

Full Length Research Paper

Assessment of phytochemical and pharmacological properties of *Mikania cordata* (Asteraceae) leaves

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The ethanol extract of the dried leaves of *Mikania cordata* (Family-Asteraceae) was investigated for its possible bioactive chemical groups and antinociceptive, cytotoxic and antibacterial activities in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 125 and 250 mg/kg body weight ($p < 0.001$) comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The crude extract produced the moderate cytotoxic activity against brine shrimp *Artemia salina* ($LC_{50}=90$ and $LC_{90}=166$ $\mu\text{g/ml}$). The extract showed antibacterial activity against some types of microorganisms upon which the extract was employed. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: Antimicrobial, antinociceptive, asteraceae, cytotoxic, *Mikania cordata*.

INTRODUCTION

Mikania cordata (Asteraceae, older name Compositae), synonym- *Mikania scandens* auct. Non L., *Eupatorium cordatum* Burm, is a common obnoxious weed native to Bangladesh. This climber small shrub with serrated leaves and many white flowers (Ahmed, 2008) is locally named as Assamlata, Germanlata and Taralata. Usually the flowers bloom in dry season. The plant has been used in traditional herbal medicine of Bangladesh to treat various ailments including pain, inflammations and some other infectious diseases by folklore people (Table 1) (Ghani, 1998a; Ahmed, 1997; Uddin, 2006). *M. cordata* has been reported to have analgesic sesquiterpene dilactone (Ahmed et al., 2001), anti-ulcerogenic effect on diclofenac sodium-induced gastrointestinal lesions in rats (Paul, 2000; Mosaddik, 2000), anticarcinogenic biological response in hepatic biotransformation systems (Bishayee, 1994a), protective effects in gastric erosions in experimental animals (Bishayee, 1994b), stimulation of hepatic protein synthesis in carbon tetrachloride-induced hepatotoxicity in mice (Mandal, 1992), inhibition of

leukotriene and platelet activating factor synthesis (Ysrael, 1990). Three chemical compounds have been isolated from it. Among them, a sesquiterpene dilactone and mikanins, sesquiterpene lactone scandenolide and a flavonol are reported (Ahmed et al., 2001; Ysrael, 1990). Antibacterial and cytotoxic activities of the plant collected from the other climate and geographical source than this study has reported (Ali et al., 2011) by using some different microorganism.

As a part of our on-going pharmacological screening of selected Bangladeshi medicinal plants (Ahmed et al., 2008, 2011; Khatun, 2006, 2008; Rahman, 2006, 2008, 2010; Sadhu, 2007a,b, 2008) we now report the possible bioactive chemical groups for the first time and the effect of *M. cordata* leaves extracts on antinociceptive, cytotoxic and antimicrobial activities which support the previous studies done on the same plant collected from different geographical sources and climates.

MATERIALS AND METHODS

Plant material collection and extraction

The leaves of *M. cordata* were collected from the campus of Khulna University, Khulna, Bangladesh, during the month of July, 2010 on

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Table 1. List of traditional uses of *Mikania cordata*.

Plant species	Family	Local name	Voucher	Traditional Uses ^a
<i>M. cordata</i>	Asteraceae	Assamlata, Germanlata	DACB- 31,267	AP- spasmolytic; L- stop bleeding from cuts and wounds, used against jaundice, septic sore, snake bite; IL- colds, influenza, fever, bronchitis in children; F- coughs, diabetes; WP- rich source of vitamin A, B and C, fish poison.

^a AP= Extract of aerial parts, L=crashed leaves, IL= infusion and decoction of leaves, F= decoction of flowers, WP=whole plant.

day time, and were taxonomically identified by experts at the Bangladesh National Herbarium (accession number: 31,267). About 150 g of powdered leaves were taken in a clean, flat-bottomed glass container and soaked in 650 ml of ethanol up to 2 inch height above the sample surface as it can sufficiently cover the sample surface. The container with its contents was sealed and kept for a period of 17 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper. After filtration the remaining portion of the plant extract was given for re-extraction for 7 days with another 150 ml of ethanol. The filtrate thus obtained was evaporated by air supplied from a continuously moving electric fan. It rendered a greenish black type residue of 3.2 g (yield 2.13%) which was designated as crude ethanolic extract of whole plants of *M. cordata*.

Drugs

Diclofenac sodium (Square Pharmaceuticals Ltd., Mohakhali, Dhaka, Bangladesh) as standard drug in antinociceptive activity using the model of acetic acid-induced writhing in mice. Kanamycin (Oxoid Ltd., UK) as standard discs (30 µg/disc) for the antibacterial activity study.

Preliminary phytochemical analysis

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 (Evans, 1989; Ghani, 1998b).

Tests for reducing sugar

Benedict's test

0.5 ml of the extract solution was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Fehling's test (standard test)

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.

Combined reducing sugar test

1 ml of the extract solution was boiled with 2 ml of diluted hydrochloric acid for 5 min. After cooling, the mixture was neutralized with sodium hydroxide solution and then Fehling's test

was performed as described earlier.

Tests for tannins

Ferric chloride test

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.

Potassium dichromate test

5 ml of the extract solution was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

Test for flavonoids

Flavonoid test

A few drop of concentrated hydrochloric acid was added to a small amount of extract of the plant material.

Test for saponins

Saponin test

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min.

Test for gums

Gum test

5 ml solution of the extract was taken and then Molisch's reagent and sulphuric acid were added.

Test for steroids

Steroid test

1 ml solution of extract was taken and then 1 ml Sulphuric acid was added.

Test for alkaloids

Mayer s test

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.

Table 2. Phytochemical properties of *Mikania cordata* leaves extract.

Compound	Alkaloids	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	+ve	+ ve	+ ve	- ve	- ve	- ve	+ve

+ve = Presence, -ve = Absence.

Dragendorff's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendorff's reagent was added.

Animals

Young Swiss-albino mice of either sex, weighing 20 to 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 to 65%, room temperature $25.0 \pm 2.0^\circ\text{C}$ and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Pharmacological studies

Antinociceptive activity

Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice (Khatun, 2006). The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 ml/kg of body weight; Group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; Groups III and IV were test groups and were treated with the extracts at dose of 125 and 250 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

Cytotoxicity test

The method of Rahman et al. (2010) was adopted to study the general cytotoxic effect of the extract. The brine shrimps used for this test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 22 h. Six doses of plant extract (10, 20, 40, 80, 160 and 320 $\mu\text{g}/\text{ml}$) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10 $\mu\text{l}/\text{ml}$. For control, same procedure was followed except for test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 12 vials with the help of a Pasteur pipette. The test tubes containing the sample and control were then kept for 24 h, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration. The procedure was repeated to avoid error.

Antimicrobial activity

Antibacterial activity study was performed by the method used by Ahmed et al. (2008). Sterile 6.0 mm diameter blank discs (BBL, Cocksville, USA) were impregnated with test substances at the dose of 500 $\mu\text{g}/\text{disc}$. These discs, along with standard discs (30 $\mu\text{g}/\text{disc}$) (Kanamycin, Oxoid Ltd., UK) and control discs were placed in Petri dishes containing a suitable agar medium seeded with the test organisms using sterile transfer loop and kept at 4°C to facilitate maximum diffusion. The Petri dishes were then kept in an incubator (37°C) to allow the growth of the bacteria. The antibacterial activities of the test agents were determined by measuring the diameter of the zone of inhibition in terms of millimeter. Antimicrobial activity was tested against *Salmonella typhi*, *Shigella Sonnei*, *Proteus* spp., *Pseudomonas aeruginosa*, *Enterococci*, *Streptococcus pyogenes*, *Shigella flexneri*, *Shigella dysenteriae*, *Staphylococcus epidermis* and *Staphylococcus aureus*.

Statistical analysis

All data obtained were expressed as mean \pm S.E.M. The Student's t-test was used to analyze data obtained from *in vivo* experiments and to determine a significant difference between the control group and experimental groups.

RESULTS

Preliminary phytochemical analysis

Results of different chemical tests on the methanol extract of the leaves of *M. cordata* showed the presence of gum, alkaloids, steroids, and tannins (Table 2).

Antinociceptive activity

Table 3 showed the effect of the ethanolic extract of *M. cordata* on acetic acid-induced writhing in mice. At dose of 125 and 250 mg/kg of body weight, the extract produced about 81.06 and 96.21% writhing inhibition in test animals respectively. The results were statistically significant ($P < 0.001$) and were comparable to the standard drug diclofenac sodium, which showed about 74.24% writhing inhibition at the dose of 25 mg/kg ($P < 0.001$).

Cytotoxic activity

In brine shrimp lethality bioassay (Table 4), the extract

Table 3. Effects of *M. cordata* leaves extract on writhing effect on acetic acid induced mice.

Treatment	Dose (mg/kg)	Mean writhing	% Inhibition	SD	P value (One way Anova)
Experimental control (1% Tween80)	10 ml/kg	26.4 ±1.33	-	5.2	P<0.001
Positive control (Diclofenac sodium)	25	6.8 ± 1.22	74.24	0.9	P<0.001
Test sample	125	5 ± 1.00	81.06	2.6	P<0.001
Test sample	250	1 ± 1.21	96.21	0.7	P<0.001

*- (VassarStats, 2009); Test sample- *M. cordata*. Crude extract. 30 min after treatment, 0.7% acetic acid was injected i.p. 10 min after injection writhing responses was recorded for 10 min. N=5.

Table 4. Brine shrimp lethality bioassay of *M. cordata* leaves extract.

Test sample	Conc. (µg/ml)	No. of alive shrimp			% mortality	LC ₅₀ (µg/ml)	LC ₉₀ (µg/ml)
		Test 1	Test 2	Average			
Ethanollic extract	10	10	10	10	0	90	166
	20	9	8	8.5	15		
	40	7	6	6.5	35		
	80	4	5	5.5	45		
	160	1	2	1.5	85		
	320	0	0	0	100		

Table 5. Effects of *M. cordata* leaves extract on bacterial strains.

Bacterial strains	Diameter of zone of inhibition in mm		
	Ethanollic extract of <i>M. cordata</i>	Blank	Control
Gram positive			
<i>Staphylococcus epidermis</i>	15	0	30
<i>S. aureus</i>	14	0	38
Gram negative			
<i>Enterococci</i>	9	0	25
<i>Salmonella typhi</i>	14	0	34
<i>Shigella sonnie</i>	13	0	30
<i>Streptococcus pyogenes</i>	13	0	27
<i>Pseudomonas aeruginosa</i>	12	0	28

showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC₅₀ and LC₉₀ were deduced (LC₅₀: 90 µg/ml; LC₉₀: 166 µg/ml) (Table 4).

Antimicrobial activity

The antibacterial activity of the extracts of *M. cordata* was evaluated against several Gram positive and Gram-negative bacteria by disc diffusion method. The results of antibacterial activity of the investigated extracts are shown in Table 5. The extracts showed moderate

antibacterial activity against both Gram -positive and Gram-negative bacteria tested in this study.

DISCUSSION

Plants are employed as important source of medication in many traditional medications (Ghani, 1998a; Ahmed, 1997; Uddin, 2006). Since *M. cordata* a common weed available throughout Bangladesh and has a wide use in traditional herbal medicine, we tried to evaluate its folklore use. Ethanol was used which has a wide range of solubility in both polar and nonpolar region. To avoid any solvent effect on the experimental animals, the solvent

was evaporated completely to dryness. Antinociceptive activity of the ethanol extract of *M. cordata* was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algnesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Khatun, 2006). Increased levels of PGE2 and PGF2 α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid. The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 3). The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result it can be concluded that the ethanolic extract of *M. cordata* might possess antinociceptive activity.

The cytotoxic activity of the ethanol extract of *M. cordata* was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc (Rahman, 2010). The extract was found to show moderate activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds. Antimicrobial activity of the extract of *M. cordata* was tested against several Gram positive and Gram-negative bacteria by disc diffusion method. It can be concluded that the extract contains antimicrobial activity against both Gram- positive and Gram-negative bacteria tested in this study.

The cytotoxic and antimicrobial activities of *M. cordata* leaves have been reported before (Ali et al., 2011). In the previous study, the plant was collected from the southern east part of Bangladesh where the soil is rocky. In our study, the plant has been collected from the southern west part of the country where the soil is saline. The climate is also quite different. From our study, the activities of *M. cordata* could be justified from the aspect of geographical and climate variation. Moreover, we report possible bioactive chemical groups in the plant for the first time which may lead to the isolate bioactive lead compound used as drug.

Conclusion

Finally, it could be suggested that the ethanolic extract of *M. cordata* leaves collected from the previous mentioned source possesses antinociceptive, cytotoxic and antimicrobial activities. These facts further indicate the scientific basis of *M. cordata* being used as a traditional medicine in Bangladesh. However, further experiments may help to determine the pharmaceutical potentialities

of the plant as a medicine.

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