



## Evaluating the Potential Cytotoxic Activity of *Acmella grandiflora* Flower and Whole Plant using Brine Shrimp Lethality Test

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### Abstract

The use of medicinal plants among indigenous people in the Philippines draws attention from the scientific community. The Department of Science and Technology in the Philippines identifies this area as a priority for research. *Acmella grandiflora* of Asteraceae family is known to contain important biocomponents such as spilanthol, acmellonate, tannins, flavonoids, and phenolic compounds. This plant is traditionally used to relieve toothache and also as stomachic, stimulant, and antidiarrheal. This research was done to evaluate the potential cytotoxic activity of *Acmella grandiflora* using the Brine Shrimp lethality Test (BSLT). The effectivity of the ethanol extract was assessed by comparing it with the decoction and 50:50 ethanol-water mixture extract. The results showed that the extraction by ethanol is the best method of extraction. The ethanol extract (WE) of the whole plant had an acute LC<sub>50</sub> of 141.25 µg/mL and the ethanol extract (FE) of the flowers exhibited the highest chronic LC<sub>50</sub> value at 28.84 µg/mL. Results indicate that *A. grandiflora* possesses potential bioactive phytochemicals worthy of further investigation specifically on its anaesthetic/sedative effect.

**Keywords:** Cytotoxicity, decoction, ethanol extract, LC<sub>50</sub>, medicinal plant.

### Introduction

Problem on people's health is one of the major concerns that goes with the rapid growth of the population in the Philippines<sup>1</sup>. Increasing cost of synthetic medicine justifies why in rural areas the use of plants for common ailments has become popular. Medicinal plants are substitute for expensive pharmaceutical drugs and are readily accessible.

The Philippine government through the Department of Science and Technology (DOST) is now addressing the need to assess scientifically the use of medicinal plants in the country. Traditional medicine is now identified as a priority area for research<sup>2</sup>.

*Acmella grandiflora* synonym *Spilanthus acmella*<sup>3</sup> is a popular folkloric herb for toothache<sup>4</sup>, stammering, and fights against throat and gum infections<sup>5</sup>. In many countries this plant has different uses. It is used traditionally as panacea in Sumatra, stimulant in Sudan, stomatitis in Java, leucorrhoea in Bangladesh, wound healing in India<sup>6</sup>, snakebite remedy and articular rheumatism in Cameroon<sup>7</sup>. Apart from its medicinal value this plant is also used as spice and additive in Brazil.

*A. grandiflora* is an interesting source of potential bioactive molecules. It contains primarily spilanthol and acmellonate that can stimulate the salivary gland and counteracts the pain caused by toothache<sup>8</sup>. Spilanthol is a pungent alkamide which is chemically N-isobutylamide. This compound has local

astriangency and anaesthetic effects<sup>9</sup> as evident by a tingling sensation and numbness in an applied area such as the gums. The study on local anaesthetic activity of this plant using guinea pig and frog showed a very strong positive result<sup>10</sup>.

The antipyretic effect and anti-inflammatory/analgesic activity of *A. grandiflora* were attributed to the presence of flavonoids<sup>11</sup>. The antioxidant property of the crude ethanol extract of the leaves is due to the presence of tannins, flavonoids, and phenolic compounds<sup>12</sup>. Other pharmacological uses as validated by many scientific researches include diuretic, pancreatic lipase inhibitor, vasorelaxant, antinociceptive, immunomodulator, and even as aphrodisiac<sup>9</sup>. Furthermore ethanol extract of the flower exhibited a high degree of insecticidal effect against *Anopheles*, *Culex*, and *Aedes* mosquitoes with 100% mortality at 7.5 ppm<sup>13</sup>.

In Mt. Matutum, South Cotabato, the local people refer to *A. grandiflora* as toothache plant. They use it extensively to relieve toothache. Fresh sample of flower is applied to the aching tooth cavity that results to numbness thus relieving the pain. Despite this, there is no reported published study in the Philippines of the said plant. This study was conducted to determine the potential cytotoxicity of *A. grandiflora* using the Brine Shrimp Lethality Test (BSLT). This test is a useful and simple bioassay which can help correlate the bioactivity of plant extract with its cytotoxic and anti-tumor potentials<sup>14</sup>. The relative toxicity of the whole plant and flower extract was compared using the LC<sub>50</sub> values. The effectivity of the most common methods of

extraction (decoction, absolute ethanol, 50:50 ethanol-water) was assessed.

## Material and Methods

**Plant Material:** Approximately three kg of fresh whole plant samples (excluding roots) of *A. grandiflora* were collected in three barangays (Dalipuga, Abuno, and Bacayo) of Iligan City, Philippines. The same amount of fresh flowers were collected at Mt. Matutum, South Cotabato. The protocol of Guevara (2005)<sup>15</sup> for plant sample preparation was followed in this study.

**Preparation of the Plant Extract:** Fresh samples were first washed with tap water and then with distilled water. One kg of whole plant samples collected from Iligan City was subjected to decoction in 2:1 water proportion in 5 minutes. The liquid was then filtered and freeze-dried. One kg of fresh flowers was also subjected to decoction. The remaining 2kg of the whole plant samples were air dried for three weeks while the flowers (2kg) were oven-dried at 30-40°C until dry and crispy. The dried samples were pulverized using electric blender and were soaked in absolute ethanol and ethanol:water mixture (50:50) for three days. The solutions were filtered and concentrated using a rotary evaporator for ethanol extract and freeze dryer for decoction. For ethanol:water extract, it was subjected first to rotary evaporator and further freeze dried to obtain a concentrated extract. Three extracts for each sample (whole plant and flowers) were produced: decoctions (WD- whole plant decoction and FD- flower decoction), ethanol extracts (WE- whole plant ethanol extract and FE- flower ethanol extract), and ethanol-water mixture extracts (WM- whole plant ethanol-water mixture extract and FM -flower ethanol-water mixture extract).

**Bioactivity of the Extracts: Brine Shrimp Lethality Test (BSLT):** The BSLT is composed of three major steps: hatching of brine shrimps, preparation of test solution, and the bioassay proper.

**Hatching of the Shrimp:** A small tank was filled with filtered sterile seawater and was divided into two compartments. The hatching chamber was covered with black cardboard before adding the eggs of *Artemia salina*. The other half of the tank was lighted with a 100-watt bulb to attract and separate the hatched shrimp from the unhatched eggs. The lighting was important to simulate the temperature of the natural habitat of the shrimp. The shrimps were then used for bioassay after 48 hours.

**Preparation of Test Solution:** The test solutions were of four concentrations; 1000 µg/mL, 500µg/mL, 100µg/mL, and 10µg/mL. To make the stock solutions, 30 mg of the extract was dissolved in 3 mL of ethanol (for WE, FE, WM, FM), filtered sterile seawater for WD and FD to produce a 10 000 µg/mL. Using a micropipette, 500µL, 250µL, 50 µL and 5µL of the stock solution were transferred to four test tubes to produce 1 000µg/mL, 500µg/mL, 100µg/mL, and 10µg/mL concentrations

upon dilution to 5.0 mL of seawater. Three replicates were prepared for the four concentrations of each extract. Five mL of filtered sterile seawater served as control. For the alcohol based extracts, they were allowed to be dried for 2 days. The dried extract was added with 150 µL of DMSO for the concentrations 1000µg/mL and 500µg/mL while 75 µL of DMSO were added for the concentrations 100µg/mL and 10 µg/mL.

**Bioassay:** Ten larvae of *A. salina* were placed in every test tube using a dropper. The volume was adjusted to 5mL by adding filtered sterile seawater. The test tubes were kept under 100-watt illumination, and the number of survivors was recorded after 6 and 24 hours.

The Reed-Muench (1938)<sup>16</sup> method was used to determine the relative toxicity of the extracts on the brine shrimp. This was done by testing the response of *A. salina* to the four concentrations of each extract. LC<sub>50</sub> represents the dose lethal to half of the population of the *A. salina*. Chronic and acute LC<sub>50</sub> refer to the dose that rendered 50% mortality of the test animals after 6-hr and 24-hr exposure, respectively.

## Results and Discussion

The cytotoxicity, antitumor, and pesticidal potential of plant sources could be assessed using brine shrimp assay<sup>17</sup>. Increasing the concentration in the different extracts shows a direct relationship with the mortality rate of the brine shrimp. The LC<sub>50</sub> (lethal concentration, 50%) values of the different extracts, (Table 1) showed that after 6 hours, the most potent extract was the whole plant ethanol extract (WE) with LC<sub>50</sub> of 141.25 µg/mL. Meanwhile after 24 hours, the ethanol extract of the *A. grandiflora* flowers (FE) exhibited the highest lethality on the test organism *A. salina* wherein a very low concentration (28.84 µg/mL) was needed to cause 50% mortality of the population. Furthermore, as indicated by the LC<sub>50</sub> values, the best method for extracting *A. grandiflora* is through ethanolic extraction for both the whole plant and the flower samples. Except for the ethanol-water mixture extract of the whole plant (WM) and the decoction of the flower (FD), all of the extracts are considered active since their LC<sub>50</sub> values are <1000 µg/mL<sup>14</sup>.

**Table-1**  
**Acute and Chronic LC<sub>50</sub> Values of Various Extracts of *Acemlagrandiflora* Whole Plant and Flowers against the Brine Shrimp *A. salina***

Extract	LC <sub>50</sub> Values, µg/mL	
	Acute (6 hr)	Chronic (24 hr)
WD	881.05	226.46
WE	141.25	55.59
WM	>1000	668.34
FD	1000	245.47
FE	389.05	28.84
FM	901.57	146.22

## Conclusion

The toxicity study conducted on *A. grandiflora* demonstrates the presence of potent bioactive components as assessed by the lethality of the extracts on the brine shrimps at different concentrations. This indicates that *A. grandiflora* contains bioactive compounds that needs further investigation specifically on its anaesthetic/sedative effect.

The bioactive components of *A. grandiflora* are best extracted by using ethanol. The ethanol extract of the flower (FE) exhibited the most lethal toxicity with LC<sub>50</sub> value of 28.84 µg/mL which may support the traditional use of the *A. grandiflora* flowers by the local people of Mt.Matutum, South Cotabato, Philippines.

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