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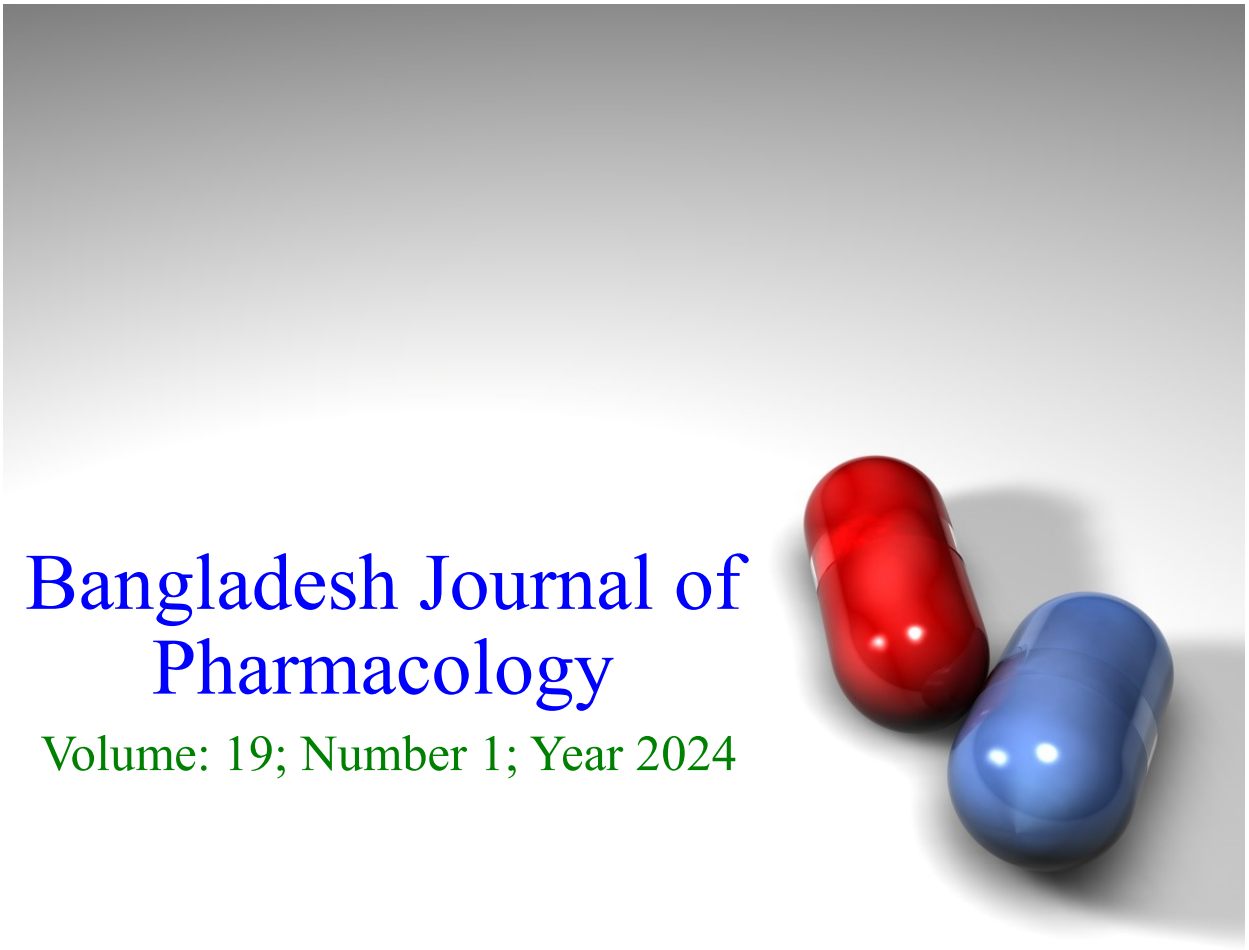

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Letter to the Editor

Anti-diabetic activity of *Calathea anulque*

Sir,

Calathea anulque is commonly known as the rattlesnake plant and originates from the tropical rainforests of South America. The foliage of this plant tends to be lanceolate in shape and color, with a distinctive rattlesnake-like pattern and a striking crimson backside. Because of the color variations and leaf patterns, the genus Calathea is widely used as an ornamental plant (Efzueni Rozali et al., 2014). *C. warscewiczii* is used in traditional Panamanian medicinal uses to treat diseases such as painful wounds and inflammation (Gupta et al., 2005). One of the plant genera, *C. panamensis,* exhibits potential anti-diabetic activity using the glucose 6-phosphatase assay and significant anti-radical activity using the DPPH trapping technique (Rodríguez et al., 2008). This study aims to evaluate the anti-diabetic properties and *in vitro* antioxidant effect of *C. anulque* leaf extracts.

The fresh and healthy plant leaves were obtained from the Thotta Kalai nursery garden, ECR, Chennai, Tamil Nadu, India. The collected leaves were washed under running tap water to remove dust particles and then rinsed with Milli-Q water. Subsequently, the leaves were left to air dry in the shade for a week without direct sunlight exposure. Using an electric blender, the dried leaves were ground into a fine powder. The extraction process involved the use of two different solvents, such as methanol and ethyl acetate. A sterile 250 mL Erlenmeyer flask was filled with 100 mL of each solvent, and 1 g of finely ground leaf powder was added. The flasks were parafilm-sealed and kept in a shaker for 48 hours at 120 rpm. The concentrates were extracted by filtering through Whatman filter paper No. 1 after 48 hours. The filtrates were evaporated using a rotary vacuum to obtain dried crude extracts.

The alpha-amylase method was carried out to determine the anti-diabetic activity of *C. anulque*. The crude extracts were dissolved in Milli-Q water to produce four different concentrations (25, 50, 75, and 100 µg/mL). As a blank, distilled water was used. As a control, a mixture of 1 µL distilled water and 500 µL amylase solution was used. About 500 µL of plant extract and amylase solution were combined in a 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M sodium chloride) and incubated at 25°C for 10 min. After pre-incubation, 500 µL of 1% starch solution was added to each tube at 5 sec intervals in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride). The reaction mixture was incubated for 10 min at 25°C. 1 mL of DNSA color reagent was added to stop the reaction. These test tubes were heated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was diluted with 10 mL of distilled water, and the absorbance was read at 540 nm using a UV-Vis spectrophotometer (Shah et al., 2020). The reference standard was acarbose. The percentage of inhibition was calculated using the following formula:

Percentage of amylase inhibition = Control absorbance – Test absorbance / Control absorbance × 100

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out to determine the antioxidant activity of *C. anulque* crude extracts according to the protocol (Al-Hadhrami and Hossain, 2016). About 1 mL of plant crude extracts of varying concentrations (25, 50, 75, and 100 µg/mL) were combined with 2 mL of 0.1 mM DPPH solution. Gallic acid was used as a reference standard. As a control, a mixture of 1 mL of methanol and 2 mL of DPPH solution was used. The mixture was shaken well and incubated in the absence of light (dark conditions) for 45 min. The absorbance of the reaction was measured at 517 nm in a UV-Vis spectrophotometer against the blank (methanol). The formula below was used to calculate the percent inhibition.

%Scavenging effect = Control absorbance - Test absorbance / Control absorbance × 100

In α-amylase assay, *C. anulque* extracts showed significant inhibitory activity. Although compared to methanolic extract, ethyl acetate extract showed more inhibition, with 87.3% at 100 µg/mL, followed by 83.8% at 75 µg/mL concentration. The standard acarbose showing 89.5% anti-diabetic activity at 100 µg/mL concentration (Figure 1A).

In comparison to the ethyl acetate extract, the *C. anulque* methanolic extract exhibits the highest radical scavenging effect of about 79.7% at 100 µg/mL, followed by 76.2% at 75 µg/mL. whereas the ethyl acetate extract of *C. anulque* exhibited a potential antioxidant activity of 50.4% at a 100 µg/mL concentration. Ascorbic acid was taken as a standard, showing 96.0% antioxidant activity

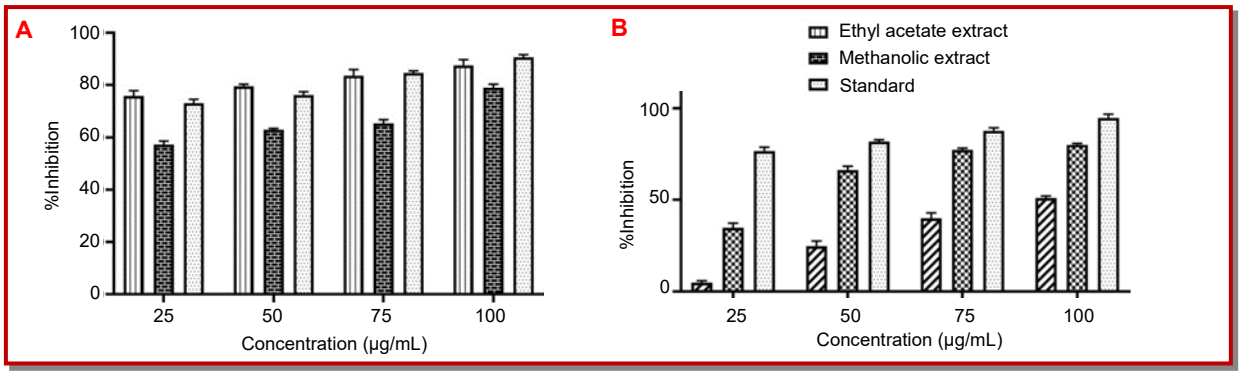

Figure 1: α-Amylase activity [anti-diabetic effect, (A)] and 2,2-diphenyl-1-picrylhydrazyl assay [antioxidant effect, (B)] of different extracts of *Calathea anulque*

Table I

Relation between anti-diabetic and antioxidant effects of different plant extracts

Names	Anti-diabetic effect (%)	Antioxidant effect (%)	References
Paederia foetida (5000 µg/mL)	46.2	63.8	Ghosh et al., 2023
Sorghum halepense (2000 µg/mL)	62.4	74.1	Shah et al., 2017
Elaeocarpus ganitrus (200 µg/ml)	53.3	78.9	Talukdar et al., 2017
Abelmoschus esculentus (250 µg/mL)	91.6	28.2	Ahmed and Kumar, 2016
Hygrophila auriculata (100 µg/mL)	78.9	53.2	Rastogi et al., 2014
Calathea anulque (100 µg/mL)	87.3	79.7	Present study
Rauwolia serpentine (0.5 µg/mL)	58.4	84.6	Chauhanet al., 2017

at 100 µg/mL concentration (Figure 1B).

C. anulque exhibits significant levels of antioxidant and anti-diabetic properties in its methanolic and ethyl acetate extracts.

Table I shows the data of some plant extracts with anti-diabetic and antioxidant effects for comparison. One might think of critical role of antioxidants in the treatment of diabetes. Some plant extracts exhibit parallel trends in both anti-diabetic and antioxidant effects, exemplified by *Calathea anulque*, others, such as *Abelmoschus esculentus*, shows a divergence with a substantial anti-diabetic effect but a relatively lower antioxidant effect.

Limitation of this study was as follows: a) the anti-diabetic effect was assessed by α-amylase activity, b) *in vivo* laboratory animal model was not done, and c) the active component within the extracts were not identified.

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