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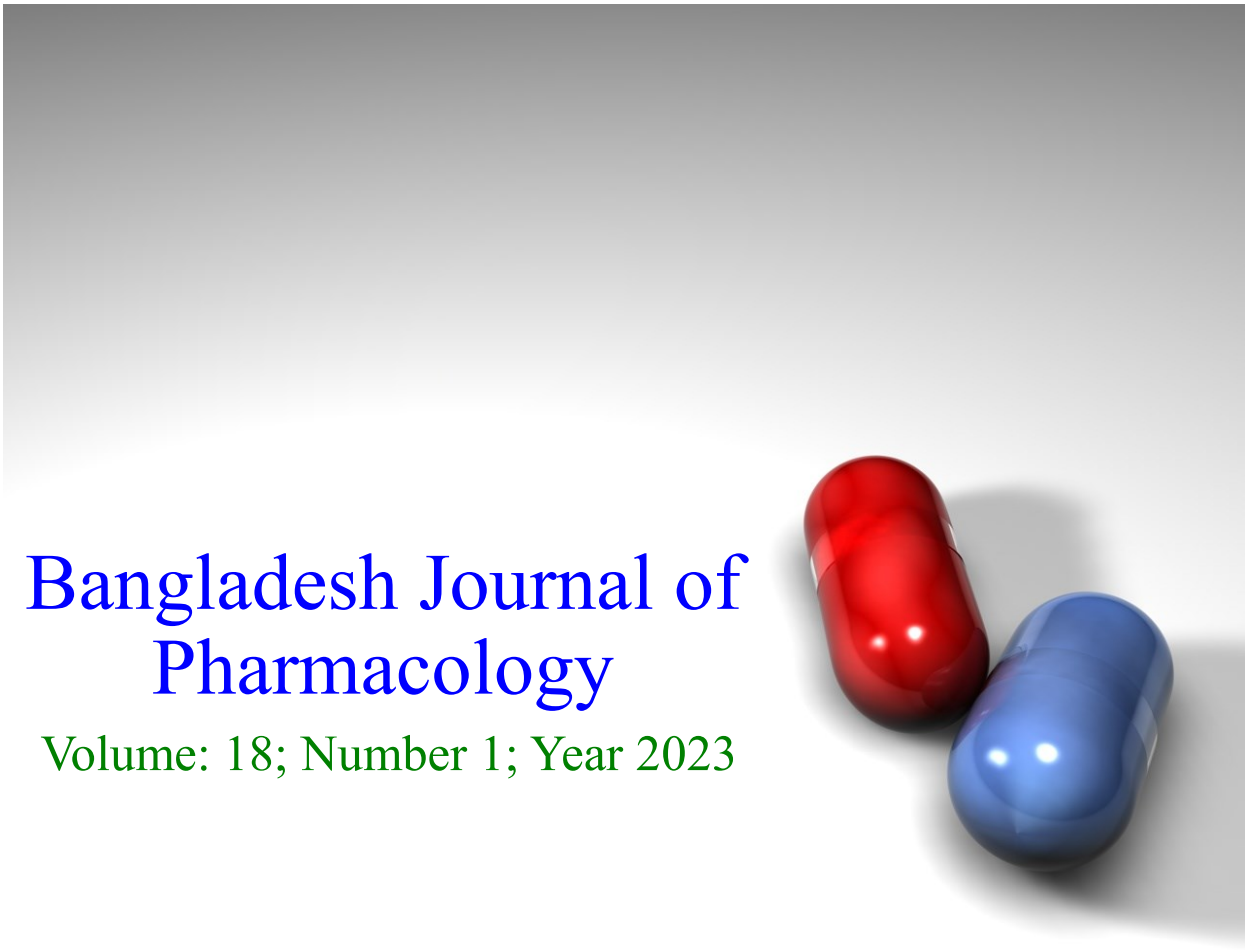

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Letter to the Editor

Cytotoxic activity of *Centrosema molle* leaf aqueous extracts

Sir,

Currently, about 39% of the approved drugs by the Food and Drug Administration are of natural origin (Boy et al. 2018). The biomedical importance of natural products especially in plants can be attributed to the presence of their diverse secondary metabolites (Atanasov et al., 2021). These secondary metabolites play a key role in biological activities, especially in their cytotoxic activity.

Cytotoxic activity is a critical factor for the success of developing novel drugs from natural products. In drug development, the tolerable level of a cell from natural products should be determined before proceeding to experiments in animal models (Bácskay et al., 2018). Moreover, the toxicity test serves as a basis for dosage selection that may involve both *in vitro* and *in vivo* setups (Maheshwari and Shaikh, 2016). Plants such as *Iphonia aucheri* (Shah et al., 2020), *Cyperus iria* (de Vera et al., 2022) had shown to have cytotoxic activities.

Centrosema molle Mart. ex Benth. is a perennial and a common climbing herb and does not have any recorded folkloric medicinal uses yet but it was noted that it has been utilized by the indigenous people in Maguindanao province, Philippines in treating wounds. In Laos, this weed had been used for treating scorpions and snakebites (Chima et al., 2013). In Nigeria, the leaves of this plant had been used for treating skin diseases (Ariwaodo et al., 2012). In addition, a study had been conducted that shows the potential of this plant for wound-healing activity (Ekpo et al., 2011). Thus, this plant shows potential for bioactive properties if proven to be non-toxic to cells.

This study determined the *in vitro* cytotoxic activity of *C. molle* leaf aqueous extract using brine shrimp lethality assay (Meyer et al., 1982). This preliminary study will help establish the dosage or concentration that will be used for future studies involving the biological activities of *C. molle* extract in both *in vitro* and *in vivo* setups. This also explores as a potential source of novel drugs in the future.

The *C. molle* plant leaves (1 kg) were collected from Pinaring, Sultan Kudarat, Maguindanao, and was authenticated at the Biology Department of Ateneo de Davao University, Davao City. *C. molle* extract was prepared by suspending 30 g of *C. molle* leaf powder in 100 mL of deionized water, then was heated at a 60°C water bath (Thermo Fisher Scientific, USA). The water bath temperature was regularly checked to maintain the desired temperature range. After 1 hour of heating, the suspension was filtered through cheesecloth and placed in a beaker. Centrifugation (Thermo Fisher Scientific, USA) at 3,000 rpm for 5 min was done, and the resulting supernatant liquid was placed in a clean amber glass bottle. *C. molle* extract concentrations were prepared by diluting different amounts of the collected supernatant liquid into different amounts of deionized water to make different concentrations. The presence of secondary metabolites was determined using standard methods by Harborne (1993).

Brine shrimp lethality assay was conducted to determine the cytotoxicity of the different concentrations of *C. molle* extract. The assay began by transferring three thousand microliters (3000 µL) of the different *C. molle* extract concentrations and control (artificial seawater) in their respective well using a Pasteur pipette. In every microwell, ten brine shrimps were pipetted. After 30 min, 6 hours, and 24 hours of exposure of brine shrimps to the samples and control, the number of them that were alive, impaired, and dead was determined. Probit analysis was employed to generate the median lethal concentrations (LC_{50}) value and 95% confidence intervals of each time of exposure of *C. molle* extract to brine shrimps (Finley, 1952). LC_{50} was obtained using a regression line by plotting the concentration against the percent mortality on a probit scale. Percent mortality was calculated using the equation:

$$Percent\ (\%)\ mortality = \frac{No.of\ dead\ brine\ shrimps}{No.of\ initial\ live\ nauplii} x\ 100$$

The presence of different secondary metabolites present in *C. molle* extract was determined. Saponins were found to be absent in the *C. molle* extract when tested using the froth test (data not shown). On the other hand, carbohydrates, reducing sugar, tannins, flavonoids, and alkaloids were present.

The percent mortality rate of the brine shrimps exposed to *C. molle* extract increased in concentration and time dependent (Figure 1). Results showed that 10,000 µg/mL concentration of *C. molle* extract had the highest percent mortality. Analysis of the results also indicated

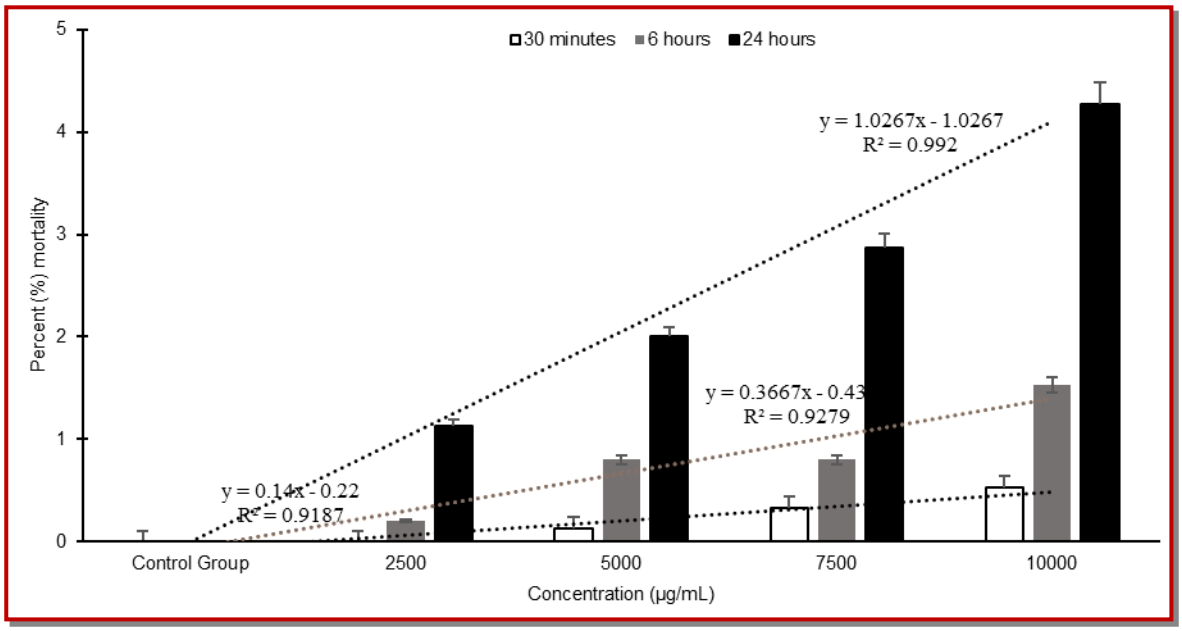

Figure 1: Percent mortality of brine shrimps at a different time of exposure to *C. Molle* leaf aqueous extract

Table I

LC_{50} of *C. Molle* at different time of exposure

Length of exposure (hour)	LC_{50} (µg/mL)	Confidence interval
0.5	62,461.4	23301.9; 167430.2
6	14,882.9	8076.4; 27425.5
24	14,842.9	8076.5; 27308.5

that the correlation coefficient of the logarithm of the concentration to the percent mortality from brine shrimps was 0.992 for the *C. molle* extract at 24 hours of exposure. This value meets the required value of >0.99 which is used to indicate an almost perfect correlation and the relationship between the ordinate and axis (Akoglu, 2018). Thus, increasing the concentration of *C. molle* extract higher than 10,000 µg/mL might also increase the percent mortality of the brine shrimps.

Data from the brine shrimp lethality assay (Table I) also shows an LC_{50} of 14,842.9 µg/mL concentration of *C. molle* extract at 24 hours of exposure. This indicates that a 14,842.9 µg/mL concentration of *C. molle* extract can kill 50% of the brine shrimps. The LC_{50} value at 14,842.9 µg/mL concentration of *C. molle* extract can be the basis for dosage or concentration selection in determining the other biological activities of *C. molle* extract such as its anti-inflammatory and anti-diabetic activities.

The *C. molle* extract at a concentration of 10,000 µg/mL showed a higher percent mortality rate among the *C. molle* extract concentrations being tested. Data from brine shrimp lethality assay also shows an LC_{50} of 14,842.9 µg/mL *C. molle* extract concentration. The correlation coefficient of the logarithm of the *C. molle* extract concentrations to the percent mortality of the brine shrimps indicated that as the concentration of *C. molle* extract increases, the percent mortality rate may also increase. Thus, this implies that the LC_{50} value of *C. molle* extract can be used to establish the dosage or concentration to be tested for future studies that involve *C. molle* extract's biological activities such as anti-inflammatory and anti-diabetic properties for both *in vitro* and *in vivo* setups.

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