



## RESEARCH PAPER

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***In vitro* phytochemical, cytotoxicity and mineral composition analyses of *Micania Cordata* (Bumr.f.) B.L. robinson leaves**

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**Abstract**

*Mikania cordata* (Family: Asteraceae) has been used in traditional medication for the ailments of human diseases. The present study was designed to explore the phytochemical constituents and cytotoxic activity of ethanolic extract of *M. cordata* (Bumr.f.) B.L. Robinson leaves. The preliminary phytochemical screening revealed the presence of reducing sugars, flavonoids, saponins, alkaloids, tannins, phlobotannins, steroids, cardiac glycosides, amino acids in the ethanolic extract of the plant leaves. The ethanolic extract did not show any noticeable toxicity in brine shrimp lethality bioassay. The LC<sub>50</sub> and LC<sub>90</sub> values were found to be 102.09 and 971.63 µg/ml respectively for the plant extract. The mineral composition of the plant leaves was also analyzed in the present study by atomic absorption spectrophotometer. The mean (±SD) content of calcium, potassium, magnesium, phosphorus, sulphur, iron, manganese and zinc was 306.00±3.60, 116.67±1.53, 54.67±1.15, 0.09±0.005, 0.58±0.02, 85.67±1.53, 5.67±0.60 and 36.00±1.73 (in µg/g) respectively in the leaves of *M. cordata*. Thus the study reveals that leave's extract contains several phytochemicals, show less cytotoxicity and the leaves also contain considerable amount of minerals. The findings of the study provide the basis for its wide use as the therapeutic both in traditional and folk medicine.

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## Introduction

Complementary and alternative medicines commonly known as traditional medicines still remain as the primary form of treatment of diseases for a majority of people in developing countries despite the availability of synthetic drugs, and the use is rapidly increasing as the scientific community finds profound nutritive and pharmacological potentials. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicines for their primary healthcare needs (WHO, 2003). Interestingly, the market demands for medicinal herbs are likely to remain high because many of the active ingredients in medicinal plants cannot yet be prepared synthetically (Thomas, 1995). *Mikania cordata* (Bumr.f.) B.L. Robinson, also known as heartleaf hempvine, belongs to the family Asteraceae, grows vines at the soil surface or tree. It is locally known as Assamlata, Jermanilata and Taralata. The plant is found throughout the tropical regions of Asia, Africa and South America. The plant has found to possess several medicinal properties such as antiulcer (Paul *et al.*, 2000), analgesic (Ahmed *et al.*, 2001), antiinflammatory (Bhattacharya *et al.*, 1992), anticancer (Bishayee and Chatterjee, 1994), antibacterial (Ali *et al.*, 2011; Nayeem *et al.*, 2011), hepatoprotective (Ahmed, 1990; Mandal *et al.*, 1992), antihelminthic and antiemetic (Bulbul *et al.*, 2013), antistress (Bishayee and Chatterjee, 1995) potentials. The leaves of this plant are traditionally used to stop external bleeding in villages of Bangladesh found by an ethnobotanical survey (Rahmatullah *et al.*, 2011).

The phytochemical constituents of the plants are important because these decide the pharmacological and biological activities such as antioxidant constituents of the plant materials may decide their role against various diseases such as coronary heart diseases and cancers (Kim *et al.*, 1994). Primarily, antioxidant effect is due to phenolic compounds such as phenolic acids, flavonoids and phenolic diterpenes and their mode of action for antioxidant compounds is due to its redox reaction properties which can

absorb and neutralize free radicals by quenching singlet and triplet oxygen (Krauss *et al.*, 2000). Besides several organic compounds, it is now well established that many minerals play a vital role in general well-being as well as in the cure of diseases (Prasad, 1993, Soetan *et al.*, 2010). Many traditional plants contain minerals that have both nutritive and curative values. Cytotoxicity analysis of crude plant extract is widely used to determine at which concentration the plant extract would cause toxicity to cells (Meyer *et al.*, 1982).

Considering the importance of phytochemicals and minerals in maintaining human health and treating various diseases, the present study was conducted to investigate the phytochemical content, cytotoxic activity and mineral composition of *M. cordata* leaves.

## Materials and methods

### Collection of plant materials

Widely available medicinal plant *M. cordata* was collected from Sathania, an upazilla of Chittagong district and in front of Saydur Rahman hall, a male student hall of University of Science and Technology, Chittagong.

### Preparation of plant extracts

The fresh leaves of *M. cordata* were separated from vine, washed with deionized water, and then shed dried at room temperature. After removing the surface water of leaves, these were dried in a hot air oven at 35°C for 3 days. Then the dried materials were placed into a grinding machine to make them a fine powder. The powdered material was subjected to the exhausted extraction using ethanol as the extraction solvent by a soxhlet apparatus. The solvent was evaporated under reduced pressure using rotary evaporator at 78-80°C. The extract was then stored at 4°C until further study.

### Preliminary phytochemical screening

The crude extract was subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test 10% (w/v) solution of

the extract in ethanol was used unless otherwise mentioned in individual test.

#### *Test for reducing sugar*

2 ml of the extract solution was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few minutes. Appearance of a reddish precipitate indicated the presence of reducing sugar.

#### *Test for tannins*

5 ml of the extract solution was placed in a test tube and then 1 ml of 5% ferric chloride solution was added to it. Appearance of a brownish green or blue black color indicated the presence of tannins.

#### *Test for phlobatannins*

5 ml of the extract was added with 10 ml aqueous 1% HCl and then boiled. Development of red precipitation indicated the presence of phlobatannins.

#### *Test for flavonoids*

Few drops of concentrated HCl were added to a small amount of the extract. Immediate development of a red color indicated the presence of flavonoids.

#### *Test for saponins*

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. Appearance of foam persisting for 10 minutes indicated the presence of saponins.

#### *Test for steroids*

5 ml of the extract was added with 10 ml water, and then 2 ml of acetic anhydride and 2 ml H<sub>2</sub>SO<sub>4</sub> were also added. The change of color from violet to blue or green indicated the presence of steroids.

#### *Test for terpenoids*

5 ml of the extract was added with 2 ml chloroform, and then 3ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added. Formation of a layer of radish brown color at the interface confirmed the presence of terpenoids.

#### *Test for alkaloids*

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added. Development of an off-white precipitation indicated the presence of alkaloids

#### *Test for cardiac glycosides*

5 ml of the extract was added with 2 ml glacial acetic acid, then 1ml concentrated H<sub>2</sub>SO<sub>4</sub> was added. Development of a brown ring at the interface indicated the presence of cardiac glycosides.

#### *Test for amino acids*

1 ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple color indicated the presence of amino acids.

#### *Brine shrimp lethality bioassay*

The brine shrimp lethality bioassay on brine shrimp nauplii was performed to predict the cytotoxicity of the ethanolic extract of *M. cordata* leaves (Meyer *et al.*, 1982). For the assay, the extract was dissolved in dimethylsulfoxide (DMSO) and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56 µg/ ml) were obtained by serial dilution technique using simulated seawater. The solutions were then added to the pre-marked vials containing 10 live brine shrimp nauplii in 5 ml simulated seawater. DMSO diluted in seawater and vincristine sulphate were used as negative and positive controls respectively. After 24 hours, 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 and control. The LC<sub>50</sub> (median lethal concentration) and LC<sub>90</sub> were then determined using regression analysis.

#### *Analysis of mineral composition*

The content of calcium, potassium, magnesium, phosphorus, sulphur, iron, manganese and zinc in *M. cordata* leaves was determined by atomic absorption spectrophotometer (AAS) following standard procedure. The samples in the powdered form were accurately weighed (1g) and digested in (3:2) mixture of nitric acid and perchloric acid. After digestion few

drops of concentrated HCl was added. The solution was heated gently and then filtered. The residue was again subjected to digestion and filtrate was collected. The entire filtrate was diluted suitably with distilled deionized water. The dilute filtrate solution was used for analysis of minerals and trace elements by an AAS (AA-7000, Shimadzu, Kyoto, Japan) using suitable hollow cathode lamps. The concentration of different mineral and trace elements was determined by relative method using A.R. grade solutions of elements of interest. Analysis for each sample was carried out in triplicate to get representative results.

## Results

### *Phytochemical screening of ethanolic extract of M. cordata leaves*

For the screening of various phytochemical compounds such as alkaloids, saponins, flavinoids, tannins, phlobatannins, steroids, terpenoids, cardiac glycosides, phytochemical screening test were performed. Data strongly suggested that ethanolic extract of *M. cordata* leaves contain most of the biologically active phytochemical compounds such as reducing sugars, flavonoids, saponins, alkaloids, tannins, phlobotannins, steroids, cardiac glycosides, amino acids except terpenoids.

**Table 1.** Mineral composition of *Micania cordata* leaves.

| Mineral    | Relative content (mean±SD) in µg/g of plant leave |
|------------|---|
| Calcium    | 306.00±3.60                                       |
| Potassium  | 116.67±1.53                                       |
| Magnesium  | 54.67±1.15  |
| Phosphorus | 0.09±0.005  |
| Sulpher    | 0.58±0.02   |
| Iron       | 85.67±1.53  |
| Manganese  | 5.67±0.60   |
| Zinc       | 36.00±1.73  |

### *Brine shrimp lethality bioassay of ethanolic extract of M. cordata leaves*

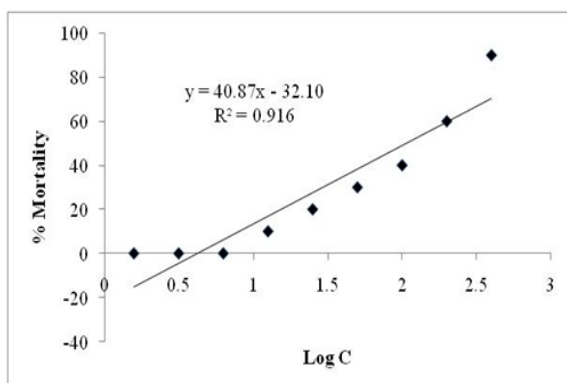
The lethality of the crude ethanolic extract of *M. cordata* leaves to brine shrimp nauplii was determined following the procedure of Meyer *et al.*, 1982. This technique was applied for the determination of general toxic property of the plant extract. From the Figure 1, the LC<sub>50</sub> and LC<sub>90</sub> values of the extract were found to be 102.09 and 971.63 µg/ml respectively. The values were compared against that for vincristine sulphate. No mortality was found in the control group, using DMSO and sea water.

Using the technique of atomic absorption spectrometry, the mineral composition of *M. cordata* leaves were determined. The data regarding mineral content are shown in Table 1. Mineral content of the plant leaves were measured as µg/g and presented as mean±SD values calculated from triplicate values.

## Discussion

The medicinal values of the plant materials may be related to their constituent phytochemicals (Varadarajan *et al.*, 2008). The preliminary phytochemical analysis revealed the presence of important phytoconstituent such as reducing sugars, flavonoids, saponins, alkaloids, tannins, phlobotannins, steroids, cardiac glycosides, amino acids in the ethanolic extract of *M. cordata* leaves. These phytochemicals have important pharmacological and/or medicinal functions such as alkaloids may be used as stimulant of the central nervous system and strong narcotic pain killers (Cordell *et al.*, 2001), steroids have hypotensive and cardiodepressant potentials (Olaleye, 2007), saponins have antimicrobial potentials (Sodipo *et al.*, 2000), flavonoids are active against bacteria (Dixon *et al.*, 1983), tannins have a wide range of anti-infective actions (Haslam, 1996), cardiac glycosides have cardioactive properties that can be used in the

treatment of congestive heart failure and cardiac arrhythmia (Riganti *et al.*, 2011), amino acids and reducing sugars may also have medicinal values since they are important biomolecules. The chemical constituents of the plant has yet not been fully elucidated, therefore further chemical analysis should be conducted to determine the chemicals present in different parts of the plant.



**Fig. 1.** Determination of LC<sub>50</sub> and LC<sub>90</sub> values for ethanolic extract of *M. cordata* leaves Mineral composition of *M. cordata* leaves

The cytotoxic activity of the ethanolic extract of *M. cordata* leaves was evaluated by the mostly used brine shrimp lethality bioassay. The LC<sub>50</sub> and LC<sub>90</sub> values of the extract were 102.09 and 971.63 µg/ml respectively. The cytotoxic activity of the extract of *M. cordata* leaves was previously done and found the similar data (Ali *et al.*, 2011; Nayeem *et al.*, 2011). But because of the geographical and climate variations, plants collected from different areas show different results. The LC<sub>50</sub> values obtained in the brine shrimp bioassay for *M. cordata* shows that the higher effect is presented by leaves extract with less toxicity. Therefore it may cause hazardous effects when intake at higher doses, otherwise at low doses is safe. The BSLB has also been used as a simple biological test for detection of antitumor compounds in plant materials (McLaughlin, 1991). LC<sub>50</sub> values <1000 ppm are considered significant for crude extracts (Meyer *et al.*, 1982). The LC<sub>50</sub> of the *M. cordata* leaves' ethanolic extract indicate that it could be regarded as promising candidate for plant-derived antitumor compounds.

The mineral composition of the *M. cordata* leaves

was determined by atomic absorption spectrophotometer and presented in Table 1. The highest amount of mineral present was calcium, followed by potassium, iron, magnesium, zinc and manganese. Phosphorus and sulphur were also detected but in very low amount. Minerals have a various functions in a number of biological processes and in ailments of diseases. Potassium is the principal intracellular cation and mainly involved in membrane potential and electrical excitation of nerve and muscle cells (Vaskonen, 2003). Magnesium, the most abundant intracellular divalent cation, serves as an essential cofactor for several enzymes necessary for physiologic processes, including neuromuscular function and maintenance of cardiovascular tone (Saris *et al.*, 2000). Calcium is essential for development of bone and teeth, muscle contraction, blood coagulation, nerve transmission, intracellular messenger, activation of enzymes etc (Wiercinsky, 1989). Iron is necessary for haemoglobin formation and also plays an important role in oxygen and electron transfer in human body (Kaya and Incekara, 2000) and normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats (Adeyeye and Otokiti, 1999). Zinc is an essential component of several enzymes and necessary for proper reproduction, wound healing, skin integrity, bone metabolism and proper functioning of taste and eyesight (Prasad, 1995).

### Conclusions

The present study reveals that *M. cordata* leaves contain several important phytochemicals, antitumor compounds and minerals which suggest its medicinal value in folklore medicinal system. However, further large scale studies are necessary to confirm these conclusions.

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### Conflict of Interests

None declared by the authors

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