

## Analgesic and Antioxidant Activity of the Hydromethanolic Extract of *Mikania scandens* (L.) Willd. Leaves

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**Abstract: Problem statement:** *Mikania scandens* (L.) Willd., a medicinal plant, is popularly used as herbal remedy for various ailments in Bangladesh. But the scientific basis for its medicinal use especially in pain and inflammation remains unknown. Therefore, the present study was designed to evaluate analgesic and antioxidant potential of the hydromethanol extract of the leaves of the plant. **Approach:** The analgesic activity was determined for its central and peripheral pharmacological actions using hotplate and tail immersion method and acetic acid-induced writhing test in mice respectively. The hydromethanol extract was also investigated for its antioxidant action using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Nitric Oxide (NO) scavenging and reducing power assays. **Results:** The extract, at the dose of 250 and 500 mg kg<sup>-1</sup>, produced a significant (p<0.05) increase in pain threshold in hotplate and tail immersion methods in a dose dependent manner. In acetic acid-induced writhing test, the extract (500 mg kg<sup>-1</sup>) produced a maximum of 53.73% inhibition (p<0.001) of writhing reaction compared to the reference drug Diclofenac-Na (76%). A dose dependent scavenging of DPPH radical and NO was observed with good reducing power with the extract. In DPPH radical scavenging assay, the IC<sub>50</sub> value of the extract was 375.40 µg mL<sup>-1</sup> while the IC<sub>50</sub> value for the reference ascorbic acid was 55.89 µg mL<sup>-1</sup>. The IC<sub>50</sub> values of the extract and ascorbic acid were 220.43 and 125.10 µg mL<sup>-1</sup>, respectively in NO scavenging assay. **Conclusion:** The findings of the study suggested that the extract from *Mikania scandens* has strong analgesic and antioxidant effects.

**Keywords:** Ethnopharmacology, medicinal plant, analgesic, antioxidant, inflammation, tail immersion.

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### INTRODUCTION

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause<sup>[1]</sup>. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency. Moreover, a number of pathologies are known to be ultimately

associated with the imbalance of pro and antioxidant factors in living systems. Exogenous antioxidant compounds may therefore exert beneficial actions upon systems which have been deprived from sufficient amounts of endogenous antioxidants as in some cardiovascular diseases, tumors, inflammation, ulcer and aging<sup>[2]</sup>. Currently, the possible toxicity of synthetic antioxidants has been criticized. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years<sup>[3]</sup>.

*Mikania scandens* (Family: Asteraceae) is a twining herb with long-petioled, opposite leaves and small

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homogamous flower-heads grows as a weed in all areas of Bangladesh<sup>[4]</sup>. Aqueous leaf extracts of this plant have been used in traditional/folk medicine to treat stomach ulcers and a variety of other diseases. In India, although the plant is consumed as a vegetable, it is thought to be efficacious in the treatment of gastric problems. The plant is a rich source of vitamin A and C and also contains vitamin B, Mikanin, friedelin, efifriedinol, some sesquiterpene, dilactones including mikanolide, dihydromikanolide, deoxymikanolide and scandenolide. Three diterpenic acids known as kaurenic acid, butyryloxykaurenic acid and benzoyloxykaurenic acid, stigmasterol and beta-sitosterin have also been isolated from this plant<sup>[4]</sup>. The plant is reported to have psychopharmacological and neuropharmacological properties; antimicrobial, anti-inflammatory, antipyretic, ulcerprotective, anticarcinogenic activities<sup>[5-8]</sup>. But still no scientific and methodical investigation has so far been reported in literature regarding its analgesic and antioxidant action. Therefore, as a part of our ongoing phytochemical and pharmacological investigations on local medicinal plants of Bangladesh<sup>[9,10]</sup>, the present study has been designed to examine the analgesic and antioxidant activity of the hydromethanol extract of the leaves of *Mikania scandens* (L.) Willd.

## MATERIALS AND METHODS

**Chemicals and drugs:** DPPH (1, 1-diphenyl, 2-picryl hydrazyl), was obtained from Sigma chemical co. USA. Ascorbic acid was obtained from SD Fine chem. Ltd., Biosar, India. Naphthyl ethylene diamine dihydrochloride was purchased from Roch-light Ltd., Suffolk, England. Sodium nitro prusside was obtained from Ranbaxy Lab., Mohali, India and potassium ferricyanide from May and Backer, Dagenham, UK. Diclofenac-Na was collected from Square Pharmaceuticals Ltd., Bangladesh and Nalbuphine was from Incepta Pharmaceuticals Ltd., Bangladesh.

**Plant material:** The whole plant with leaves, stems and roots was collected from Savar, Dhaka in February 2008 and was identified by Prof. Dr. Abdul Ghani, Stamford University Bangladesh. The plant was thoroughly washed with water; roots and stems were discarded and the leaves were dried in hot air woven at 55°C for 3 days and at 40°C for the next 4 days.

**Extraction:** The dried leaves were coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 50°C. The solvent was completely removed and obtained dried crude extract which was used for investigation.

**Animal:** For the experiment Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRDB). Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

**Phytochemical screening:** The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable foam and steroids with Libermann- Burchard reagent. Gum was tested using Molish reagent and concentrated sulfuric acid; reducing sugars with Benedict's reagent. These were identified by characteristic color changes using standard procedures<sup>[4]</sup>.

### Analgesic screening:

**Hot plate method:** The animals were divided into four groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 mL kg<sup>-1</sup> body weight), animals of Group II received Nalbuphine at 10 mg kg<sup>-1</sup> body weight while animals of Group III and Group IV were treated with 250 and 500 mg kg<sup>-1</sup> body weight (p.o.) of the crude extract of *M. scandens*. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5°C. A cut off period of 15 s, was observed to avoid damage to the paw<sup>[11]</sup>. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples<sup>[12-14]</sup>.

**Tail immersion test:** The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice<sup>[14]</sup>. The animals were treated as discussed above. From 1-2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and

the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs.

**Acetic acid-induced writhing test:** The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min<sup>[15]</sup>.

**Tests for antioxidant activity:**

**DPPH radical scavenging activity:** The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by<sup>[16]</sup>. Plant extract (0.1 mL) was added to 3 mL of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated from  $[(A_0 - A_1)/A_0] \times 100$ , where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the extract/standard. The inhibition curves were prepared and  $IC_{50}$  values were obtained by Probit analysis<sup>[17]</sup>.

**Nitric oxide scavenging assay:** Nitric oxide radical scavenging was estimated on the basis of Griess Illosvoy reaction using method followed by Govindarajan *et al.*<sup>[18]</sup>. In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and *M. scandens* extract (5-250  $\mu\text{g mL}^{-1}$ ) or standard solution (ascorbic acid, 0.5 mL) was incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

**Reducing power:** The reducing power of *M. scandens* was determined according to method followed by Srinivas *et al.*<sup>[19]</sup>. Different concentrations of *M. scandens* extract (50-250  $\mu\text{g}$ ) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and  $\text{FeCl}_3$  (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

**Statistical analysis:** Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. p-values < 0.05, 0.001 were considered to be statistically significant.

**RESULTS**

**Phytochemical screening:** Phytochemical analyses of the crude extract revealed the presence of flavonoid steroid, alkaloid, tannin, gum and saponin (Table 1).

**Analgesic screening:**

**Hot plate method:** Result of hotplate test is shown in Table 2. Both doses of the extract produced a dose dependent increase in latency time when compared with the vehicle. The result was found to be statistically significant (p < 0.05-0.001).

**Tail immersion test:** The tail withdrawal reflex time following administration of the extract of *M. scandens* was found to increase with increasing dose of the sample. The result was statistically significant (p < 0.05-0.001) and was comparable to the reference drug Nalbuphine (Fig. 1).

**Acetic acid-induced writhing test:** Table 3 shows the effects of the extract of on acetic acid-induced writhing in mice. The oral administration of both doses of *M. scandens* extract significantly (p < 0.001) inhibited writhing response induced by acetic acid in a dose dependent manner.

Table 1: Result of chemical group tests of the methanol extract of *Mikania scandens*

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
ME of <i>M. scandens</i>	+	+++	-	++	+	+++	+

ME: Methanolic extract; (+): Present; (-): Absent

Table 2: Effects of the hydromethanol extract of *Mikania scandens* on latency to hotplate test

Groups	Dose (mg kg <sup>-1</sup> )	Mean latency (s) before and after drug administration				Percentage of inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Group-I	Vehicle	2.26±0.219	2.45±0.225	2.16±0.197	2.58±0.261	-	-	-
Group-II	10	2.34±0.088	5.62±0.624**	7.97±0.649**	11.67±0.1.007**	64.8	71.5	80.4
Group-III	250	2.21±0.073	3.27±0.264*	4.57±0.747*	4.30±0.383*	44.1	46.7	44.5
Group-IV	500	2.07±0.217	3.57±0.850**	5.66±0.546**	5.45±0.469**	56.9	62.1	52.8

Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg kg<sup>-1</sup> body weight, Group III and Group IV were treated with 250 and 500 mg kg<sup>-1</sup> body weight (p.o.) of the crude extract of *M. scandens*. Values are mean ±SEM, (n = 5); \*: p<0.05, \*\*: p<0.001 Dunnet test as compared to control

Table 3: Effects of the hydromethanol extract of *Mikania scandens* on acetic acid-induced writhing in mice

Groups	Dose (mg kg <sup>-1</sup> )	No. of writhing	Percentage of protection
Group-I	Vehicle	40.2±1.271	-
Group-II	10	10.4±0.940**	76.13
Group-III	250	26.1±.994**	35.07
Group-IV	500	18.6±0.906**	53.73

Values are mean ± SEM, (n = 5); \*\*: p<0.001, Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg kg<sup>-1</sup> body weight, Group III and Group IV were treated with 250 and 500 mg kg<sup>-1</sup> body weight (p.o.) of the crude extract of *M. scandens*

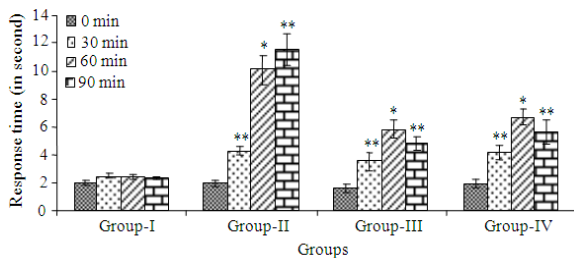


Fig. 1: Effects of the hydromethanol extract of *Mikania scandens* on tail withdrawal reflex of mice induced by tail immersion method. Values are mean ±SEM, (n = 5); \*: p<0.05, \*\*: p<0.001, Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg kg<sup>-1</sup> body weight, Group III and Group IV were treated with 250 and 500 mg kg<sup>-1</sup> body weight (p.o.) of the crude extract of *M. scandens*

**DPPH radical scavenging activity:** The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *M. scandens* is shown in Fig. 2. This activity was found to increase with increasing concentration of the extract. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color.

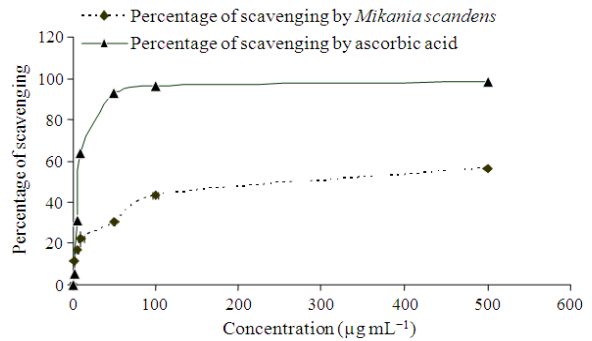


Fig. 2: DPPH radical scavenging activity of the hydromethanol extract *Mikania scandens*. Values are the average of duplicate experiments and represented as mean ±SD

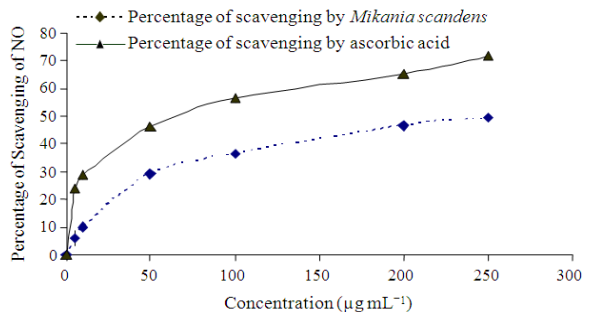


Fig. 3: Nitric oxide scavenging activity of the hydromethanol extract *Mikania scandens*. Values are the average of duplicate experiments and represented as mean ±SD

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC<sub>50</sub> value of the extract was 375.40 µg mL<sup>-1</sup>, as opposed to that of ascorbic acid (IC<sub>50</sub> 55.89 µg mL<sup>-1</sup>), which is a well known antioxidant.

**Nitric oxide scavenging assay:** The hydromethanol extract of the *M. scandens* exhibited dose dependent scavenging of nitric oxide (Fig. 3) with an IC<sub>50</sub> value of 220.43 µg mL<sup>-1</sup> compared to 125.10 µg mL<sup>-1</sup> which was the IC<sub>50</sub> value for the reference ascorbic acid.

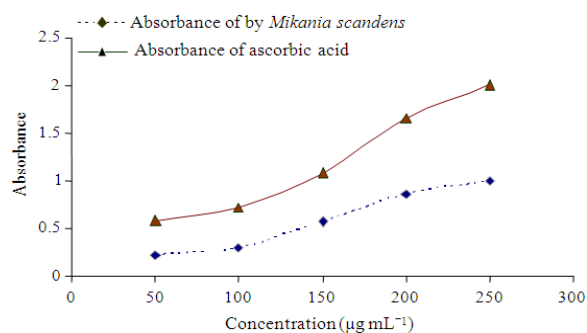


Fig. 4: Reducing power of the hydromethanol extract *Mikania scandens*. Values are the average of duplicate experiments and represented as mean  $\pm$ SD

**Reducing power:** The hydromethanol extract displayed remarkable reducing power which was found to rise with increasing concentration as shown in Fig. 4.

### DISCUSSION

The hotplate method and tail immersion test are considered to be selective to examine compounds acting through opioid receptor; the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain<sup>[20,21]</sup>. The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic.

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid<sup>[22]</sup>. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells<sup>[23]</sup>, acid sensing ion channels<sup>[24]</sup> and the prostaglandin pathways<sup>[25]</sup>.

Preliminary phytochemical screening reveals the presence of flavonoid steroid, alkaloid, tannin, gum and saponin in the plant extract. So, the observed analgesic activity may be attributed to these compounds. Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species form phagocytes invading the inflammation sites<sup>[26]</sup>. There are also reports on the role of flavonoid, a powerful antioxidant<sup>[27,28]</sup>, in analgesic activity primarily by targeting prostaglandins<sup>[29,30]</sup>. There are also reports on the role of tannins in antinociceptive activity<sup>[31]</sup>. Again the plant extract

demonstrated good antioxidant action in the tested models. So it can be assumed that cyclooxygenase (COX) inhibitory activity together with antioxidant activity may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relieve pain-sensation.

Polyphenolic compounds, like flavonoids, tannins and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity<sup>[27,28,32,33]</sup>. Flavonoids and tannins present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action. NO scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions<sup>[34]</sup>. A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported<sup>[35]</sup>. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom<sup>[36]</sup>. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals<sup>[37]</sup>.

### CONCLUSION

Based on the results of the present study, we conclude that the plant extract possesses strong analgesic and antioxidant potential. However, further studies are necessary to examine underlying mechanisms of analgesic and antioxidant effects and to isolate the active compound (s) responsible for these pharmacological activities.

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