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Original Article

Investigation of analgesic potential and *in vitro* antioxidant activity of two plants of Asteraceae family growing in Bangladesh

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ABSTRACT

Background: The present study was designed to investigate analgesic potential and *in vitro* antioxidant activity of crude ethanolic extract of two plants (*Ageratum conyzoides* L. and *Mikania cordifolia* L.) of Asteraceae family growing in the coastal region of Bangladesh.
Methods: The analgesic potentials of the plants were evaluated using acetic acid induced writhing in mice and for investigating the antioxidant activity, four complementary test methods namely DPPH free radical scavenging assay, reducing power assay, ferrous ion chelating ability assay, and total phenolic content determination were carried out.
Results and discussion: The analgesic activities of the crude extracts were found significant at dose of 500 mg/kg-body weight inhibition ($p < 0.001$) in comparison with standard Diclofenac sodium. The results of the antioxidant tests showed that *A. conyzoides* is fairly strong with antioxidant properties in comparison with standards used, whereas *M. cordifolia* got moderate antioxidant potentiality.
Conclusion: The study tends to suggest potent analgesic activity of crude ethanolic extract of leaves of *A. conyzoides* L. and *M. cordifolia* L., and justify their traditional use for antioxidant and analgesic treatment; however, *in vivo* antioxidant activity and active component(s) of the extract for antioxidant and analgesic potentiality are yet to be discovered.

1. Introduction

Asteraceae is a large family of flowering plants containing more than 25,000 species and 1000 genera.[1] The species in this family are generally featured due to their antioxidant, anti-inflammatory, analgesic and antipyretic activity.[2] In this study we have selected two different plants (*Ageratum conyzoides* L. and *Mikania cordifolia* L.) from Asteraceae family to evaluate their antioxidant and analgesic activity. *A. conyzoides* leaves are used as styptic and antiseptic, applied to wounds, prevent tetanus, fever, cough and colds, hepatitis, dysentery, neurasthenia, snake bites.[3,4] *M. cordifolia* may contribute a

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major role in controlling and preventing sexually transmitted diseases.[5] The molecules which are capable of hindering the oxidation of other molecules are literally known as antioxidants. Synthetic antioxidants may have adverse biological effects on human body; therefore, much attention has been put toward natural antioxidants.[6] Now a day, foods contain antioxidants for preventing fats and oils from foaming rancid products. Packaged foods containing vegetable oils or animal fats may have antioxidants added.[7] Plants are potential sources of natural antioxidants.

By acting in the CNS or on the peripheral pain mechanism, analgesic compounds selectively relieves pain without significant alteration of consciousness. Actually analgesics are applied when the noxious stimulus cannot be removed or as adjuvants to more etiological approach to pain.[8] The basic goal of our study was to investigate and compare the analgesic and antioxidant potentials of the crude ethanolic extracts of two widely growing plants of Asteraceae family, and to justify their use in traditional remedies.

2. Materials and methods

2.1. Plant materials collection and extraction

Leaves of two plants of Asteraceae family named *A. conyzoides* L. and *M. cordifolia* L. were collected by the authors from the surrounding area of Noakhali, a coastal region of Bangladesh, in November, 2010. The plants were identified and authenticated by expert botanist of Bangladesh National Herbarium (DACB Accession no. 39526 and 34527, respectively), Mirpur, Dhaka.

500 g of the dried and powdered samples were soaked each in 2500 ml of 80% ethanol (Merck KGaA, Darmstadt, Germany) in clean, sterilized and flat-bottomed glass container. The container with its contents was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material and Whatman® filter paper no. 1. The resultant filtrates were then evaporated in water bath maintaining 40 °C to dryness and thus greenish-black (*A. conyzoides*) and blackish (*M. cordifolia*) semisolid mass of the extracts were obtained.

2.2. Test animals and drug

For the screening of *in vivo* analgesic potential of crude ethanolic extract of *A. conyzoides* and *M. cordifolia* leaves, young Swiss-albino mice aged 4–5 weeks (either sex), average weight 20–25 g were used. They were collected from the Animal Resources Branch of ICDDR, B (International Centre for Diarrheal Disease and Research, Bangladesh). After collection, they were kept in favorable condition for one week for adaptation and fed rodent food and water *ad libitum* formulated by ICDDR, B. The experiment was carried out according to the protocol approved by the Animal Ethics Committee of NSTU Research Cell, Noakhali Science and Technology University, and maintaining the internationally recognized principles for laboratory animal use and care.

In the experiment, Diclofenac Sodium (donated by Opsonin Pharma Ltd., Bangladesh) was used as standard. Tween 80 and acetic acid used were of analytical grade (Merck KGaA, Darmstadt, Germany).

2.3. Chemicals for antioxidant assay

1,1-Diphenyl-2-picryl hydrazyl (DPPH), Trichloroacetic acid (TCA), L-Ascorbic acid, Butylated Hydroxy Anisole (BHA), gallic acid, Folin–Ciocalteu phenol reagent, phosphate buffer (pH 6.6), potassium ferricyanide [$K_3Fe(CN)_6$] (1%), distilled water, EDTA, ferrozine, $FeCl_2$ and $FeCl_3$ (0.1%) were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.4. Analgesic potential

Analgesic potential of the ethanolic extract of *A. conyzoides* and *M. cordifolia* leaves were tested using the model of acetic acid induced writhing in mice.[9,10] Experimental animals ($n = 5$) were randomly selected and divided into four groups denoted as group I, group II, group III, group IV. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Each group received a particular treatment i.e. control, positive control (standard Diclofenac Na) and two doses (250 and 500 mg/kg-body weight) of the extract solution. Positive control group was administered at the dose of 25 mg/kg-body weight and control group was treated with 1% Tween 80 in water at the dose of 15 ml/kg-body weight. Test samples, standard drug and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

2.5. Antioxidant assay

There are various well known methods, which are followed to determine the antioxidant properties of plant extracts. The antioxidant activities of ethanol extract of the leaves of *A. conyzoides* and *M. cordifolia* L. were evaluated using determination of DPPH free radical scavenging activity, determination of reducing power, determination of ferrous ion chelating ability, and total phenolic content determination methods.

2.5.1. Determination of DPPH free radical scavenging activity

The stable DPPH radical scavenging activity was measured using the modified method described by Chang et al[11] Stock solution (1 mg/ml) of the ethanol extract of the leaves of *A. conyzoides* and *M. cordifolia* were prepared in ethanol from which serial dilutions were carried out to obtain the concentrations of 5, 10, 20, 40, 60, 80, 100 μg/ml. In this assay, 2 ml of 0.1 mm ethanolic DPPH solution was added to 2 ml of extract solution at different concentrations and the contents were stirred vigorously for 15 s. Then the solutions were allowed to stand at dark place at room temperature for 30 min for reaction to occur. After 30 min, absorbance was measured against a blank at 517 nm with a double beam UV spectrophotometer (UV-1800, Shimadzu, Japan). The percentage of DPPH radical scavenging activity of each plant extract was calculated as:

$$\text{DPPH radical} - \text{scavenging activity}(I\%) = [(A_0 - A)/A_0] \times 100$$

Table 1 – Effect of ethanolic extract of leaves of *Ageratum conyzoides* and *Mikania cordifolia* on acetic acid induced writhing mice.

Animal group	Treatment	*Ageratum conyzoides*		*Mikania cordifolia*	
		Writhing count (Mean ± SEM) (%writhing)	% Writhing inhibition	Writhing count (Mean ± SEM) (%writhing)	% Writhing inhibition
Group I $n = 5$	1% Tween 80 solution in water (orally)	12.95 ± 0.68 (100)	–	12.95 ± 0.68 (100)	–
Group II $n = 5$	Diclofenac sodium (25 mg/kg) (orally)	6.9 ± 0.85** (53.23)	46.77	6.9 ± 0.85** (53.23)	46.77
Group III $n = 5$	Ethanol extract (250 mg/kg) (orally)	9.7 ± 0.58* (74.90)	25.10	9.86 ± 0.84* (76.13)	23.87
Group IV $n = 5$	Ethanol extract (500 mg/kg) (orally)	7.1 ± 0.79**(54.82)	45.18	7.51 ± 0.76** (57.99)	42.01

Values are expressed as mean ± SEM (Standard Error Mean); * indicates $p < 0.01$; ** indicates $p < 0.001$, one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice.

where A_0 is the absorbance of the control solution (containing all reagents except plant extracts); A is the absorbance of the DPPH solution containing plant extract. The concentration of sample required to scavenge 50% DPPH free radical (IC_{50}) was calculated from the plot of inhibition (%) against the concentration of the extract. Ascorbic acid and BHA were used as positive control standard.

2.5.2. *Determination reducing power*

This assay was determined according to the method reported by Oyaizu[12] with slight modifications. Briefly, 1 ml of extract solution of different concentrations (5, 10, 20, 40, 60, 80, 100 μg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). The mixture was incubated at 50 °C for 20 min. The reaction was terminated by adding 2.5 ml of Trichloroacetic acid (10%, w/v), then the mixture was centrifuged at 3000 rpm for 10 min. The supernatant solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1% w/v) solution. Then the absorbance was measured at 700 nm against a blank using UV spectrophotometer. Increased absorbance value of the reaction mixture indicates increased reducing power. Three replicates were made for each test sample and average data was noted. Here, ascorbic acid and BHA were used as positive control standard, too.

2.5.3. *Determination of ferrous ion chelating ability*

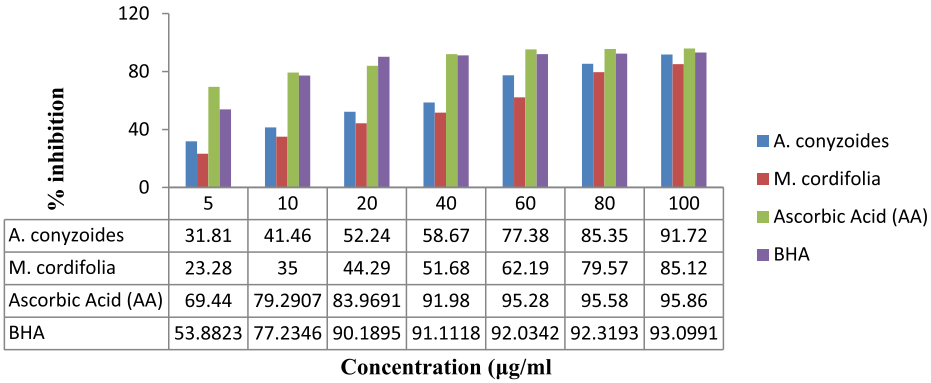
The ferrous ions chelating activity of ethanol extract of the leaves of *A. conyzoides* and *M. cordifolia*, and standards was investigated according to the method of Dinis et al[13] Briefly, ethanol extracts (5 ml) was added to 0.1 ml solution of 2 mM $FeCl_2$ and ethanol. Then, the reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine and mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in UV spectrophotometer, wherein the Fe^{+2} chelating ability of extracts was monitored by measuring the ferrous ion–ferrozine complex. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given in the underneath formula.

$$\text{Ferrous ions chelating ability}(\%) = [(A_0 - A)/A_0] \times 100$$

Where, A_0 is the absorbance of the control solution (containing all reagents except plant extract); A is the absorbance in the presence of the sample of plant extracts. Three replicates were made for each test sample and average data was noted. Here, EDTA was used as positive control standard.

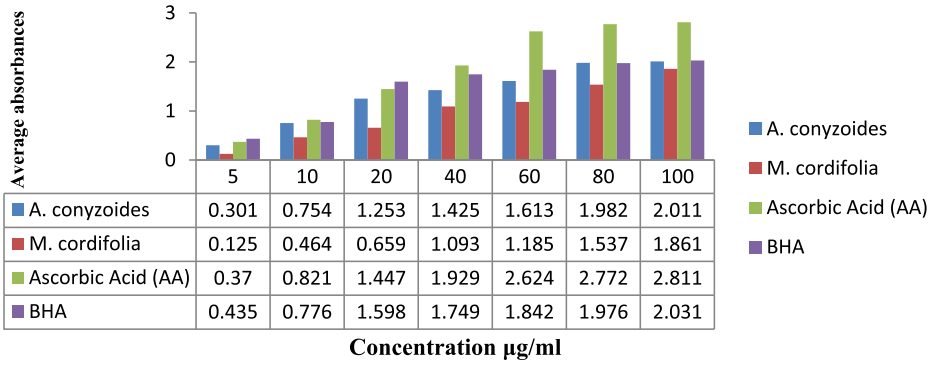
2.5.4. *Determination of total phenolic content*

The total phenolic contents of the extracts were determined by the modified Folin–Ciocaltu method.[14] Briefly, 0.5 ml of each extract (1 mg/ml) was mixed with 5 ml Folin–Ciocaltu reagent (1:10 v/v distilled water) and 4 ml (75 g/L) of Sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 30 min at 40 °C for color development .The absorbance was read at 765 nm with a spectrophotometer (UV-1800,



	5	10	20	40	60	80	100
A. conyzoides	31.81	41.46	52.24	58.67	77.38	85.35	91.72
M. cordifolia	23.28	35	44.29	51.68	62.19	79.57	85.12
Ascorbic Acid (AA)	69.44	79.2907	83.9691	91.98	95.28	95.58	95.86
BHA	53.8823	77.2346	90.1895	91.1118	92.0342	92.3193	93.0991

Fig. 1 – Comparative DPPH radical scavenging activity of ethanol extract of the leaves of *Ageratum conyzoides*, and *M. cordifolia* with standards of ascorbic acid and butylated hydroxy anisole.



	5	10	20	40	60	80	100
A. conyzoides	0.301	0.754	1.253	1.425	1.613	1.982	2.011
M. cordifolia	0.125	0.464	0.659	1.093	1.185	1.537	1.861
Ascorbic Acid (AA)	0.37	0.821	1.447	1.929	2.624	2.772	2.811
BHA	0.435	0.776	1.598	1.749	1.842	1.976	2.031

Fig. 2 – Comparative reducing power assay of ethanol extract of leaves of *Ageratum conyzoides* and *M. cordifolia* with standard AA and BHA.

Shimadzu, Japan). Total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve.

2.6. Statistical analysis

For antioxidant determination, data were presented as mean ± Standard deviation (SD). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons using SPSS 16.0 for Windows®. The results obtained were compared with the control group. p values < 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Analgesic potential

A dose-dependent analgesic potential was showed by the crude extract of *A. conyzoides* and *M. cordifolia* leaves (Table 1). The analgesic activities of both plants were significant ($p < 0.05$) at the dose of 500 mg/kg-body weight in comparison with control animals; however, the activity was less than that of diclofenac Na (standard). In the study, *A. conyzoides* extract was found more effective than that of *M. cordifolia* L.

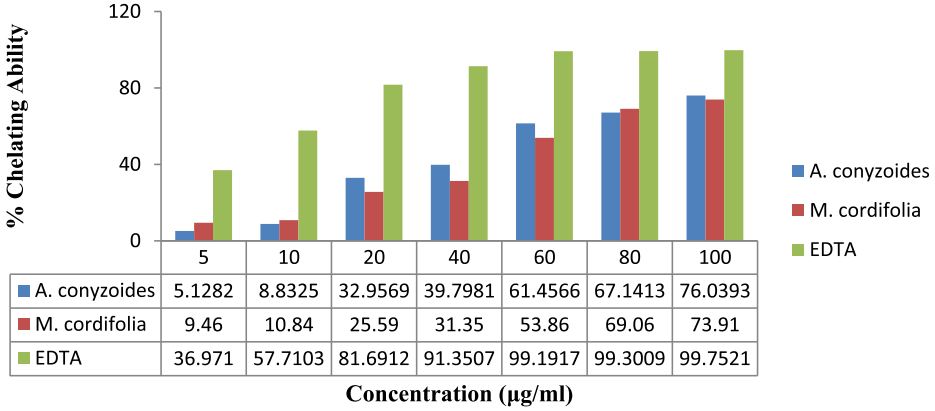
3.2. Antioxidant activity

3.2.1. DPPH free radical scavenging activity

The investigation shows that DPPH free radical scavenging activity of crude ethanolic extracts of *A. conyzoides* and *M. cordifolia* leaves were found to be increased with the increase of concentrations of the extracts (Fig. 1). The extracts exhibited 91.72 ± 0.053% and 85.12 ± 0.087% inhibition respectively at the concentration of 100 µg/ml, whereas standard Ascorbic acid (AA) and BHA showed 95.86 ± 0.031% and 93.099 ± 0.019% inhibition respectively at the same concentration. In the study, if the IC_{50} value is less than 30 µg/ml, be considered as strong scavenging activity; $30 \leq IC_{50} \leq 100$ µg/ml as moderate, and $IC_{50} > 100$ µg/ml be considered as weaker activity.[15] Therefore, it can be revealed that *A. conyzoides* got strong free radical scavenging activity (IC_{50} (µg/ml) = 18.91 ± 0.085), whereas *M. cordifolia* got moderate scavenging activity (IC_{50} (µg/ml) = 39.81 ± 0.081).

3.2.2. Reducing power assay

In this study, it is seen that the reducing power activity of the leave extracts increases with the increase of concentrations of the extract solutions (Fig. 2). *A. conyzoides* and *M. cordifolia* exhibited 2.011 ± 0.0009 and 1.861 ± 0.021 average absorbance at 700 nm respectively in 100 µg/ml concentration, whereas AA and BHA exhibited 2.811 ± 0.0013 and 2.031 ± 0.0009



	5	10	20	40	60	80	100
A. conyzoides	5.1282	8.8325	32.9569	39.7981	61.4566	67.1413	76.0393
M. cordifolia	9.46	10.84	25.59	31.35	53.86	69.06	73.91
EDTA	36.971	57.7103	81.6912	91.3507	99.1917	99.3009	99.7521

Fig. 3 – Comparative Fe^{2+} ion chelating ability of ethanol extract of leaves of *A. conyzoides* and *M. cordifolia* with standard EDTA.

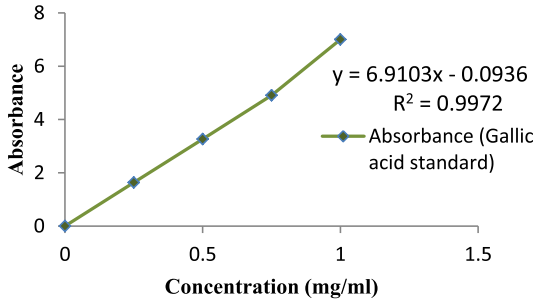


Fig. 4 – Total phenolic content of gallic acid standard.

average absorbance in the same concentration. Therefore, the reducing power of crude ethanolic extract of leaves of *A. conyzoides* is higher than that of *M. cordifolia*.

3.2.3. *Ferrous ion chelating ability*

Fig. 3 reveals the ferrous ion chelating ability of ethanolic extracts of *A. conyzoides* and *M. cordifolia*. The leave extracts exhibited 76.0393 ± 0.041% and 73.91 ± 0.016% chelating ability respectively, whereas EDTA (standard) showed 99.75 ± 0.011% chelating ability at 100 μg/ml concentration. The IC_{50} values of *A. conyzoides* and *M. cordifolia* leave extracts as percentage (%) Fe^{2+} ion chelating ability were found 16.28 ± 0.05 μg/ml and 32.67 ± 0.021 μg/ml respectively, whereas EDTA showed 8.87 ± 0.035 μg/ml. Therefore, the ferrous ion chelating ability of *A. conyzoides* was found better than that of *M. cordifolia*.

3.2.4. *Total phenolic content determination*

The ethanolic extracts of *A. conyzoides* and *M. cordifolia* were tested for total phenolic content. Based on the absorbance values of the extract solutions the colorimetric analysis of the total phenolics of extracts were determined and compared with that of standard solution (Fig. 4) of gallic acid equivalents. Result (Table 2) shows that the total phenolic amount calculated for *A. conyzoides* was quite better than that of *M. cordifolia*.

4. Conclusion

In the context of the above discussion, it can be revealed that the crude ethanol extract of leaves of *A. conyzoides* possess significant analgesic and antioxidant activity, whereas *M. cordifolia* possess significant analgesic potential and moderate antioxidant activity. However, it would be interesting to investigate the *in vivo* antioxidant activity, anti-inflammatory and antinociceptive activity as well, and find out causative component(s), and mechanism for antioxidant and analgesic potentiality by different parts of the plants *A. conyzoides* and *M. cordifolia*.

Table 2 – Total phenolic content of the crude ethanol extract of *A. conyzoides* and *M. cordifolia* leaves.

Crude ethanol extract	Avg. absorbance at 765 nm	Total phenolic content (mg of GAE/gm) of extracts
Mikania cordifolia	1.115 ± 0.059	225.82 ± 0.590
Ageratum conyzoides	2.521 ± 0.0021	378.37 ± 0.92

Conflicts of interest

All authors have none to declare.

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