



If you did not document it, you did not do it.



Antibacterial and Cytotoxic Activity of Ethanol Extract of *Mikania Cordata* (Burm.F.) B.L. Robinson Leaves

Md. Sekendar Ali, Md. Saiful Islam, Md. Masudur Rahman, Md. Rabiul Islam, Mohammed Aktar Sayeed and Md. Rafikul Islam*

Department of Pharmacy, International Islamic University Chittagong, Bangladesh

ABSTRACT

The purpose of the present study to examine the antibacterial and cytotoxic properties of ethanol extract of leaves of *Mikania cordata* (Burm.f.) B.L. Robinson. To determine antibacterial activities, the extract was tested against four Gram positive and six Gram negative bacteria at three concentrations (500, 800, 1000 $\mu\text{g disc}^{-1}$) through disc diffusion method. The extract showed moderate antibacterial actions and that was increased by increasing the concentration of the sample. The maximum antimicrobial potential was obtained against *Shigella flexneri* and no sensitivity was found for *Klebsiella* sp. Comparatively gram-positive bacteria demonstrated more susceptibility to the extract than gram-negative bacteria. Cytotoxic property of the sample was done using Brine shrimp lethality bioassay where it did not show noticeable toxicity. So, our present study reveals that the leaves extract of *M. cordata* possess considerable antibacterial properties along with lesser amount of cytotoxicity.

KEY WORDS:

Mikania cordata, Ethanol, Antibacterial, Cytotoxic

received on 09-04-2011

modified on 05-05-2011

accepted on 06-05-2011

available online 15-05-2011

www.jbclinpharm.com

INTRODUCTION

Infectious diseases have been, in the past and continue to remain, a leading cause of morbidity, disability and mortality in the world. Approximately 15 million people die each year due to infectious diseases – nearly all live in developing countries [1]. Their control is a constant challenge that faces health workers and public health officials in both industrialized and developing countries. Only one infectious disease, smallpox, has been eradicated and stands as a landmark in the history of the control of infectious diseases. The international community is now embarked on the path to eradicate poliomyelitis and dracunculiasis (guinea worm infection) by the close of this century. Other infectious diseases, such as malaria and tuberculosis, have foiled eradication attempts or control efforts and are re-emerging as

increasing threats in many countries. Some infectious diseases, such as tetanus, will always be a threat if control measures are not maintained. Drugs that once seemed invincible are losing their effectiveness for a wide range of community-acquired infections, including tuberculosis, gonorrhoea, pneumococcal infections (a leading cause of otitis media, pneumonia, and meningitis), and for hospital-acquired enterococcal and staphylococcal infections [2]. Indiscriminate and unconcerned use of antibiotics has led to increased microbial resistance. Consequently, newer agents have been brought in at increased economic costs to the patient but they too have become inefficacious in due course and pose a great threat to human health in world. So, noble, emerging and re-emerging infectious diseases have become a focus for the development of new cost-effective drug in both industrialized and developing countries.

Nature has been a source of medical treatments for thousands of years and today plant based systems

*Corresponding Author:
Email: rafik_ruph@yahoo.com

continue to play an important role in the primary health care of 80% of the world's population [3, 4]. According to a study by Grifo and colleagues, 84 of a representative 150 prescription drugs in the United States fell into the category of natural products and related drugs [5]. They were prescribed predominantly as anti-allergic/pulmonary/respiratory agents, analgesics, cardiovascular drugs, and for infectious diseases. The increasing prevalence of multidrug resistance in pathogenic microorganisms, as well as, undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. Common side effects are allergic reaction or toxic reactions or mutagenic substances. Therefore, medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [6]. As natural products have been elaborated within living systems, they are often perceived as showing more "Drug likeness" and biological friendliness than totally synthetic molecules," making them good candidates for further drug development [7-9]. In continuation of this effort, we study antibacterial potential of *Mikania cordata* and its cytotoxic properties.

The medicinal plant, *Mikania cordata* (Burm. f.) B. L. Rob is also known as heartleaf hempvine belongs to the family Asteraceae. It is perennial, fast-growing, creeping or twining vine with ribbed stems 3-6 m long; leaves opposite, heart-shaped. The plants are found throughout the tropical regions of Asia, Africa and South America.

It is widely used as traditional medicine against gastric ulcer and a variety of other diseases. There are several scientific reports on different parts of the plant. Mandal PK *et al.* (2006) reported that the root extract of the plant possessed hepatoprotective activity [10]. The leaves of *Mikania* species are used in folk medicine have antispasmodic, antiulcerogenic and antirheumatic agents [11, 12]. The plant is also used to treat asthma, respiratory problems and infections. Haque *et al.*, (2011) reported that among three fractions (methanol, methylene chloride and n-hexane) of aerial parts of the *Mikania cordata*, methylene chloride fractions showed maximum antimicrobial activity [13]. But there is no scientific report on antibacterial activity of ethanol extract of leaves of the plant. So, the aim of our ongoing research is to investigate the antibacterial and cytotoxic properties of the ethanol extract of *Mikania cordata* leaves.

MATERIALS AND METHODS

Preparation of Extract

Ethanol extract of *Mikania cordata* leaves was used in the present study. The matured leaves were collected in the month of October 2010 from Chittagong, Bangladesh. The leaves were dried under shade and pulverized in a mechanical grinder. The powder was extracted with ethanol. The extracted juice was filtered by using a clean cloth and then filter paper. The extract was concentrated first in Rotary vacuum evaporator and then on water bath. The extract was then kept in a refrigerator for antimicrobial and cytotoxic properties.

Antibacterial Assay

The disc diffusion method was used to test antibacterial activity against ten bacteria [14]. The antimicrobial activity of ethanol extract of *Mikania cordata* leaves were determined at three concentration (500, 800, 1000 $\mu\text{g disc}^{-1}$). Solutions of known concentration (10 mg/ml) of the test sample were made by dissolving measured amount of the samples in calculated volume of solvent. Dried and sterilized filter paper discs (5.5 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the pathogenic test microorganisms. Standard antibiotic discs (Kanamycin 30 $\mu\text{g disc}^{-1}$) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4°C) for 1 h to allow diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37°C for 12 h to allow growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the mean of the reading was taken.

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [15, 16]. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquari-

um shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. The test sample (extract) were prepared by dissolving them in DMSO (not more than 50 μ L in 5 mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 μ g/ml. A vial containing 50 μ L DMSO diluted to 5 mL was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\text{Mortality} = \frac{N_t}{N_0} \times 100$$

where, N_t = Number of killed nauplii after 24 hrs of incubation,

N_0 = Number of total nauplii transferred i.e 10.

The LC_{50} (Median lethal concentration) was then determined using Regression analysis.

RESULTS

Antimicrobial Test

The antibacterial activities of the extract obtained from the plant under study by the disc diffusion method are shown in Table 1. The extract of the plant screened at three concentration (500, 800, 1000 μ g disc⁻¹) against four Gram positive and six Gram negative bacteria and showed various levels of antimicrobial activities with the different zone of inhibition. Larger zone of inhibition was obtained with the increasing concentration of the sample and all tested microorganisms were more sensitive at 1000 μ g disc⁻¹. So, the antimicrobial activity is directly proportional to the concentration of the extract. At 500 μ g disc⁻¹, the extract showed no sensitivity against *Bacillus subtilis*, *Bacillus megaterium*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Klebsiella sp*. The extract showed maximum inhibition against *Staphylococcus aureus* and *Shigella flexneri* whereas no activity was found against *Klebsiella sp* at all three concentrations. Kanamycin disc (30 μ g/disc) was used as standard whose zone of inhibition ranged from 25-38 mm. No zone of inhibition was found for negative control disc.

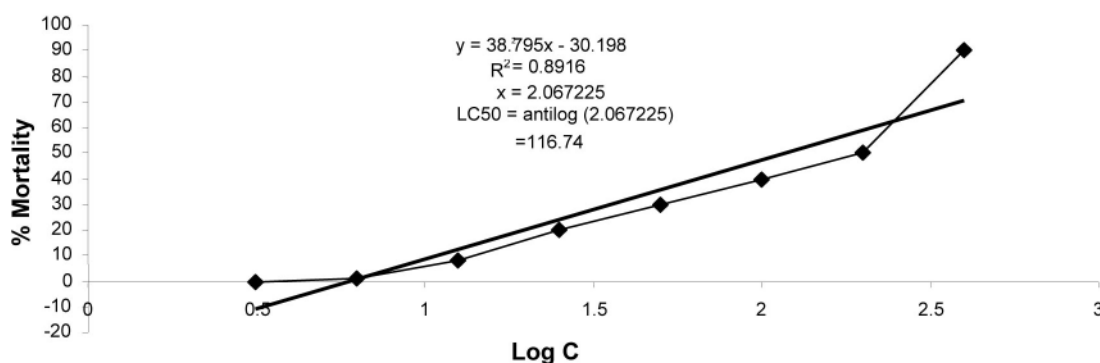
Brine shrimp lethality bioassay

Following the procedure of Meyer, the lethality of the crude extract of *Mikania cordata* leaves to brine shrimp was determined on *Artemia salina* after 24 h

Table 1: Antibacterial effect of ethanol extract of *Mikania cordata* leaves

Name of the Bacteria	Zone of Inhibition (mm)			
	Kanamycin (30 μ g/disc)	Leaves		
		500 μ g/disc	800 μ g/disc	1000 μ g/disc
Gram positive				
<i>Staphylococcus aureus</i>	38	15	16	17
<i>Bacillus subtilis</i>	27	-	7	10.5
<i>Bacillus cereus</i>	28	7	10	11
<i>Bacillus megaterium</i>	30	-	6.5	6.5
Gram negative				
<i>Salmonella typhi</i>	27	7	11	15
<i>Shigella dysenteriae</i>	30	-	7	9
<i>Pseudomonas aeruginosa</i>	35	-	7	14
<i>Escherichia coli</i>	25	7	10	12
<i>Klebsiella sp</i>	30	-	-	-
<i>Shigella flexneri</i>	35	12	17	20

Figure 1: Determination of LC₅₀ value for *Mikania cordata* leaves from linear Correlation between log concentrations versus % mortality



of exposure the samples, the positive control and vincristine sulphate. This technique was applied for the determination of general toxic property of the plant extract. From the Figure 1, we have observed that the LC₅₀ value of the extract was 116.74 µg/ml and that for standard vincristine sulphate was 9.5 µg/ml. No mortality was found in the control group, using DMSO and sea water.

DISCUSSION

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has regained public attention over the past 20 years as this type of medicine is easily accessible in some regions. A postulation denoted that by 2010, at least two-thirds of Americans will opt for alternative therapies [17]. The non prescription use of medicinal plants is cited today as an important health problem, in particularly their toxicity to the kidney [18]. So, if the plant extract found to show significant antimicrobial activities must take into account acceptable levels of toxicity.

The results obtained in our present study indicated that the crude ethanol extract of *Mikania cordata* leaves showed better antibacterial activities at higher concentrations against both the Gram positive and Gram negative bacteria. The extract showed maximum and minimum activities against *Shigella flexneri* and *Klebsiella* sp respectively; both of which are Gram negative bacteria. All bacteria except *Klebsiella* sp showed better sensitivity to the plant extract at 800 and 1000 µg/disc while no activity was found against five bacteria among ten at 500 µg/disc concentrations. The extract did not

cause any apparent *in vitro* toxicity in brine-shrimp lethality bioassay comparing standard drug vincristine sulphate. But the extract demonstrated antibacterial action at higher concentration that may cause hazardous effect to the patient. So, further studies are needed to understand the mode of action of the individual compounds after separation from the extract and this may lead to develop a new antimicrobial agent in future.

REFERENCE

1. WHO. The global burden of disease: 2004 update. World Health Organization, 2008. Available at www.who.int/healthinfo/global_burden_disease/2004_report_update/en/index.html. Accessed Feb 26, 2011
2. Robert J Kim-Farley. New, Emerging and Re-emerging Infectious Diseases. Regional Health Forum WHO South-East Asia Region, 2006. Available at http://www.searo.who.int/en/Section1243/Section1310/Section1343/Section1344/Section1350_5239.htm. Accessed Feb 26, 2011
3. Farnsworth NR, Akerele O, Bingel AS, et al. Medicinal plants in therapy. Bull WHO. 1985; 63(6): 965–981.
4. Duraipandiyar V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India, BMC Comp. Alter Med. 2006; 6: 35-41.
5. Grifo F, Newman D, Fairfield A, et al. The origins of prescription drugs. In: Biodiversity and Human Health. Grifo F, Rosenthal J, eds. Washington DC: Island Press; 1997:131- 163.
6. Machado TB, Leal ICR, Amaral ACF, et al. Brazilian phytopharmaceuticals evaluation against hospital bacteria. Phytother Res. 2005; 19: 519-525.
7. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov. 2005; 4: 206-220.
8. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci. 2005; 78: 431–441.
9. Drahl C, Cravatt BF, Sorensen EJ. Protein-reactive natural products. Angew Chem Int Ed Engl. 2005; 44: 5788–5809.
10. Ahmed M. Further dilactones from *Mikania cordata*. Pharmazie. 1990; 45: 697.
11. Mandal PK, Bishayee A, Mukherjee JR, et al. *Mikania cordata* root extract in the inhibition of lipid peroxidation and reduction of enzyme leakage in mice with carbon tetrachloride induced liver damage. Phytotherapy Research. 1992; 6(4): 227–229.

12. Pereira NA, Pereira BMR, Nascimento MC, et al. Pharmacological screening of plants recommended by folk medicine as snake venom antidotes, IV. Protection against jararaca venom by isolated constituents. *Planta Med.* 1994; 60: 99–100.
13. Mosaddik MA, Alam KM. The anti-ulcerogenic effect of an alkaloidal fraction from *Mikania cordata* on diclofenac sodium-induced gastrointestinal lesions in rat. *J Pharm Pharmacol.* 2000; 52 (9), 1157–1162.
14. Chowdhury NS, Alam MB, Zahan R, et al. Antimicrobial and toxicity studies of different fractions of the aerial parts of the *Mikania cordata*. *Int J of Pharm and Life Sci.* 2011; 2 (3): 592-598.
15. Bauer AW, Kirby WMM, Sherris JC, et al. Antibiotic susceptibility testing by a standardized disc diffusion method. *Am J Clin Pathol.* 1966; 45: 493-496.
16. Meyer BN, NR Ferrigni, JE Putnam, et al. Brine shrimp: A convenient general bioassay for active plants constituents. *J Med Plant Res.* 1982; 45: 31-34.
17. Zhao G, Hui Y, Rupprecht JK, et al. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. *J Nat Prod.* 1992; 55: 347-356.
18. Humber JM, The role of complementary and alternative medicine: accommodating pluralism. *J Am Med Assoc.* 2002; 288: 1655-1656.
19. Mendonça-Filho RR. Bioactive Phytochemicals: New Approaches in the Phytosciences. In: *Modern phytomedicine: Turning medicinal plants into drugs.* Ahmad I, Aqil F and Owais M, eds. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA; 2006: 1-24.