Chapter in Book

Phytochemical Screening Analysis of *Eleutherine Bulbosa* Extracts and Its Potential as Antibacterial and Antioxidant Activity

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Abstract: *Eleutherine bulbosa or bawang dayak in Malay is a well-known member of the Iridaceae family with a wide range of therapeutic possibilities. The Dayak population has historically utilized the Eleutherine bulbosa as a folk remedy to treat a variety of illnesses including diabetes, breast cancer, nasal congestion, and infertility issues. E. bulbosa is a revolutionary in both medicine and drug discovery and development due to its naturally occurring antioxidants and antibacterial constituents. However, there were limited studies on the E. bulbosa in Malaysia. Therefore, the present study was conducted to identify the phytochemical constituents in E. bulbosa extract using preliminary phytochemical screening analysis, to investigate the antibacterial activity in E. bulbosa extracts using the disc diffusion method and to examine the antioxidant abilities in E. bulbosa extract using the DPPH radical scavenging assay performance. The dried bulbs of E. bulbosa was extracted using maceration extraction in 95% ethanol. The percentage yield obtained was 2.22%. Meanwhile, the phytochemical screening showed the presence of flavonoids, alkaloids, tannins, and saponins. For antioxidant activity, the E. bulbosa extract inhibits scavenging activity, lower than standard reference, ascorbic acid. Thus, it proved that E. bulbosa bulb extract has good antioxidant activity and potential to acts as natural antioxidants. As for antibacterial study, the present study showed that the E. bulbosa extract was active against the gram negative (E. coli) and resistance against gram positive (B. licheniformis). The inhibition zones of E. bulbosa extract against E. coli in three different concentrations (1.25%, 5%, 15%) were 9.7 mm, 12.7 mm, and 17.0 mm, respectively, while there was no inhibition zone against B. licheniformis. This might be due to the error when conducting the antibacterial study.*

Keywords: Eleutherine bulbosa, antioxidant, antibacterial

1. INTRODUCTION

Eleutherine bulbosa or known as *lagrimas de la virgin* ('tears of virgin') is an herbaceous, everlasting flowering plant, mostly found in the Amazon rainforest, widely in South America and Africa region (Insanu *et al.*, 2014), Mexico, and several parts in Africa and Asia, such as central Borneo, Indonesia. *Eleutherine bulbosa* or bawang dayak in Malay, was originally under the Iridaceae family, a genus of Eleutherin, and it also lies in the order of Asparagles. *E. bulbosa* has white or pink and five to six petals of flowers and the length of the leaves is approximately 25 cm. *E. bulbosa* is a perennial rhizomatous flowering plant, with 5 to 7 cm long and 3 to 4 cm widths of the bulb, and the bulb is almost the same as an onion, in which the bulb of *E. bulbosa* has purple-red wine color, long and ellipsoid shape, compared to an onion which has round and spherical shape (Kamarudin *et al.*, 2021).

The bulb of *E. bulbosa* has a variety of therapeutic potential that can be used to treat several diseases such as cancer, heart, uric acid, fertility problems, diabetes, and nasal congestion (Kamarudin *et al.*, 2021). Phytochemical compounds from plant extract can provide as natural antioxidants to reduce reactive oxygen species (ROS) and shield against oxidative stress (Claudya *et al*., 2021). The antioxidant activity of *E. bulbosa* extract demonstrated its potential as a source of natural antioxidants due to the presence of bioactive compounds such as flavonoids, alkaloids, saponins and tannins (Shi *et al*., 2019). The bioactive compounds such as flavonoids, phenols, glycosides, triterpenoids, and anthraquinones from *E. bulbosa* extract also may contributing in antibacterial activities towards the gram-positive and gram-negative bacteria (Novaryatiin *et al.*, 2019). Thus, the present study was conducted to identify the phytochemical compounds of ethanolic *E. bulbosa* extract as well as its potential as antioxidant and antibacterial activity.

2. METHOD & MATERIAL

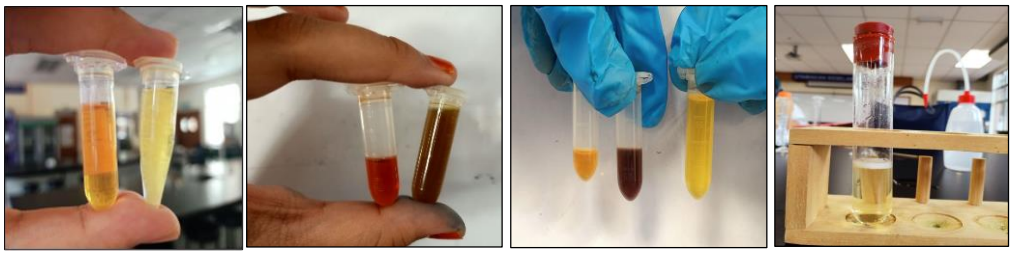
The fresh bulbs of *Eleutherine bulbosa* were washed properly to remove all the dust debris and soil with double distilled water and dried in a hot air oven at 60°C for 48 hours. The bulbs were then made into powder using an electric blender. To prepare the extract, the dried powdered bulbs was extracted using maceration technique with 95% ethanol as the solvent for 24 hours. The filtrate was then concentrated to evaporate the ethanol completely using rotary evaporator. The percentage yield for the extract was calculated with respect to the starting material. The crude ethanolic bulbs extract was screened qualitatively for the phytochemical constituents such as alkaloids, flavonoids, tannins, and saponins utilizing standard methods of analysis (Novaryatiin *et al.*, 2019). The antioxidant activity of bulbs extract was then examined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay by using ascorbic acid as standard references (Pratiwi *et al.*, 2013). The antibacterial activity of bulbs extract was done in comparisons with standard antibiotic gentamicin as positive control and distilled water as negative control using disc diffusion methods against *Bacillus licheniformis* as gram-positive and *Escherichia coli* as gram-negative bacteria. The zone of inhibition (mm) was measured in each agar to identify the potential bulbs extract to inhibit the growth of bacteria.

3. FINDINGS

The maceration extraction stage using 95% ethanol is the first step in analysing the components in *Eleutherine bulbosa* extract. The crude extract of *E. bulbosa* has a viscous liquid, rubbery colour, a familiar odour, and a bitter flavour. The percentage yield of the bulbs extract was 2.22%.

3.1 Phytochemical screening analysis

The preliminary phytochemical screening of ethanolic extract of *E. bulbosa* was studied in a specific qualitative test, which revealed the presence of alkaloids, flavonoids, tannins, and saponins. Table 1 shows the qualitative analysis of phytochemicals constituents in *E.e bulbosa* ethanolic extract. The observation results of each test showed in Figure 1.



(a) (b) (c) (d)

Figure 1. Results of preliminary phytochemical test (a) alkaloids (b) flavonoids (c) tannins (d) saponins

Table 1. Qualitative analysis of phytochemicals content in *Eleutherine bulbosa*'s ethanolic extract

Phytochemicals	Test	Reaction	Results
Alkaloids	Mayer's test	Turbidity and no formation of precipitate	+
Flavonoids	Shinoda test	Intensive yellow colour, and converted into yellowish-colourless when added few drops of acetic acid	+
Tannins	Ferric chloride test	Green-black colour	+
Saponins	Foam test	Stable foam layer	+

3.2 Antioxidant activity

For the antioxidant test, the ethanolic extract of *E. bulbosa* was examined by using the DPPH scavenging assay test. Based on Figure 2, when the samples reacted with the DPPH solution after 30 minutes of incubation under a dark room, the colour of the samples were changed from purple to paler. Table 2 shows the percentage inhibition of *E. bulbosa* extract and ascorbic acid for the antioxidant activity. From the result obtained, the percentage of inhibition increases as the concentration of each extract sample and standard sample increases. Besides, the percentage of inhibition for *E. bulbosa* ethanolic extract comparable to the standard reference of ascorbic acid. Thus, it proved that *E. bulbosa* extract has higher antioxidant activity and potential to act as natural antioxidants.

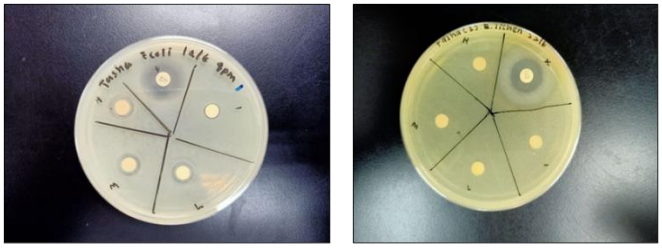
Figure 2. Color changes of samples extract after addition of DPPH solution

Table 2. Percentage inhibition of *Eleutherine bulbosa* extract and Ascorbic acid (%)

Concentration (mg/ml)	Ascorbic acid %	Concentration (mg/ml)	*Eleutherine bulbosa* %
2	20.16	**20**	30.08
4	29.12	**40**	32.64
6	31.36	**60**	37.92
8	34.08	**80**	42.40
10	43.68	**100**	48.32

3.3 Antibacterial activity

Figure 3 showed the zone of inhibition for antibacterial activity using *E. bulbosa* extract for both gram-positive (*Escherichia coli)* and gram-negative bacteria (*Bacillus licheniformis)*. Based on Figure 3, the gram-negative bacteria do have zone of inhibition for each concentration, while for gram-positive bacteria do not have any zone of inhibition. This might be due to the incorrect settings and caused the bacteria resistance to the extract. Table 3 showed the zone of inhibition diameter of *E. bulbosa* extract of against gram-positive and gram-negative bacteria including 6 mm disc paper.



(a) (b)

Figure 3. Inhibition zone from antibacterial activity assay using disc diffusion method (a) *Escherichia coli* (b) *Bacillus licheniformis*

Table 3. Inhibition zone diameter of ethanol extracts of the *Eleutherine bulbosa* against bacteria by disk diffusion method (including 6mm disc paper)

Strains	Diameter of the Inhibitory Zones (mm)			Positive Control	Negative control
	Concentration (%)				
	1.25	**5.0**	**15.0**		
Escherichia coli	9.7	12.7	17.0	21.0	7.0
Bacillus licheniformis	-	-	-	19.0	7.0

4. DISCUSSION

For the plant extraction process, the secondary metabolites in the *E. bulbosa* sample were taken into consideration when selecting the maceration procedure, due to avoid losing the thermolabile chemicals found in *E. bulbosa* which are typically lost during maceration due to the elevated temperatures (Wicaksono *et al.*, 2018). The 95 % ethanol was used as solvent due to it has short carbon chain and was able to dissolve both polar and nonpolar secondary metabolites (Mustarichie *et al.*, 2020).

According to Table 1, alkaloids, flavonoids, tannins and saponins were shown the positive results in phytochemical screening analysis of the *E. bulbosa* extract. Ravi, 2016 stated that in the plant's

defensive mechanism against infections, saponins functions as a chemical barrier and can result in the release of some proteins and enzymes from bacterial cells. Tannins was responsible for the noticed antibacterial properties, since tannins compound was available in *E. bulbosa* (Novaryatiin, 2019)

For the antioxidant test, the ethanolic extract of *E. bulbosa* was performed by using DPPH scavenging assay test. Previous research has shown that *E. bulbosa* extract has a substantial antioxidant activity, implying that the extract is extremely likely to block the free radicals (Agustin *et al*., 2016). The antioxidant test was conducted on five concentrations: 20, 40, 60, 80, 100 mg/ml for the extracts, and 2, 4,6,8,10 mg/ml for the ascorbic acid, respectively. Due to ascorbic acid is regarded as one of the most potent antioxidants, the concentration of the sample and the standard varies. Furthermore, the strong suppression of antioxidant activity makes the DPPH free radical scavenging assay extremely sensitive, allowing it to detect ascorbic acid at low concentrations (Ahmad and Abdullah, 2013).

Based on the result in Table 2, the *E. bulbosa* ethanolic extract does have higher antioxidant activity comparable to the standard of ascorbic acid. As the concentration of each extract sample and standard sample increases, the percentage of inhibition also increases. Hence, due to its antioxidant action, *E. bulbosa* extract might have the ability to prevent the free radical formation. Some studies stated that the presence of flavonoids, phenolics, and tannins contributed to the antioxidant activity because they are phenolic compounds. These phenolic substances are capable of donating hydrogen atoms to decrease the DPPH radicals to a more stable form, which it could do the placement of phenolic hydrogen in phenolic compounds and affects their ability to scavenge free radicals.

Antimicrobial activity refers to all active principles that inhibit the growth of bacteria, prevent the formation of microbial colonies, and may destroy microorganisms. *Eleutherine bulbosa* are frequently used as an antibacterial agent to treat numerous infectious disorders in traditional medicine (Wicaksono *et al*., 2018). All the concentration *E. bulbosa* extract are intermediate form in *E. coli*, while resistance form in *B. licheniformis*. It proves that the higher concentration of extracts, the higher yield of zone inhibition towards the bacteria. Some researchers also stated that increased concentrations would lead to a larger composition of bioactive chemicals in the extract, which would result in a stronger ability to suppress bacterial growth (Harlita *et al*., 2018).

The *E. bulbosa* extract contains secondary metabolite substances such as flavonoids, tannins, saponins, quinones, steroids, and triterpenoids (Munaeni *et al*., 2019). Many phenolics and flavonoids chemicals found in plants extracts have the potential to prevent the growth of both gram-negative and gram-positive bacteria (Negi, 2012). Hence, it manifested that in preliminary phytochemical test and antioxidant test, flavonoids do shows in positive side and contributes a lot in each test, including antibacterial activity. Not only that, tannins also play a part in each test, especially in antibacterial activity. Several studies stated that tannins can bind to proline-rich proteins and prevent the creation of new proteins. Other than tannins and flavonoids, alkaloids able to harm the cell wall, which are known to have potential as an antibacterial agent. The bacterial cell becomes susceptible to osmotic pressure because it lacks peptidoglycan components, and a high osmotic pressure will cause the bacterial cell to lyse (Harlita *et al.*, 2018).

5. CONCLUSION

As conclusion, the present study has been conducted to enhance the study of *Eleutherine bulbosa* ethanolic extract. The *E. bulbosa* was successfully extracted out. From the preliminary phytochemical screening analysis, the extracted *E. bulbosa* revealed the presence of alkaloids, flavonoids, tannins, and saponins. As for antioxidant activity, the *E. bulbosa* extract can act as a natural antioxidant due to the ability of *E. bulbosa* extract *to* scavenge DPPH radical as good as ascorbic acid. Lastly, the *E. bulbosa*

extract also has the ability to inhibit the gram-negative bacteria, thus potential to act as antibacterial agent. Therefore, the benefit of *E. bulbosa* extract has potential to be commercialized as one of the organic ingredients for pharmaceutical or cosmeceutical products.

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