Flavonoids	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	−	+	+	−	+	+	−
Saponin	−	−	−	−	−	−	−	−	−

Quinones	+	+	−	+	+	−	+	+	−

Ethanolic peel extract (PE), 50% ethanolic peel extract (P50E), Peel water extract (PW),
Ethanolic flesh extract (FE), 50% ethanolic flesh extract (F50E), Flesh water extract (FW),
Ethanolic seed extract (SE), 50% ethanolic seed extract (S50E), Flesh seed extract (SW).
(+) and ((−) indicate the presence and absence of phytochemical compounds, respectively.

Different parts of *E. conferta* fruit extracts contained some phytochemical compounds including acidic compounds, alkaloids, flavonoids, phenol, tannin, terpenoids, quinones, except saponins. Meanwhile, some of the phytochemical compounds were absent in the water extracts of the samples. The ethanolic extracts of peel, flesh and seed had shown the presence of acidic compound, alkaloids, Betacyanin, flavonoids, phenol, tannin, quinones, and terpenoids. Saponin was not found in all the extracts. The efficacy of ethanolic extracts showing the presence of more phytochemicals is high compared to that of the water extracts of the different parts of the plants [15]. This could be due to the poor solubility of these phytochemicals in water. Thus, it concludes water is not eligible to be used as phytochemical extraction solvent from different parts of *E. conferta* fruit.

3.5. Toxicity Screening

Among the different parts of *E. conferta*, the lowest and highest percentage of mortality was found in the flesh and seed samples, respectively (Table 5). Besides, the ethanolic extracts had the highest toxic compared to that of the 50% ethanolic and water extracts.

Table 5. The percentage of brine shrimp lethality after treated with different extraction solvents on the different parts of *E. conferta* fruit using different concentration (ppm) for 24 hours.

		Percentage of Mortality (%)		
	Concentration	**Ethanol**	**50% ethanol**	**Water**
Control	-	0.00	0.00	0.00
Peel	500 ppm	23.33	16.67	13.33
	1000 ppm	26.67	23.33	16.67
Flesh	500 ppm	16.67	13.33	6.67
	1000 ppm	23.33	16.67	10.00
Seed	500 ppm	66.67	53.33	36.67
	1000 ppm	70.00	56.67	43.33

Although the extraction solvents used were different, the toxicity level showed a low percentage of mortality for the low concentration (500 ppm) and a high percentage of mortality for the high level of concentration (1000 ppm). The results showed that the different parts of the sample with different extraction solvents at different concentration levels will impact the mortality and larval toxicity. The flesh sample has less toxicity ($p<0.05$) as compared to other parts of the samples. Hence, the flesh sample is considered safe to consume or for other uses. Meanwhile, the ethanolic extract has a high toxicity level, followed by the 50% ethanolic extract and water. This may be attributed by the content of ethanol in the extract since the probability of survival of the brine shrimp is less in the organic solvent. Thus, brine shrimp has a high probability of survival in the water extracts. The chemical constituents that might be acidic could also contribute to the fruit's toxic content. This statement is in agreement with the past studies conducted by [1] which reported that the *E. conferta* fruit extracts at different maturity stages contain three types of organic acids (ascorbic acid, mallic acid, and oxalic acids).

4. Conclusion

This study was conducted to determine phytochemicals from the peel, flesh and seed of *E. conferta* Toxicity levels of the plant was also investigated. The results revealed that the mixture of solvent like 50% ethanol as extraction solvent led to the maximum extraction of yield in terms of TFC, and TPC. The flesh extract showed the highest yield whereas the lowest yield belonged to the seed extract. The highest TPC and TFC were found in the peel. Phytochemical screening showed that the presence of bioactive compounds were significantly different in different parts of *E. conferta.* Nevertheless, the toxicity screening ensured that the toxicity level is high in the seed part while low toxicity was shown in the flesh of the fruit. In a nutshell, the bioactive compounds found in the fruit are known to be biologically active and therefore may provide medicinal advantages beyond basic nutrients.

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