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Comparative Cytotoxicity of Selected *Mikania* species using Brine Shrimp Lethality Bioassay and Sulforhodamine B (SRB) Assay

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Abstract

This study aims to assess the comparative cytotoxic activity of the ethanolic extract of *Mikania cordata* (MC), *Mikania micrantha* (MM) and *Mikania scandens* (MS) (Family: Asteraceae) using brine shrimp lethality bioassay and colorimetric sulforhodamine B assay method. In SRB assay, A549 human lung carcinoma, SK-Mel-2 skin melanoma and B16F1 mouse melanoma cell lines were used. In brine shrimp assay, the LC_{50} for MC, MM, MS and vincristine were found to be 29.04, 15.84, 32.35, and 1.2 µg/ml, respectively. The results indicate that all the *Mikania* species showed moderate lethality against nauplii. In SRB assay, 50% cell growth inhibition concentration (IC_{50}) of MC, MM, MS and cisplatin against B16-F1 were 33, 15, 39 and 6.8 µg/ml, respectively. Similar data were observed for other cell lines indicating moderate cytotoxicity of the extracts. Among the species, *M. micrantha* showed relatively potent cytotoxic effect followed by *M. cordata* and *M. scandens.* Similar data were observed for brine shrimp lethality bioassay and the results suggest that *M. micrantha* possesses highest cytotoxic potentials among all the species.

Key words: *Mikania cordata*, *Mikania micrantha*, *Mikania scandens*, Cytotoxicity, Brine shrimp lethality bioassay, SRB assay.

Introduction

Plants have been served as excellent sources of drug discovery since several decades due to less or no harmful side effects on health, and can be used in day-to-day life (Parekh and Chanda, 2007; Mohamed *et al.*, 2012). In developing countries including Bangladesh, about 75% of the populations rely on different forms of traditional medicine for their primary health care (Bastos *et al.*, 2008; Haque *et al*., 2018). To reveal the potential of folkloric reputed medicinal plants available in Bangladesh, we selected *Mikania* genus since the genus is the largest of its kind in the Eupatorieae (Family Asteraceae) tribe with around 400 species (Ferreira and Oliveira, 2010; Ittiyavirah and Sajid, 2013). *Mikania* species are well-known folkloric reputed plants used to treat cancer, fever, inflammation, rheumatism, oxidative stress, spasmolytic, respiratory diseases as well as snake bites (Hajra *et al.*, 2010; Ittiyavirah and Sajid, 2013; Khatun *et al*., 2020). However, only a few of the species have been studied for biological activity.

A variety of biological activities such as antibacterial, antiviral, anti-inflammatory, antispasmodic, antioxidant, cytotoxic etc. are reported for *Mikania* species (Luciane *et al.*, 2012; Hoult and Payá, 1996) and most of the reported activity are limited to *Mikania micrantha* and *Mikania cordata* though there are a lot of available species. In Bangladesh *M. cordata*, *M. micrantha* and *M. scandens* are widely available. Previously we reported comparative phenolic contents and antioxidant activity (Khatun *et al*., 2020),

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antimicrobial activity (Khatun *et al*., 2017a), and anti-inflammatory and thrombolytic activity (Khatun *et al*., 2017b) of these three species. A number of published research showed cytotoxic activity of various *Mikania* species including *Mikania cordata* (Ali *et al.*, 2011), *Mikania micrantha* (Saikia *et al*., 2020) and *Mikania scandens* (Ahammed *et al*., 2020). However, comparative cytotoxicity evaluation of these species was not reported before. So, the current study was conducted to evaluate comparative cytotoxicity study of three commonly available *Mikania* species, *Mikania cordata*, *Mikania micrantha* and *Mikania scandens* using brine shrimp lethality bioassay and colorimetric sulforhodamine B assay.

Materials and Methods

Collection and identification of plants: The whole part of *M. cordata* (MC), *M. micrantha* (MM) and *M. scandens* (MS) were collected from Rajshahi (northern part of Bangladesh), Barisal (Southern part) and Kushtia (western part), respectively, during the month of August 2017 and were identified by Dr. AHM Mahbubur Rahman, associate professor and taxonomist, Department of Botany, University of Rajshahi, Bangladesh.

Extraction and fractionation: The plants labelled as MC, MM and MS were air dried for several days and then oven dried at 45^{o}C for 24 hours. The dried plants were crushed separately into course powder. About 170 gm powdered plant materials were taken separately in an amber-colored extraction bottle and were soaked with 70% ethanol (90 mL × 3 times) for 7 days with occasional shaking. The extracts were filtered through cotton and Whatman No. 1 filter, concentrated with rotary evaporator under reduced pressure at 45°C and preserved at 4 $^{\circ}$C. The percentage of MC, MM and MS extract (w/w) were 11.00%, 10.5% and 9.3%, respectively (Khatun *et al*., 2020).

Cytotoxicity assay:

Brine shrimp lethality bioassay: The experiment was carried out by the method described by Meyer *et al*. (1982). In brief, *Artemia salina* Lech. (brine shrimp eggs) was allowed for 48 hours in simulated seawater to hatch and mature as nauplii (Larvae) at 25 ^{o}C. Serially diluted extracts from MC, MM and MS were added to the simulated sea-water (5 mL) containing 20 nauplii. 120 µl of DMSO was added to each of the three remarked glass vials containing 5 ml of simulated sea water and 20 shrimp nauplii to use as negative control group. After 24 hours incubation at 25 ^{0}C, the number of survivors was counted. The LC_{50} (50% lethal concentration, µg/ml) were determined from triplicate experiments. Different concentrations of vincristine sulfate were taken as positive control.

Cancer cell line cytotoxicity assay (SRB assay): Cytotoxicity was measured by SRB (colorimetric sulforhodamine B assay) method (Skehan *et al.*, 1990) using human lung carcinoma (A549), skin melanoma (SK-Mel-2), and mouse melanoma (B16F1) cell line. Human lung cancer cell line A-549, human melanoma SK-MEL-2, and mouse melanoma B16-F1 were collected from Korean Cell Line Bank (Chongno-gu, Seoul, Korea). Briefly, exponentially growing cells were harvested and suspended in the culture medium (100 mL, RPMI-1640) in a 96-well plate. Following seeding density was used: 1×10^5, 1×10^5, and 2×10^4 cells/ml for A-549, SK-MEL-2, and B16-F1, respectively. After 24 h incubation at 37 ^{0}C under humidified 5% CO_2, serially-diluted test solutions (100 ml in RPMI medium) were added to the wells and incubated further for 48 h. The cells were fixed with 50% trichloroacetic acid and stained for 30 min with SRB solution. Unbound dye was removed by 1% acetic acid (four times) and protein-bound dye was then extracted with 10mM tris base (pH 10.5) for 5 min. Optical density of the released dye was measured at 520 nm in a microplate reader (Tecan Sunrise microplate reader). The results were expressed in terms of IC_{50} value. Cisplatin was used as a reference.

Statistical analysis: All analyses were carried out in triplicates. Statistical comparisons were performed

using Microsoft Excel, 2019. Mean values ± S.D. were calculated for the parameters where applicable.

Results and Discussion

The ethanolic extract of *M. cordata*, *M. micrantha*, *M. scandens* were evaluated for lethality against brine shrimp nauplii (Meyer *et al*., 1982). The result of the lethality bioassay is shown in Table l where mortality rate varies with concentration of sample and was increased with increasing concentration of the sample. The median lethal concentration (LC_{50} in µg/ml) was determined by extrapolation from graph and the values for MC, MM, MS and standard vincristine sulfate were found to be 29.04, 15.84, 32.35, and 1.2 µg/ml, respectively. Compared to positive control, the results indicate that all the *Mikania* species showed moderate lethality against brine shrimp nauplii and the activity of *M. micrantha* is two times more than that of *M. cordata* and *M. scandens* (Figure 1A). Since, the brine shrimp lethality bioassay is a preliminary toxicity screening of plant extracts (Oberlies *et al.*, 1998), the findings had the potential for further cytotoxicity investigation.

Table 1. Results of *in-vitro* brine shrimp lethality bioassay of various *Mikania* species.

Extract	Conc. (µg/ml)	Log C	% of Mortality	Probit value (From % of Mortality)	LC_{50} (µg/ml)
M. cordata	200	2.30	100		29.04
	100	2	90	6.28	
	50	1.7	75	5.67	
	25	1.4	25	4.33	
	12.5	1.1	15	3.96	
	6.25	0.8	10	3.72	
	3.125	0.5	10	3.72	
	1.56	0.1	2	2.95	
M. micrantha	200	2.30	90	6.28	15.84
	100	2	90	6.28	
	50	1.7	75	5.67	
	25	1.4	60	5.25	
	12.5	1.1	40	4.75	
	6.25	0.8	30	4.48	
	3.125	0.5	10	3.72	
	1.56	0.1	15	3.96	
M. scandens	200	2.30	75	5.67	32.35
	100	2	50	5	
	50	1.7	60	5.25	
	25	1.4	45	4.87	
	12.5	1.1	40	4.75	
	6.25	0.8	40	4.75	
	3.125	0.5	30	4.48	
	1.56	0.1	10	3.72	

Data for standard vincristine sulfate (IC_{50} 1.2 µg/ml) is not shown in the table

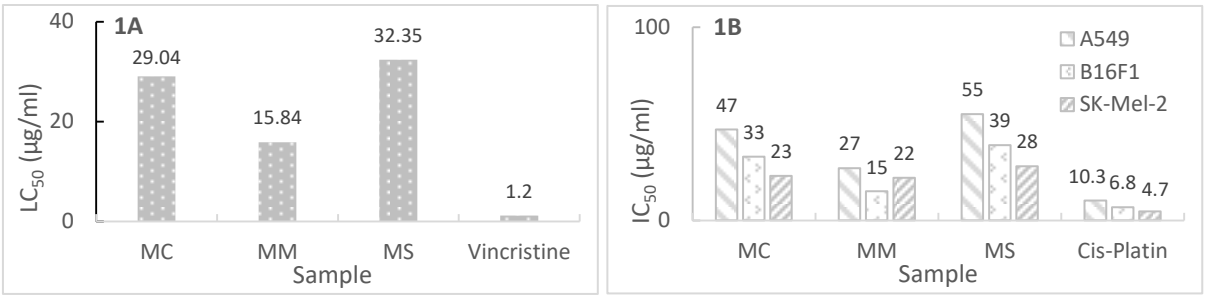

Figure 1. Comparison of (**A**) LC_{50} values from brine shrimp lethality bioassay and (**B**) IC_{50} values from SRB assay of extracts from *M. cordata* (MC), *M. micrantha* (MM) and *M. scandens* (MS).

Table 2. Results of *in vitro* cytotoxicity assay (SRB assay) of various *Mikania* species.

Extract	IC_{50} against cancer cell lines (µg/ml)		
	A549	B16F1	SK-Mel-2
M. cordata	47	33	23
M. micrantha	27	15	22
M. scandens	55	39	28
Standard Cisplatin	10.3	6.8	4.7

The extracts were further evaluated for cytotoxicity against human lung cancer cell line A-549, human melanoma SK-MEL-2, and mouse melanoma B16-F1 using SRB (colorimetric sulforhodamine B assay) method (Skehan *et al.*, 1990). Results are shown in Table 2 and Figure 1B. The IC_{50} value of MC, MM, MS and standard Cisplatin against cancer cell lines A-549, B16-F1 and SK-MEL-2 were 47, 33 and 23 µg/ml; 27, 15 and 22 µg/ml; 55, 39 and 28 µg/ml, and 10.3, 6.8 and 4.7 µg/ml, respectively. The result demonstrated that *M. micrantha* showed potent cytotoxic effect followed by *M. cordata* and *M. scandens.* Similar data were observed for brine shrimp lethality bioassay and the results distinctly proved that *M. micrantha* might possess highest percentages of phytochemicals responsible for cytotoxic activity.

Mikania is an important plan genus commonly known as guaco found in the tropics of America and Asia (Ferreira and Oliveira, 2010). We have identified three commonly available species in Bangladesh: *Mikania cordata*, *Mikania micrantha* and *Mikania scandens*. Among these, *Mikania cordata* has been used in traditional herbal medicine for a long to treat various ailments including cancer by folklore people (Uy *et al*., 2015). Several reporters have also shown the cytotoxic activity of *Mikania* species (Saikia *et al.*, 2020; Ahammed *et al.*, 2020).

In our present study, we have evaluated lethality potentials of the selected species using brine shrimp lethality bioassay and cytotoxic potential using colorimetric sulforhodamine B assay. The shrimp lethality assay is a useful tool for preliminary evaluation of plant extract toxicity as well as for screening diverse compounds (Carballo *et al.*, 2002), whereas SRB assay determines the inhibition of cell proliferation as well as cytotoxicity based on the measurement of cellular protein content (Bhagat *et al.*, 2014). Though the extracts gave a higher IC_{50} value by SRB assay when compared to LC_{50} value by lethality bioassay, there was good correlation between IC_{50} and LC_{50} values. The activity of *Mikania* extracts followed a similar trend by both lethality bioassay and SRB assay where *M. micrantha* showed highest activity followed by other two species.

Conclusion

The present observation established that the ethanolic extracts of *M. cordata*, *M. micrantha* and *M. scandens* have potent cytotoxic activity *in vitro*. The results validate the use of the plants in folkloric medicine and will provide scientific foundation for rational development and utilization of these plants. From the observation it was found that *M. micrantha* was more active than other species. Hence, the study can easily identify a more potent species that could be helpful for developing new leads. Further investigations in this direction can be attempted to find promising anti-cancer phytochemicals.

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