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Research Article

Toxicity study of *Kyllinga brevifolia* and *Scurrula parasitica* using brine shrimp lethality test

Mohd Kamal N.H. [1], Ihsan Safwan K. [1], Zaridah M.Z. [1], Susilatul Ziana A.[2], Azman M. [1], Muhammad Syahmi A. [1], Ahmad Tirmizi A. [3], Aida Adrieanna B.H. [3], Alia Shafika B. [3], Nur Khairah A.K. [3]

[1] *Natural Product Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Malaysia*
[2] *Department of Diagnostics and Allied Health Sciences, Management and Science University, 40100 Shah Alam, Malaysia*
[3] *Department Medical Science and Technology, University College MAIWP International (UCMI), 68100 Batu Muda, Malaysia*

*Correspondence: mohdkamal@frim.gov.my

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Abstract: *Kyllinga brevifolia* is an herb whose rhizome is used in traditional medicine as a refreshing drink and is said to have digestive, diuretic, sedative, tonic, antispasmodic, and diaphoretic properties. *Scurrula parasitica* has neuroprotective, sedative, anticancer, immunomodulatory, antiviral, diuretic, antihypertensive, antioxidant, antimutagenic, antiviral, antihepatotoxic, and antiviral effects. The use of these two herbs in health products needs to be studied more carefully as the demand for herbal products is increasing nowadays. Thus, the main purpose of this study is to determine the toxicity of the aqueous extract of *K. brevifolia* and *S. parasitica* using the brine shrimp lethality test (BSLT). The leaves of the two plants were dried in an oven at a temperature of 55°C for 48 hours. The dried leaves were ground and boiled at 100°C for 1 hour. BSLT test was performed with 8 replicates at 100, 50, 25, 12.5, 6.25, 3.125, 1.562 0.781, 0.39, 0.195, and 0.097 mg/mL. *K. brevifolia* was not significantly different from the untreated controls and had no lethal concentration 50 (LC_{50}). In contrast, the LC_{50} of *S. parasitica* was 50 mg/mL.

Keywords: *Kyllinga brevifolia*, *Scurrula parasitica,* brine shrimp lethality test, *Artemia salina*, water extract

INTRODUCTION

Kyllinga brevifolia is an herbaceous plant that has stems with grass-like leaves. It grows from long and slender rhizomes that creep underground (Rodiyati, 2003). The length of the stem ranges from 10 to 50 cm. It is found in ponds and open, moist areas. Traditionally, this herb is used as a medicinal (Hellión-Ibarrola et al., 2016). There are reports of consumption of the rhizome as a cooling drink in traditional medicine in Paraguay for its digestive, diuretic, sedative, tonic, antispasmodic, and diaphoretic effects (Basualdo and Zardini, 1995). The leaf contains an essential oil that could be useful for health (Paudel et al., 2012). Previous pharmacological studies have shown that *K. brevifolia* is anxiolytic (Hellión-Ibarrola et al., 2012) and antidepressant (Hellión-Ibarrola et al., 2016). In addition, Ho et al., (2012) reported that *K. brevifolia* has potential antioxidant properties. Extracts of *K. brevifolia* have also been reported to have anti-inflammatory, sedative, and analgesic properties (Hellioen-Ibarrola et al., 1999). Even if an herb has good efficacy for human health, it is useless if it can cause toxic effects that are likely to damage vital organs.

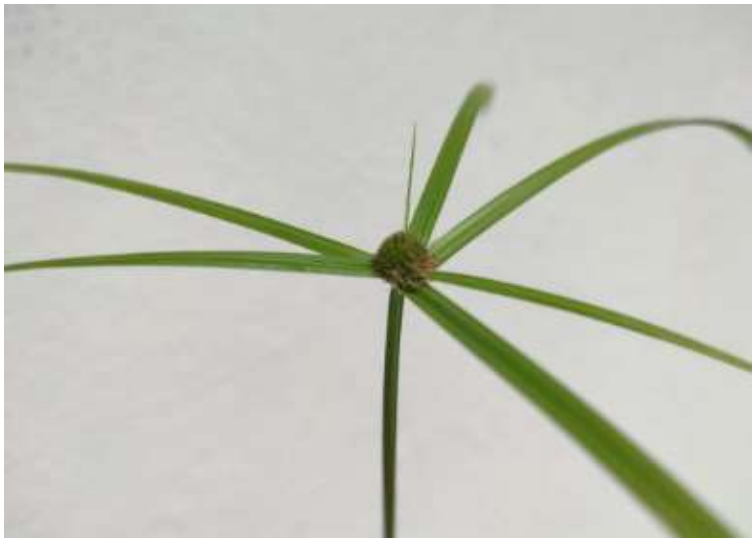

Figure 1: *K. brevifolia*

Meanwhile, *S. parasitica* is also one of the herbal trees that are said to have many health benefits (Moghadamtousi et al., 2014). Its local name is cinnamon mistletoe or known as 'Pokok Benalu Api' in Malaysia. *S. parasitica* is a 0.5-1 m tall shrub with densely hairy branches and leaves when young (Lim et al., 2016). Several previous studies reported that *S. parasitica* has neuroprotective, sedative, anticancer, immunomodulatory, antiviral, diuretic, antihypertensive, antioxidant, antimutagenic, antiviral, antihepatotoxic, and anti-nephrotoxic effects (Muhammad et al., 2019; Moghadamtousi et al., 2014). In addition, *S. parasitica* is of great benefit in the traditional medicine of Asian communities (Lim et al., 2016). However, there are many recent reports that the herbal extract has potentially toxic effects when used at a high concentration or over an extended period (Ekor, 2014; Brima, 2017). Therefore, a simple screening study is required to ensure that any

herbal extract produced is not toxic. BSLT screening test is rapid and very suitable for performing herbal extracts.

Figure 2: *S. parasitica*

The BSLT approach using shrimp larvae (*Artemia salina*) is one of the most cost-effective toxicity screening tests for evaluating the toxicity of herbal extracts (Harwig and Scott, 1971; Carballo et al., 2002). This technique is used to screen the toxicity of plant extracts or newly discovered synthetic compounds before more expensive cell culture testing and more difficult animal testing (Wu, 2014). Cost-effectiveness and speed make this test an excellent choice for beginning the study of natural products (Sarah et al., 2017). This preliminary toxicity screening test is based on the ability of the plant extract to kill shrimp larvae (Nerdy et al., 2021). The toxicity of the extract was determined by counting the number of shrimp that died after exposure to the extract. This method is simple, inexpensive, and requires a small amount of test material (Kolbeck and Tintjer, 2016). Despite numerous studies on *K. brevifolia* and *S. parasitica*, their toxicological properties remain unclear. Although there are many previous research reports on *K. brevifolia,* the studies conducted focus only on rhizome samples. The toxicity of the leaves and stems of this plant has not been thoroughly studied. In addition, *S. parasitica* has been traditionally used by decoction with water. However, most published scientific reports on toxicology have used an organic solvent extract. Therefore, the current study focuses on water extract samples of the two herbs. Both samples will be used for BSLT to determine the toxic effects on brine shrimp, which can be used

for extrapolation to higher-level biological system models such as cell culture and laboratory animals.

METHODS

The extraction process of K. brevifolia and S. parasitica: K. brevifolia and *S. parasitica* leaves were collected at the Forest Research Institute of Malaysia (FRIM). *K. brevifolia* and *S. parasitica* leaves were washed, dried in an oven at 55oC for 48 hours, and ground into powder. For the extraction process, 1000 mL of distilled water was added into a beaker containing 100 g of each herbal powder. The sample was boiled at a temperature of 100oC for 1 hour. Then, it was filtered and evaporated using a rotary evaporator at a temperature of 55°C. After that, the water extract was stored in a -20°C freezer until used.

Preparation of Artemia salina and BSLT: Water extracts of *K. brevifolia* and *S. parasitica* were tested as described by Mohd Kamal et al., (2022). First, brine shrimp eggs were artificially hatched in seawater prepared from commercial sea salt by dissolving 25 g of sea salt in 100 mL of water. Then, the salt solution was placed in a beaker and 2 g of shrimp eggs were added. After 24 hours, the hatched shrimp were used for BSLT. Each extract was dissolved in water to obtain an extract concentration of 100 mg/mL. Serial dilutions were then performed to produce extract concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 0.781, 0.39, 0.195, and 0.097 mg/mL. Salted water was added to the plate at a volume of 90 µL per well. Then, 10 µL of larval suspension containing approximately 10 - 20 larvae were added to the wells. Then, 100 µL of each sample was added to a 96-well plate and incubated for 24 hours. After 24 hours, the number of dead larvae in each well was observed and counted using the Dino-Lite digital microscope (Figure 3). Larvae that did not move were considered dead. The test was performed with 8 replicates for each concentration, and the test was also repeated on 3 different trials/days. The lethal concentration 50 (LC_{50}) was determined as indicated in the result interpretation.

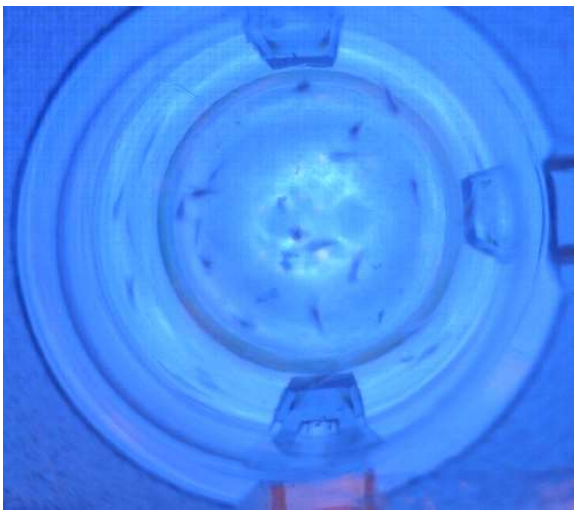

Figure 3: Brine shrimp observed under Dino-lite microscope

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Calculation of the percentage of survival was done using the formula:

% Death = Total larvae – Number of dead larvae × 100/ Total larvae

Statistical analysis: All of the presented results are the means (± standard deviation) of eight repetitions in each experiment for each *K. brevifolia* and *S. parasitica* extract concentration. There were three independent experiments on different days performed. Charts and statistical analysis were performed by Graph Pad PRISM version 6. Statistical significance was observed using a One-Way ANOVA.

RESULTS

Figure 4 shows that the extract of *K. brevifolia* at the concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 0.781, 0.39, 0.195, and 0.097 mg/mL was not significant compared to the control. However, the result of the concentration of 1.562 mg/mL of *K. brevifolia* extract showed a slightly significant result compared to the control group. However, no LC_{50} was observed in the graph. This indicates that all *K. brevifolia* extracts did not cause death in 50% of all brine shrimp populations.

Figure 5 shows that all concentrations of *K. brevifolia* extract of 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.39, 0.195, and 0.097 mg/mL were without significance compared to the control. However, no LC_{50} was detected in the graph.

From 6, all concentrations of *K. brevifolia* extract 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.39, 0.195 and 0.097 mg/mL showed no significant difference compared to control.

Figure 7 shows that the concentrations of *S. parasitica* extracts of 100 and 50 mg/mL have high significance compared to the control. While 0.781 mg/mL showed low significance compared to the control. In addition, *S. parasitica* extracts with concentrations of 25, 12.5, 6.25, 3.125, 1.562, 0.39, 0.195, and 0.097 mg/mL did not show significant differences compared to the control. In this graph, the LC_{50} of *S. parasitica* can be detected at a concentration of 50 mg/mL.

According to Figure 8, the result shows that *S. parasitica* extract at concentrations of 100, 50, and 12.5 mg/mL were highly significant compared to the control. On the other hand, the concentrations of 25 and 6.25 mg/mL of *S. parasitica* were not very significant compared to the control. Moreover, the other concentrations 3.125, 1.562, 0.781, 0.39, 0.195, and 0.097 showed no significant difference compared to the control. In this graph, the LC_{50} of *S. parasitica* can be detected at a concentration of 50 mg/mL.

According to Figure 9, the results show that the concentrations of *S. parasitica* 100, 50 and 25 mg/mL were very significant compared to the control. The concentration of 1.562 mg/mL was significant, and the concentration of 3.125 mg/mL was slightly significant compared to the control. Moreover, the other concentrations of 12.5, 6.25, 0.39, 0.195 and 0.097 mg/mL showed no significant differences compared to the control group. In this diagram, the LC_{50} of *S. parasitica* can be detected at a concentration of 50 mg/mL.

DISCUSSION

Toxicity testing is the first step in the investigation of a drug before it is used as a product to be used for human consumption (Reymon et al., 2022). An effective and a simple method for determining toxicity is the BSLT (Carballo et al., 2002).

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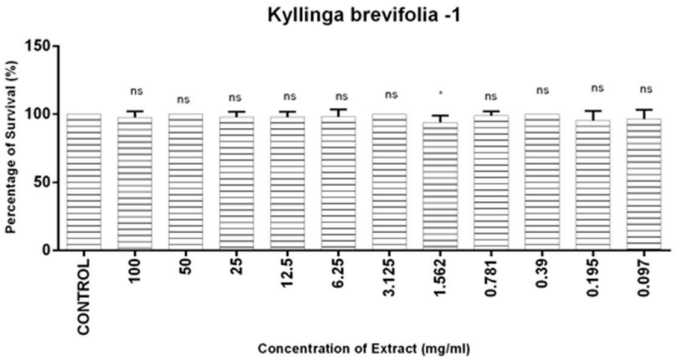

Figure 4: The effect of *K. brevifolia* on brine shrimp viability on day 1.
ns; not significant, * $p<0.05$ compared to control.

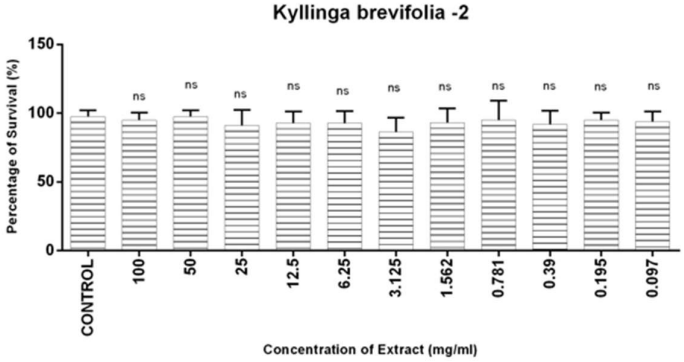

Figure 5: The effect of *K. brevifolia* on brine shrimp's viability on day 2.
'ns' denoted as not significantly different ($p<0.05$) compared to the control

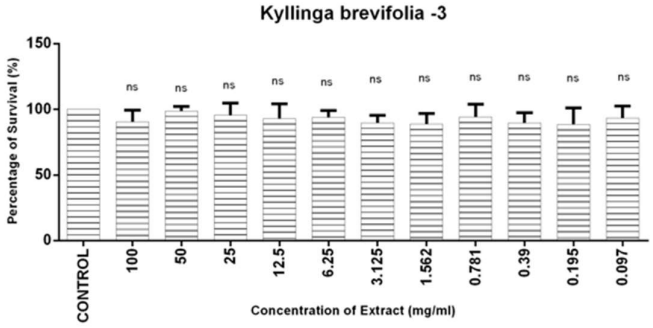

Figure 6: The effect of *K. brevifolia* on brine shrimp's viability on day 3.
'ns' denoted as not significantly different ($p<0.05$) compared to control

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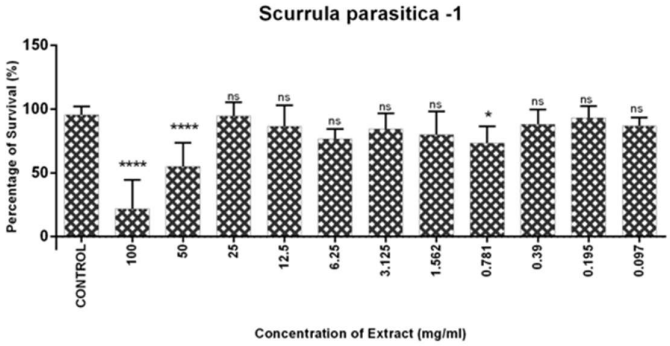

Figure 7: The effect of *S. parasitica* on brine shrimp's viability on day 1.
ns; not significant different, * p<0.05, **** p< 0.0001 compared to control

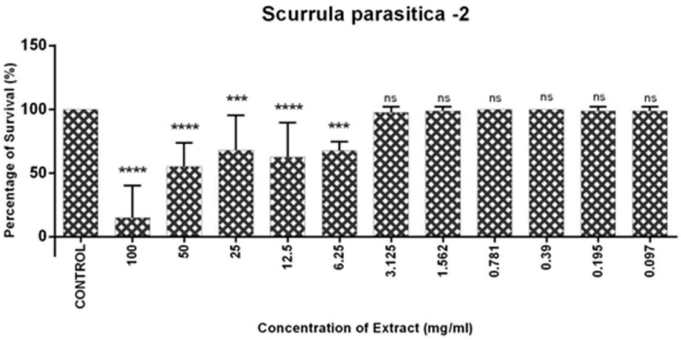

Figure 8: The effect of *S. parasitica* on brine shrimp's viability on day 2.
ns; not significant different, *** p<0.001, **** p<0.0001 compared to control

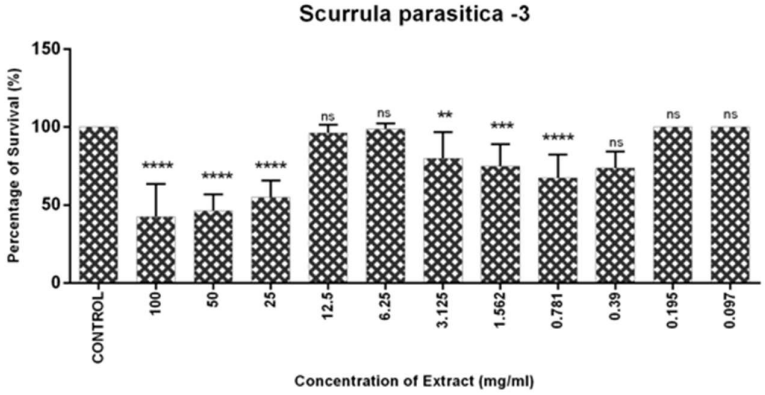

Figure 9: The effect of *S. parasitica* on brine shrimp's viability on day 3.
ns; not significant different, ** p<0.01, *** p<0.001, **** p<0.0001 compared to control

The method determines the LC_{50} value of active compounds or extracts (Aksono et al., 2022). In this study, the concentrations used for each extract were 100, 50, 12.5, 6.25, 3.125, 1.562, 0.781, 0.39, 0.195, and 0.097 mg/mL, which provides a wider range for determining the toxicity of the samples.

Based on the results the LC_{50} in *K. brevifolia* was above 100 mg/mL. To date, there are no similar previous toxicity studies with water extracts of *K. brevifolia* to compare with these results. However, Hidayah et al., (2020) conducted a toxicity test almost identical to this current study and concluded that the methanol extract of *K. brevifolia* is potentially toxic. This could be due to the different content of the two extracts. The water extract of *K. brevifolia* could be more harmless than the extract of *K. brevifolia* prepared with organic solvents.

The LC_{50} of *S. parasitica* was consistently 50 mg/mL. This result agrees with that of Saifa, (2018) who reported that *S. parasitica* is toxic to *Artemia salina* as the LC_{50} is 43 mg/mL. Elsyana et al, (2016) reported that the toxic activity of plants from the Loranthaceae family depended on the host plants and the extraction solvent. In many cases, the flavonoids and triterpenoids from the extract were toxic to brine shrimp (Elsyana et al., 2016). Therefore, the toxicity of *S. parasitica* to brine shrimp could be due to these compounds.

The toxicity profile of herbal extracts cannot be evaluated by BSLT alone. This is because BSLT is too simple to be compared with the effects of toxicity of plants on the human body and organs. It is proposed to conduct studies on the toxicity of *K. brevifolia* and *S. parasitica* in human cell cultures and laboratory animals, which will provide more information on the toxicity of the two herbs.

CONCLUSION

In summary, the water extract of *K. brevifolia* was consistently found to be non-toxic in the BSLT, as no LC_{50} was detected in all three experiments. However, the water extract of *S. parasitica* had a consistent LC_{50} value of 50 mg/mL, which could be potentially toxic.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

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