

## Sedative, anxiolytic and analgesic effects of *Urena sinuata* L. leaf extract in animal models

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### Abstract

The sedative and analgesic potential of *Urena sinuata* L. was investigated for the first time in this study. The crude methanol extract of *Urena sinuata* L. leaves was evaluated for its central nervous system (CNS) depressant effect using rodent behavioral models. Methanol extract of *Urena sinuata* at a dose of 400 mg/kg body weight, displayed a suppressive effect on motor activity, exploratory behavior (in hole cross and open field tests) and prolongation of thiopental induced sleeping time in mice. In the elevated plus-maze (EPM) test, the same dose of methanol extract significantly ( $p < 0.05$ ) increased the time spent by the treated mice in EPM open arms. Analgesic potential of the extract was also evaluated for centrally acting analgesic activity using formalin induced licking response model and for peripheral analgesic action using acetic acid-induced writhing test and tail immersion tests. In formalin induced licking response model, a significant ( $p < 0.05$ ) inhibition of pain compared to reference drug diclofenac sodium was observed. In acetic acid-induced writhing test and tail immersion test, the extract at 200 mg/kg body weight produced a significant reduction of writhing response and pain respectively. These results evidenced the potential sedative and analgesic effects of *Urena sinuata* leaves.

### Keywords

*Urena sinuata*

Neuropharmacology

Anxiety

Sedative

Anxiolytic

Analgesic

Elevated plus-maze

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### Introduction

Anxiety disorders are the most common emotional disorders affecting people worldwide. Anxiety is reflected as natural emotion but becomes a problem when it occurs too often. More than twenty percent of the adult population suffers from these conditions at some stages of their life throughout the world (Arne, 2000; Abid *et al.*, 2006; Wattanathorn *et al.*, 2007; Susan, 2011). According to the U.S. National Institute of Mental Health (NIMH), anxiety disorders can be related to mental disorders, including depression, traumatic events and other physical illnesses. These illnesses include endocrine disorders such as thyroid disorders and glucose metabolic disorders such as diabetes and hypoglycemia. Although medications can not fully cure anxiety disorders, they can relieve the symptoms and reduce their occurrences to a great degree. Prescription drugs which are commonly used in treating anxiety disorders may include benzodiazepines (commonly known as anxiolytics) and several types of antidepressants, especially those from the group of selective serotonin reuptake inhibitors (SSRI). Beta-adrenergic blocking drugs, to be more precise, could also be prescribed for reducing the peripheral symptoms such as palpitations and tremors. However, these sorts of synthetic drugs can frequently cause drug dependence and some

others adverse effects. Therefore, the surveillance of alternative natural and herbal drugs is still desirable.

*Urena sinuata* L. (Family *Malvaceae*), also known as *U. lobata* or *U. morifolia*, is a wild shrubby plant with some folk medicinal uses in its native areas. It is widely grown in tropical and subtropical areas throughout the world (Ghani, 2003). Where is used as natural remedy for several ailments. The roots of the plant are sweet, slightly cooling, antirheumatic and antipyretic (Abdullah *et al.*, 2011). Moreover, root decoction is used in the treatment enteritis, dysentery, rheumatic pains, tonsillitis and alongside the stem of the plant is used in Brazil as a remedy of severe windy colic (Browner, 1985; Cheryl, 2007). Poultice prepared from the roots and leaves is used as an emollient and is given for snake bites, sprains and bruises. The root is also used as an external application for lumbago in India and for reproductive purposes for both genders in the Pacific, Trinidad and Tobago, China and India (Nadkarni, 1976). In Philippines, the root is considered as emollient, refrigerant and maturant (Anon, 1976; Ahmed *et al.*, 2009).

The flowers are used as expectorant in dry and inveterate chronic coughs. Furthermore, an infusion of the flowers is used in gargle and throat bronchitis (Kirtikar and Basu, 1965). On the other hand, the leaves are prescribed in inflammation of the intestines and the bladder. The plant, as a whole, is considered as

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medicinal as well as economic plant to produce fiber (Aramina fiber) for various purposes in Madagascar, Nigeria and Western Sudan, Chad, Central African Republic, Zaire and Gabon (Anon, 1976; Ahmed *et al.*, 2009). Above mentioned documentations show a number of biological activities, but the anxiolytic action of *U. sinuata* is yet to be studied. As we are interested in psychophysiological importance of traditionally used plants, we attempted to investigate the sedative, anxiolytic and analgesic effects of the methanolic extract of *U. sinuata* leaves.

## Materials and Methods

### Drugs and chemicals

Diazepam and diclofenac sodium were kindly donated by Square Pharmaceutical Ltd., Bangladesh. Thiopental sodium (G-Thiopental, Gonoshasthaya Pharmaceuticals Limited, Bangladesh), Tween solution (TWEEN® 80, Sigma-Aldrich Co. LLC, USA), acetic acid (Merck, Germany), methanol (Merck, Germany) and nalbuphine hydrochloride hydrate (98%, Sigma-Aldrich, India) were purchased for the study. All chemicals and solvents used in this research were of analytical grade.

### Collection of plant material and identification

*Urena sinuata* L. (Family: *Malvaceae*) leaves were collected from University of Chittagong campus, Chittagong, Bangladesh, in April-June, 2011. The plant was taxonomically identified and authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate professor, Department of Botany, University of Chittagong. A voucher specimen (K-2341) of the plant sample has been preserved for future reference. The research has been jointly conducted in the Laboratory of Phytomedicine and Industrial Research of the Department of Biochemistry and Molecular Biology, University of Chittagong and the BGS Trust University and the University of Chittagong during August to December 2012.

### Preparation of extract

The leaves were thoroughly washed with water, air dried for a week at 35-40°C and ground into fine powder using electric grinder (Miyako 3 in one grinder, China). The powder (500 g) was successively extracted in methanol (55-60°C) for 10 days in a two days interval. The extracts were dried by using rotary evaporator (RE 200, Bibby Sterling Ltd., UK) under reduced pressure. A 27.0 g (yield 5.4 % w/w) of greenish-black semisolid crude extract was found and preserved at 4°C for further use.

### Experimental animals

Six-week-old Swiss albino mice weighing 25-35 g were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B). The animals were housed under standard laboratory conditions (relative humidity 55-60%, room temperature  $23.0 \pm 2.0^\circ\text{C}$  and 12 h light : dark cycle) ensuring a noise free isolated animal house to be acclimatized for 7 days. During the entire period of study the animals were caged individually and supplied with standard pellet diet with water ad libitum. Animal experimentations were maintained and carried out with the guidelines of the Institutional Animal Ethics Committee (CUAE, Reference no IIUC/AE 05).

### Assay of sedative effect

#### Hole board test

The experiment was carried out by established method as described by Takagi *et al.* (1971). A steel partition was fixed in the middle of a cage of  $30 \times 20 \times 14 \text{ cm}^3$ . A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The animals were divided into control, positive control, and test groups containing five mice each. The test groups received the extract of *U. sinuata* orally at a dose of 400 mg/kg body weight whereas the normal control group received vehicle (1% Tween-80 in water orally). The number of passages of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the extract. Diazepam (1 mg/kg body weight i.p.) was used as positive control in the hole board test.

#### Open field test

The experiment was carried out according to the methods described by Gupta *et al.* (1971). In the open field test, the animals were divided into control, positive control and test groups containing five mice each. Similar doses mentioned in previous section were used for both test and control groups. The floor of a half square meter open field (Royce, 1977) was divided into a series of squares each alternately colored black and white. The apparatus had a 40 cm height wall. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drug.

#### Thiopental sodium induced sleeping time test

The experiment was conducted through the method described by Ferrini *et al.* (1974). The animals

were randomly divided into three groups consisting of five mice each. Similar doses of extract for test group and diazepam (1 mg/kg body weight, i.p.) for positive control group was used. Thirty minutes later, thiopental sodium (40 mg/kg body weight) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep i.e. time between the loss and recovery of righting reflex.

#### *Elevated plus maze (EPM) test*

The method initially suggested by Handley and Mithani (1984) was employed with minor modifications by Lister (1987). The apparatus consists of two open arms ( $5 \times 10 \text{ cm}^2$ ) and two closed arms ( $5 \times 10 \times 15 \text{ cm}^3$ ) radiating from a platform ( $5 \times 5 \text{ cm}^2$ ) to give the apparatus a plus sign appearance. The apparatus was placed 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The maze floor and walls were constructed from dark opaque wood. Sixty minutes after the treatment (400 mg/kg body weight orally for test group, 1% Tween-80 in water orally for normal control and Diazepam 1 mg/kg body weight i.p.), each animal was placed at the center of the maze facing one of the enclosed arms. During the 5 min test period, the number of open arms entries was recorded. Entry into an arm was defined as the point when the animal places all four paws onto the arm. The procedure was conducted in a sound attenuated room; observations made from an adjacent corner.

#### *Assay of analgesic effect*

##### *Acetic acid-induced writhing test*

This was based on the method described by Koster *et al.* (1959). The mice were divided into three groups of five animals each. The extract 200 mg/kg body weight orally, vehicle (1% Tween-80 in water orally) and diclofenac sodium (40 mg/kg body weight, i.p.) were administered to the respective group 30 min before intraperitoneal injection of 0.1 ml/10 g acetic acid solution (0.7%). Immediately after administering acetic acid, mouse were observed and the number of writhing or stretches were counted for 15 min. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect. The percent inhibition (% analgesic activity) was calculated by,

$$\% \text{ inhibition} = \{(A-B)/A\} \times 100$$

Where, A = average number of writhing of the control

group; B = average number of writhing of the test group.

#### *Formalin test*

The method was done according to the method described by Sharma *et al.* (2010). A 20  $\mu\text{l}$  of 1.0% v/v formalin was injected subcutaneously into the right hind paw of mice. The time (in sec) spent in licking the paw and biting responses of the injected paw were taken as an indicator of pain response. The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. Extract (200 mg/kg body weight, orally) and diclofenac sodium (0.5 mg/kg body weight, i.p.) were administered 30 min prior to formalin injection. Control animals received 10 ml/kg body weight of distilled water orally.

#### *Tail immersion test*

The experiment was performed according to the methodology depicted by Toma *et al.* (2003). Mice were closely restrained in a wire mesh cage and the tails (1/3rd of the tail) were then dipped in the water bath thermo-statistically maintained at  $55 \pm 0.5^\circ\text{C}$ . The time in second to withdraw the tail clearly out of the water was taken as the reaction time. All the animals were screened and those that failed to respond within 60 sec were not used for the assay. Measurement of threshold was made just before (0 min) and at 30, 60 and 90 min interval after administration of the extract (400 mg/kg body weight, orally) or nalbuphine (10 mg/kg body weight i.p.). Vehicle (1% tween-80 in water, 10 mg/kg p.o) served as the control.

#### *Statistical analysis*

All data were expressed as mean  $\pm$  SEM with 95% confidence intervals (CI). Statistical significance between the groups were tested using one-way analysis of variance (ANOVA) followed by Dennett's post-hoc test (unpaired t-test) by the statistical software "Statistical Package for Social Science" (SPSS, Version 18.5, IBM Corporation, NY). The quantitative values were compared with the positive (vehicle) control group and were considered statistically significant at  $p < 0.05$ .

## **Results**

### *Assay of sedative activity*

#### *Hole board test*

In the hole cross test, the extract showed a

Table 1. Effect of *U. Sinuata* leaf extract on EPM test albino mice.

Animal group	Dose/Route	% number of entry into open arm (Mean ± SE)	% time (in seconds) spent in open arm (Mean ± SE)
I (Control)	10 ml/kg, p.o	55.88 ± 2.13 <sup>a</sup>	51.93 ± 8.24 <sup>a</sup>
II (Diazepam)	1 mg/kg, p.o	76.28 ± 1.84 <sup>b</sup>	79.39 ± 5.74 <sup>b</sup>
III (MEUS)	400 mg/kg, p.o	39.37 ± 6.56 <sup>c</sup>	23.25 ± 9.00 <sup>c</sup>

MEUS represents methanol extract of *U. sinuata*. Values with superscript letters are significant ( $p < 0.05$ ) to each other.

Table 2. Analgesic activity of *U. sinuata* leaf extract by acetic acid induced writhing method in mice.

Group	Treatment	Dose	No. of writhing (Mean ± SE)	Percent inhibition
Control	1% Tween-80 in water	10 ml/kg, p.o	49.25 ± 2.59 <sup>a</sup>	-
Standard	Diclofenac Sodium	40 mg/kg, i, p	19.75 ± 1.10 <sup>b</sup>	59.9
Test	MEUS	200 mg/kg, p.o	25.00 ± 1.41 <sup>c</sup>	49.3

MEUS represents methanol extract of *U. sinuata*. Values with superscript letters are significant ( $p < 0.05$ ) to each other.

Table 3. Analgesic activity of the methanolic extract of *U. sinuata* by tail immersion response

Group	Treatment	Dose	Basal reaction time (Sec)	Reaction Time (Sec)		
				30 min	60 min	90 min
Control	Tween-80 in water	10 ml/kg, p.o	2.21 ± 0.10	2.50 ± 0.18 <sup>a</sup>	2.90 ± 0.37 <sup>a</sup>	2.39 ± 0.19 <sup>b</sup>
Standard	Nalbuphine	10 mg/kg, i, p	2.27 ± 0.14	6.05 ± 0.49 <sup>b</sup>	9.23 ± 0.40 <sup>b</sup>	11.93 ± 1.00 <sup>b</sup>
Test	MEUS	200 mg/kg, p.o	2.77 ± 0.32	6.26 ± 0.26 <sup>b</sup>	5.53 ± 0.17 <sup>b</sup>	5.84 ± 0.18 <sup>b</sup>

Values with superscript letters are significant ( $p < 0.05$ ) to each other.

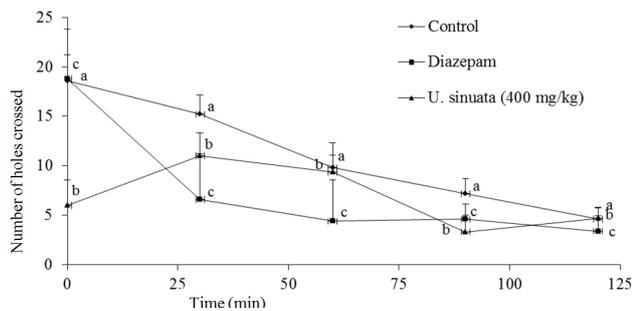


Figure 1. Effect of *U. sinuata* leaf extract on hole board test in mice. Data are shown as Mean ± SE of five animals in each group. Values with superscript letters are significant ( $p < 0.05$ ) to each other.

decrease in locomotion to the test animals from the second observation period as evident by the reduction in number of hole crossed by the treated mice compared to the control group (Figure 1). The result was comparable to the reference drug diazepam and was statistically significant ( $p < 0.05$ ).

#### Open field test

In the open field test, the number of squares traveled by the mice was suppressed significantly in the test group throughout the study period. The CNS depressant activity obtained for extract was more than that of standard drug and the result was statistically significant (Figure 2).

#### Thiopental sodium induced sleeping time test

In the thiopental sodium induced sleeping time test, the test group treated with the extract at 400 mg/kg body weight showed significant ( $p < 0.05$ ) decrease in onset of action and increased the duration

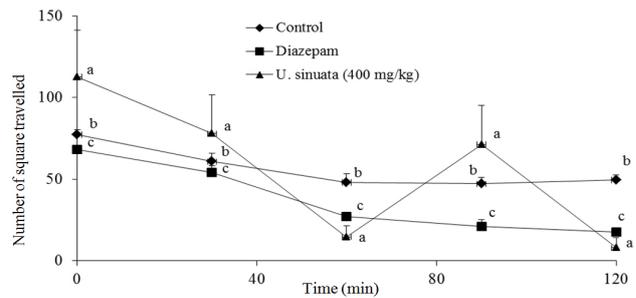


Figure 2. Effect of *U. sinuata* leaf extract on open field test in mice. Data are shown as Mean ± SE of five animals in each group. Values with superscript letters are significant ( $p < 0.05$ ) to each other.

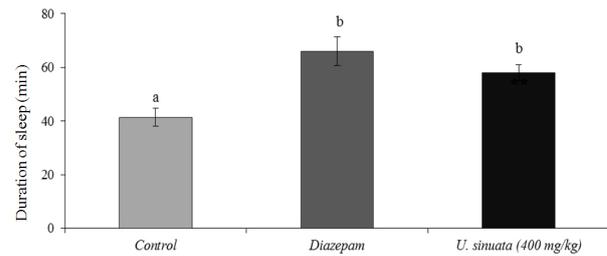


Figure 3. Effect of *U. sinuata* leaf extract on thiopental induced sleeping time in mice. Data are shown as Mean ± SE of five animals in each group. Values with superscript letters are significant ( $p < 0.05$ ) to each other.

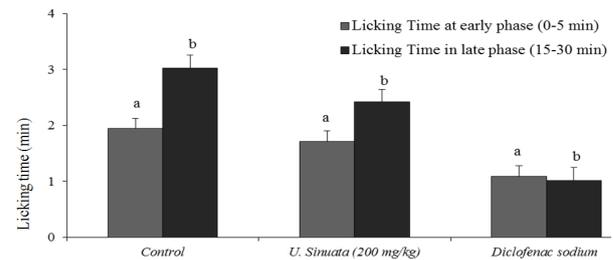


Figure 4. Effect of *U. sinuata* leaf extract on hind-paw licking in the formalin test in mice. Data are shown as Mean ± SE of five animals in each group. Values with superscript letters are significant ( $p < 0.05$ ) to each other.

of sleep. In addition, the dose dependently prolonged the duration of sleeping time in test animals compared to controls (Figure 3).

#### Elevated plus maze (EPM) test

The methanol extract of *U. sinuata* at the dose of 400 mg/kg body weight significantly decreased the percentage of entries of mice into the open arms and the percentage of time spent in the open arms of the EPM. Result of EPM test is presented in Table 1.

#### Assay of analgesic activity

##### Acetic acid induced writhing test

Table 2 shows the effects of the extract on acetic acid induced writhing in mice. Oral administration of the extract significantly ( $p < 0.05$ ) inhibited writhing response induced by acetic acid which was

comparable to the reference drug.

#### Formalin test

The methanol extract of *U. sinuata* significantly suppressed formalin-induced pain response in mice, with a more potent effect on the second phase than the first one. In the late phase (15-30 min) of this test, the extract exerted 20.08% inhibition whereas 66.45% inhibition was obtained for diclofenac sodium against pain. The results were dose dependent and statistically significant ( $p < 0.05$ , Figure 4).

#### Tail immersion test

There was a significant increase of the tail withdrawal reflex time following administration of the extract at dose of 200 mg/kg body weight. The result was statistically significant ( $p < 0.05$ ) compared to the reference drug nalbuphine (Table 3).

### Discussion

The present study revealed the effect of *U. sinuata* leaf extract on induced behavioral changes in mice model. Hole board and open field methods are two common ways to investigate the locomotor activity, which is often used to assess the depressant effects of crude extract (Bhattacharya and Statyan, 1997; De Sousa *et al.*, 2005). Locomotor activity is considered as an increase in alertness and decrease in locomotor activity which eventually indicates a sedative effect (Verma *et al.*, 2010). The plant extract displayed a central nervous system depressant activity as indicated by the decrease in locomotor activity in the hole board, open field and EPM test.

Thiopental basically a hypnotic agent, given at appropriate dose, induced hypnosis by potentiating gamma aminobutyric acid (GABA) mediated postsynaptic inhibition through allosteric modification of GABAA receptors. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both (Nyeem *et al.*, 2006). The extract potentiated sleep induced by thiopental suggesting that the leaves of the plant possess a sleep inducing property. The marked sedative effect of the extract was also found by the reduction in sleeping latency and increase of thiopental sodium induced sleeping time. This in turn hyperpolarizes the postsynaptic membrane at a level below what spike generation is possible. And for this reason some GABAA agonists are frequently used for their hypnotic effects. It is reported that GABA is the major inhibitory neurotransmitter in the central nervous system (Davies, 2003). CNS depressant drugs mainly exert their action through GABAA receptor (Kolawole *et al.*, 2007). Therefore,

the extract of *U. sinuata* may contain compounds that can act by hyperpolarization of the CNS via GABA receptor or benzodiazepine receptor located adjacent to the GABA receptor.

GABAA-benzodiazepine receptors are the most abundant inhibitory receptor (Squires *et al.*, 1997) system in the CNS and binding of a benzodiazepine agonist to its recognition site results in increased chloride ion flux (Trofimiuk *et al.*, 2005). The compounds identified from the leaves of *U. sinuata* include ursolic acid and several other compounds like eicosanol, farnesol and  $\beta$ -sitosterol (Srinivasan *et al.*, 2009). These act as GABAA agonists and this agonistic property could be attributed to the CNS depressant effect of *U. sinuata* leaves although it is not determined which of the substances are exactly responsible for these effects.

The elevated plus maze test is considered as a valuable model to predict anxiolytic or anxiogenic effects of drugs in rodents (Lister, 1987). Anxiolytic compounds typically increases the percentage of open arms entries as well as the time spends in open arms. Additionally, the number of the enclosed entries is used as a parameter to reflect the general motor activity. Confirming the previous results (Bhattacharya and Mitra, 1991), methanol extract of *U. sinuata* at the dose of 400 mg/kg body weight decreased the percentage of entries of mice into the open arms and the percentage of time spent in the open arms of the EPM suggesting a sedative-like effect.

In this experiment, the methanol extract (200 mg/kg body weight) was also evaluated in acetic acid-induced writhing test, formalin test and tail immersion test for its analgesic activity. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesic action. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels (Voilley, 2004; Hossain *et al.*, 2006). Therefore, the significant pain reduction of the plant extract may be due to the presence of chemical components acting on the prostaglandin pathways or interfering with other mediators responsible for peripheral pain.

The formalin test is another reliable model of analgesic which is better correlated with clinical pain (Tjolsen *et al.*, 1992; Ghannadi *et al.*, 2005). This method elucidates central and peripheral activities. The response of early phase is supposed to represent a direct chemical stimulation of pain, due to the irritant effect of formalin on sensory C fibers (Tjolsen *et al.*, 1992). The late phase response is most likely secondary to the development of an inflammatory response and the release of allergic

mediators (Hunskar and Hole, 1987). Inhibition of licking response of the test drugs in the early phase and late phase signifying the analgesic effect of the extract in the formalin test.

The tail immersion method was used in this research to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena (Pal and Pawar, 2011). But the extend of activity shown by the crude extracts are less than that of the standard drug nalbuphine but many fold more than that of the control group, which justifies its activity. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while nonsteroidal anti-inflammatory drugs inhibit only peripheral pain (Elisabetsky *et al.*, 1995; Pal *et al.*, 1999). The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic.

## Conclusion

The neuropsychological profiles of the present investigation of the methanol extract of *U. sinuata* indicate the strong CNS depressant and analgesic activities of the extract which decreased locomotion & onset of sleep and increased duration of sleep, inhibition of central & peripheral pain of mice in different experimental models. The results of this study showed that *U. sinuata* leaf possesses a promising sedative, anxiolytic and analgesic potential. Further research is suggested to find out the mechanisms involved in these effects.

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