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ASSESSMENT OF PHYTOCHEMICAL, CYTOTOXIC, ANTHELMINTIC AND THROMBOLYTIC ACTIVITY OF *MIKANIA MICRANTHA* LEAVES: A NEW ADDITION IN PHYTOMEDICINE

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Abstract

The aim of the study was to find out the phytochemical evaluation, cytotoxic, anthelmintic & thrombolytic activity from the leaves of *Mikania micrantha*. *Mikania micrantha* (Asteraceae), commonly known as mile-a-minute weed is an extremely fast-growing, perennial creeping weed. To study for Phytochemical evaluation was determined through qualitative analysis. The cytotoxic activity was determined by using brine shrimp lethality bioassay. Anthelmintic activity by the study of paralysis and death time and was compared with albendazole as the reference standard, Thrombolytic activity by clot disruption. Phytochemical evaluation indicates the presence of chemical constituents including flavonoids, steroids, saponins & some amount of alkaloids & glycosides. In cytotoxicity assay, the LC50 values of the sample were (7.51) µl/ml where the LC50 values of the standard potassium dichromate were (124.13) µl/ml as a positive control. In the anthelmintic assay, there is no paralyzed and death worm through ethanol extract whereas standard albendazole showed paralyzed & dead worm. The extract shows (86.87) % for 200mg and (84.15) % for 100mg clot lytic whereas standard streptokinase shows (87.33) % for 30,000 I.U and (82.11) % for 15,000 I.U clot lytic activity in thrombolytic activity assay. This study shows that the ethanol extract of *Mikania micrantha* has some bioactivity but further compound isolation is necessary to confirm the activities of individual compounds.

Keywords: *Mikania micrantha, Cytotoxicity, Anthelmintic, Thrombolytic, Phytochemical evaluation.*

Introduction

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases [1]. An herbal-based traditional medical practice that uses various plant materials in modalities considered both preventive and therapeutic. Phytomedicine or the use of herbal medicine with therapeutic properties has played a significant role throughout history [2, 3].

Studies on the natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential therapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs [4, 5].

A number of modern drugs like aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine, tubocurarine, and artemisinin are examples, which were originally discovered through observations of traditional cure methods of indigenous people [6]

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies [7]. In developing countries including Bangladesh, about 75% of the populations rely on different forms of traditional medicine for their primary health care [8]. The high cost of imported conventional drugs or inaccessibility to western health care facility, imply that traditional model of health care is the main form of health care that is affordable and available to our rural people [5]. However, the potential benefits of herbal medicines could lie in their high acceptance by patients, efficacy, relative safety and low costs [9].

Mikania micrantha (Asteraceae), commonly known as mile-a-minute weed, is an extremely fast-growing, perennial creeping weed. It's natively distributed in Central and South America. Since the 1980s it has been present in south China. Invasive Species Specialist Group of IUCN listed the weed as one of the world's 100 worst invasive alien species [10].

Different parts of the plant *Mikania micrantha* are used to treat jaundice, fever, colds, dysentery, rheumatism, respiratory diseases, and scorpion stings and also reported as good haemostatic agents. It is also used to evaluate the analgesic and anti-inflammatory activity of the ethanolic extracts of leaves of *Mikania micrantha* [11].

Mikania micrantha is a widespread weed which has various ethno pharmacological uses. It is used to stop minor external bleeding and heal cuts but its medicinal properties are still not fully discovered. In Bangladesh and India, it is used to treat the gastric ulcer and as a local antiseptic. From the literature survey, it seems that *Mikania micrantha* have some biologically active compound. Some chemical & pharmacological works have been done on this plant. The work is an attempt to evaluate the possible biochemical, pharmacological & toxicological activities of the crude extract.

Antimicrobial constituents of the leaves of M. micrantha were isolated using bioactivity- guided fractionation. The inhibit hypha growth method and inhibit spore germination method is used to evaluate the antifungal activity of the isolated compounds. Antibacterial activity was assessed using the minimum inhibitory concentrations and the minimum bactericidal concentrations (MBCs). The result of bioassay showed that all of the isolated compounds were effective against tested strains and deoxymikanolide showed the strongest activity [12].

Mikania micrantha has a beneficial effect in the treatment of diabetes mellitus and can form a part of therapy in its management [13].

Evaluation and screening of thrombolytic activity of the crude ethanolic extract of the powdered plant. The purpose of a fibrinolytic drug is to dissolve thrombin in acutely occluded coronary arteries thereby to restore blood supply to ischemic myocardium, to limit necrosis and to improve prognosis [14]. The thrombolytic activity of the leaf extracts of *Mikania micrantha* has not been evaluated. Accordingly, this prompted us to investigate the thrombolytic activity of *Mikania micrantha*.

Most anthelmintics were discovered and developed for use in the veterinary field, where helminths significantly impact health and productivity. Few companies are searching for new compounds for use in human medicine, but commercial competition has produced a steady supply of new products for the veterinarian [15]. This study is also designed for anthelmintic activity test.

Chemoprevention by natural products may be considered a promising approach to cancer control and management [16]. Many studies have demonstrated antiproliferative, cytostatic and cytotoxic activities of phytochemicals against cancer cells [17]. The antitumor activity of this plant extract was tested for cytotoxic activity.

The ethyl acetate extracts used in Fungicidal efficacy and trypsin-inhibiting activity of the whole plant, flowers, and leaves. Fourteen phenolic compounds, including two new ones, were isolated from the aerial parts of M. micrantha [18, 19]

In the present study, we evaluate the phytochemical, cytotoxic, anthelmintic and thrombolytic activity of *Mikania micrantha* leaves.

Material and Method

Collection of Plants and identification

The plant leaves were collected from Feni month of November at day time. During collection, the plant leaves were not washed or cleaned by water due to the chance of hydrolysis, oxidation and other types of chemical degradation. The dust that was attached to the plant leaves were removed by handshaking. During collection, any type of adulteration was strongly prohibited. The plant leaves were identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The registration number is 45748 (DACB).

Extraction and Isolation

The plant leaves were dried by shed drying for about twenty days. The leaves were cut into small pieces and the shade-dried materials were ground into a fine powder which had been made completely devoid of any water content. Then 200 gm materials were macerated into sufficient ethanol for 15 days and kept at room temperature. After filtration through Whatman filter paper, the filtrates were concentrated at 400°C with rotary evaporation. The concentrated extracts were then preserved in individual containers in the refrigerator.

Phytochemical screening

Phytochemical screening is the extraction, screening, and identification of medicinally active compounds found in plant parts e.g., leaves, stems, barks, roots, etc. or whole plant. Flavonoids, alkaloids, tannins, antioxidants, glycosides, etc. are different major compounds having medicinal properties in a plant. The collected plant extracts of *Mikania micrantha* was subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents including alkaloids, glycosides, steroids, gums, and tannins.

Cytotoxic activity

Cytotoxic activity of the extract was carried out according to the Meyer method (Meyer et al. 1982). Artemia salina leach (brine shrimp eggs) was used as the test organism. It was hatched in simulated sea water. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. Different concentration of extract (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/mL) was prepared using dimethyl sulfoxide (DMSO) in seawater. A set of seven test tubes were used where 10 shrimps were taken and a solution of different concentration was applied to it. At last, the final volume was adjusted with saline water and kept for 24 h. Vincristine sulfate was used as a standard. The lethal concentration LC50 of the test samples after 24h was obtained by a plot of percentage of the shrimps killed against the sample concentration.

Anthelmintic activity

Preparation of drug & extract solutions

The standard drug albendazole was received from Lazz pharma, kalabagan, Dhaka. Standard drug Albendazole and ethanol extract of *Mikania micrantha* were prepared as 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, and 100 mg/ml concentrations using water [23].

Procedure

Earthworms were placed in Petri dishes containing extract and concentrations, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, and 100 mg/ml of solutions. Albendazole solution was used as reference standard drug and distilled water as a control and distilled water as negative control. If any worm did not move the Petri dishes content in the wash basin and allowing the worms to move freely. By tapping the end of each worm with the index finger and applying a bit of pressure, the worms that were alive showed motility and those dead were non mobile. The motile worms were returned to the respective Petri dishes containing drug solutions, and the process was carried out again. The time is taken for paralysis, motility activity of any sort and death time of worms were observed and recorded after ascertaining that the worms did not move either when shaken vigorously nor when dipped in warm water (50°C)

Thrombolytic activity

Preparation of extract dose

Extract Concentration, Stock solution = 100mg/10ml. Standard: Streptokinase 1500000, IU/5ml, Dose: 30000 IU in 100µl [24].

Procedure

In vitro clot lysis activity of the leaves was carried out according to the method with minor modifications. With ethical considerations, and aseptic precaution, 5 ml of venous blood was drawn from healthy volunteers (n = 3) having no history of smoking, taking lipid-lowering drugs, oral contraceptive or anticoagulant therapy and transferred to the different pre-weighed sterile micro-centrifuge tube (1 ml/tube) [25]. The micro-centrifuged tubes were subjected to incubation at 37°C for 45 min. After the formation of a clot, serum was completely removed from the tubes (carried out without disturbing the clot formed) and each tube having clot was again weighed to determine the weight of the clot

(clot weight = weight of clot containing tube – the weight of tube alone)

To each micro-centrifuge tube containing pre-weighed clot, 100 µl solution of different extracts, concentration 1 mg/mL, were added accordingly. As a positive control, 100 µl of streptokinase and as a negative thrombolytic control, 100 µl of sterilized distilled water were separately added to the control tubes numbered. Then all the tubes were incubated again at 37°C for 90 min and observed for clot lysis. After incubation, the obtained fluid was removed from the tubes and they were again weighed to observe the difference in weight after clot disruption. At last, the difference obtained in weight was calculated and the result was expressed as the percentage of clot lysis following the underneath equation.

% of clot lysis = (wt. of lysis clot /initial clot wt.) × 100

Statistical Analysis

All values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA and the statistically significant differences were analyzed using a paired t-test. $p<0.05$ was considered statistically significant.

Result and Discussion

Chemical group test evaluation

A wide range of chemical test was done for the identification of major classes of therapeutically important phytochemicals. After these chemical group test, it is evident that in the plant *Mikania micrantha* leaves have flavonoids, steroids, saponins & some amount of alkaloids & glycosides. The following table represents the phytochemicals present in *Mikania micrantha* leaves **[Table-1].**

Cytotoxicity evaluation

The lethal concentration (LC50) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best -fit line was obtained from the curve data by means of regression analysis. Potassium dichromate was used as positive control and the LC50 compared with the negative control. The crude ethanolic extract, standard, and control show this effect **[Table-2], [Figure 1].**

The lethal concentration (LC50) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration and the best -fit line was obtained from the curve data by means

of regression analysis. Potassium dichromate was used as a positive control. The LC50 of the extract is 7.51µg/ml whereas the LC50 of the standard is 124.13µg/ml.

Anthelmintic activity assay

The ethanolic extract of plant *Mikania micrantha* caused paralysis but there is no dead earthworm. The reference drug albendazole showed the time of paralysis and death. Drug concentrations, Paralyzing time and death time of ethanolic extract, standard and control are shown in **[Table-3].** Evaluation of anthelmintic activity was compared with reference standard albendazole. Albendazole showed effective paralyzing & death time in a dose-depended manner. Since there was no death of any earthworm through the extract, so we can say that the extract does not have anthelmintic activity.

Thrombolytic activity assay

The thrombolytic activity was determined through two concentrations of extract & compared with two concentration of the standard. The lysis of clot for extract (200mg) & extract (100mg) are 86.87% and 84.15% compared whereas the clot lysis for standard streptokinase (30,000IU) & streptokinase (15,000 IU) are 87.33% & 82.11%.and the lysis by the control is very negligible 2.22% & 3.30% **[Table-4].** $p<0.05$ was considered statistically significant & the result of significance is $p< 0.00002$. Since the extract solution showed enough clot lysis compared with the standard it strongly noticed that the extract has significant thrombolytic activity.

Conclusion

The experiment showed that the ethanol extract of leaves of M. micrantha shows the presence of flavonoids, steroids, saponins and some amount of alkaloids and glycosides through phytochemical screening. The extract also has the thrombolytic activities enough cytotoxicity and don't have any anthelmintic activity. These compounds show these activities because the biological activities of plants may be due to the presence of a diverse group of chemical compounds. Alkaloids show the cytotoxicity [26]. Flavonoids show the thrombolytic activity [27]. Hence this study was conducted by crude extract, further advanced studies will be carried out for compound isolation and the necessary test will be performed to observe which compounds are actually responsible for specific effects.

References

[1] Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research. 2007;10(2).

[2] Mohamed IN, Shuid AN, Borhanuddin B, Fozi NF. The application of phytomedicine in modern drug development. The Internet Journal of Herbal and Plant Medicine. 2012;1(2):1-9.

[3] Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola L*. fruit. J Herb Med Toxicol. 2008;2(2):51-4.

[4] Ramesh BN, Mahalakshmi AM, Mallappa SH. Towards a better understanding of updated ethnopharmacology of *Celosia argentea L*. Int J Pharm Pharm Sci. 2013;5(3):54-9.

[5] Nidavani RB, Mahalakshmi AM. Teak (Tectona grandis Linn.): a renowned timber plant with potential medicinal values. Inter J Pharm Pharm Sci. 2014;6(1):48-54.

[6] Nidavani RB, Mahalakshmi AM, Seema M, Krishna KL. PHARMACOLOGY OF CELOSIA ARGENTEA L. Journal of Atoms and Molecules. 2014;4(1):635.

[7] Mahalakshmi AM, Nidavani RB. *Physalis angulata L.*: An ethnopharmacological review. Indo American Journal of Pharmaceutical Research. 2014.

[8] Bastos GN, Silveira AJ, Salgado CG, Picanço-Diniz DL, Do Nascimento JL. *Physalis angulata* extract exerts anti-inflammatory effects in rats by inhibiting different pathways. Journal of Ethnopharmacology. 2008;118(2):246-51.

[9] Hsieh WT, Huang KY, Lin HY, Chung JG. *Physalis angulata* induced G2/M phase arrest in human breast cancer cells. Food and Chemical Toxicology. 2006;44(7):974-83.

[10] Chinmoyee Deori, Geetamoni Dutta, Swarnamoni Das, Daisy Phukan, Gaurav Gogoi. To Evaluate the Anti-Inflammatory Activity of Ethanolic Extract of Leaves of Mikaniamicrantha On

Experimental Animal Models. J. Evolution Med. Dent. Sci. 2017;6(50):3818-3821, DOI: 10.14260/Jemds/2017/825.

[11] Chinmoyee Deori, Geetamoni Dutta, Swarnamoni Das, Daisy Phukan. Analgesic Activity of Ethanolic Extract of Leaves of Mikaniamicrantha on Experimental Animal Models. International Journal of Pharmaceutical Science.2016; 7(3): 168.

[12] Li Y, Li J, Li Y, Wang X-x, Cao A. Antimicrobial Constituents of the Leaves of *Mikania micrantha*. H.B.K. PLoS One. 2013; 8(10); 76725.

[13] W.H.Wan Nurhayati, T. Norli Arlizan, S.Nurdiana, ShahAlam, Selangor. Effect of *Mikania micrantha* leaf extract on the level of blood glucose and hepatic glycogen in the normal and alloxan-induced diabetic rats. NPAIJ, 9(10), 2013 [398-402].

[14] Laurence DR, Bennett PN. Clinical Pharmacology. Churchill Livingstone; 1992; 7(1); p. 483.

[15] George A. Conder, in Antibiotic and Chemotherapy (Ninth Edition), 2010.

[16] Karikas et al. Anticancer and chemopreventive natural products: some biochemical and therapeutic aspects. J Buon. 2010; 15: 627-638.

[17] Gordaliza et al. Natural products as leads to anticancer drugs. ClinTranslOncol. 2017; 9; 767-776

[18] Madhavankutty, Jyothilakshmi, Mathew Jyothis, MukalelSankunniLatha. Antidermatophytic Activity of Mikaniania micrantha Kunth: An Invasive Weed. Pharmacognosy research. 2015; 7(Suppl 1); 20-25.

[19] Li-Mei Dong, Xu-Chao Jia, Qing-Wen Luo, Qiang Zhang, Bi Luo, Wen-Bin Liu, Xu Zhang, Qiao-Lin Xu, and Jian-Wen Tan. Phenolics from *Mikania micrantha* and Their Antioxidant Activity. Molecules. 2017; 22(7); 1140.

[20] Kavitha Sama and Rajeshwari Sivaraj. Pharmacognostical and phytochemical screening of fruit and leaves of *Cissus arnottiana*. Asian Journal of Pharmaceutical and Clinical Research. 2012; Vol 5, Suppl 2.

[21] Md. Irfan Amin Chowdhury, MitaliDebnath, Md. Foyez Ahmad, Mohammad Nazmul Alam, Arif Mohammad Saleh, Sudipta Chowdhury, Rajesh Barua, Muhammad Moin Uddin Mazumdar, Abu Hena Mustafa Kamal. Potential Phytochemical, Analgesic and Anticancerous Activities of Cymbopogon citrates leaf. American Journal of Biomedical Research. 2015; Vol. 3, No. 4, pp 66-70.

[22] Avijitdey, Mohammad Ahsanul Akbar, S, M Abdur Rahman. Biological Investigation of the plant Bohervia Repens. Lambert Academic Publishing. 2012; 47-51.

[23] S. S. Das, Monalisha Dey, and A. K. Ghosh. Determination of Anthelmintic Activity of the Leaf and Bark Extract of Tamarindus Indica Linn. Indian J Pharm Sci. 2011 Jan-Feb; 73(1): 104–107.

[24] Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of Fagoniaarabica (Dhamasa) on in vitro thrombolysis. BMC Complement Altern Med. 2007; 7:36.

[25] Ali Ramjan, Marjan Hossain, Jannatul Ferdous Runa, Hasanuzzaman Md, and Islam Mahmodul. Evaluation of the thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna J Phytomed. 2014; 4(6); 224-462.

[26] Vital PG, Rivera WL. Antimicrobial activity, cytotoxicity, and phytochemical screening of Voacanga globosa (Blanco) Merr. leaf extract (Apocynaceae). Asian Pac J Trop Med; 2011; 4(10); 824–828.

[27] Mohammad Sekendar Ali, Mohammad Ruhul Amin, Chowdhury Mohammad Imtiaz kamal, and Mohammad Ahossain. In vitro antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the A. philippense L. leaves. Asian Pac J Trop Biomed. 2013; 3(6): 464–469.

Table-1: List of phytochemicals found in *Mikania micrantha leaves*

Chemical groups	Name of the Tests	Findings	Result
Alkaloids	Mayer's test	Yellow color precipitation	+
	Dragendroff's test	Orange brown color precipitation	+
Saponins	Frothing test	Produced foam	+
Glycosides	Sodium hydroxide test	The yellow color was not considered	–
	Fehling's test	No red brick red precipitation	+
Steroids	Sulphuric acid test	The red color was formed	+
Gums	Molishc's test	No red violet ring	–
Reducing Sugar test	Benedict's test	No red color precipitation	–
	Fehling's test	No red brick precipitation	–
Flavonoids	Hydrochloric acid test	red color formed	+

Table-2: Test result of the cytotoxic activity of different samples

Sample	Concentration (µg/ml)	No. of nauplii taken	No. of dead nauplii	% Mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Extract	400	10	10	100	7.51*	248.91
	200	10	9	90		
	100	10	9	90		
	50	10	8	80		
	25	10	8	80		
	12.5	10	7	70		
	6.25	10	5	50		
	3.13	10	3	30		
	1.57	10	2	20		
	0.78	10	1	10		
Potassium dichromate	400	10	7	70	124.13**	477.48
	200	10	7	70		
	100	10	6	60		
	50	10	6	60		
	25	10	4	40		
	12.5	10	4	40		
	6.25	10	4	40		
	3.13	10	3	30		
	1.57	10	2	20		
	0.78	10	2	20		
Control	10	10	0	0	-	-

*Linear equation: y = 0.1657x + 48.755
**Linear equation: y = 0.1132x + 35.949

Table-3: Concentration, paralyzing time & death time sample, standard & control

Group	Concentration (mg/ml)	Paralyzing time (min)	Death time (min)
Distilled Water	-	NA	NA
Ethanolic Leaf extract	6.25	NA	NA
	12.5	NA	NA
	25	NA	NA
	50	NA	NA
	100	NA	NA
Albendazole	6.25	29	36
	12.5	26	34
	25	23	30
	50	18	24
	100	15	20

Table-4: % of clot lysis in different concentrations of standard, sample, and control

Treatment	Concentrations	% of clot lysis
Extract	200 mg	86.87
	100 mg	84.15
Standard	30,000 IU	87.33
	15,000 IU	82.11
Control	-	2.22
	-	3.30

Figure 1: Comparison between the cytotoxic effect of sample and standard