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Evaluation of Analgesic, Neuropharmacological and Anti-diarrheal Potential of *Jatropha gossypifolia* (Linn.) Leaves in Mice

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The aim of the study was to evaluate the possible analgesic, neuropharmacological and anti-diarrheal activities of methanol extract of leaves of *Jatropha gossypifolia* Linn. (Euphorbiaceae) in mice. Acetic acid induced writhing inhibition test was used to measure the analgesic activity of the extract. The neuropharmacological activity of the extract was assessed by hole cross, hole board and elevated plus-maze tests and the anti-diarrheal activity was evaluated by castor oil induced diarrhea inhibition test. The extract demonstrated highly significant ($p<0.001$) analgesic activity with % inhibitions of writhing response at doses 200 and 400 mg kg^{-1} b.wt. were 67.56 and 65.14%, respectively. In hole cross test, the extract at both doses showed significant ($p<0.05$) sedative effect in mice. In hole board test, the extract showed highly significant ($p<0.001$) anxiolytic activity at dose 200 mg kg^{-1} b.wt. whereas, the same activity was observed at dose 400 mg kg^{-1} b.wt. in elevated plus-maze test. The extract also showed highly significant ($p<0.001$) anti-diarrheal activity in castor oil induced diarrhea inhibition test. The present findings suggest that the plant possess significant analgesic, neuropharmacological and anti-diarrheal properties and could be a prominent source of medicinally importance phytoconstituents.

Key words: *Jatropha gossypifolia*, analgesic, anxiolytic, sedative, anti-diarrheal

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INTRODUCTION

Jatropha gossypifolia Linn. (Euphorbiaceae) is a bushy, extroverted shrub of about 1.8 m in height and commonly known as Bellyache bush (English) (Oduola *et al*., 2005a), Lal Bheranda, Laljeol, Aar kocha (Bengali), Karachuni (Marma), Kander (Garo) (Uddin, 2006). The shrub is native to tropical America (Bullangpoti *et al*., 2012) but it is also commonly seen in road sides and fallow lands in Bangladesh.

J. gossypifolia is a medicinally important shrub as it is rich source of diverse phytoconstituents. The bark of the shrub contains jatrophine (an alkaloid) (Oduola *et al*., 2005b) whereas the stem contains jatroiden (a lignin) (Oduola *et al*., 2005a). The phytoconstituents previously reported to be found in the plant were saponin, lignan, tannin, phenolic compounds, flavonoid, curcin, triterpenes, diterpene, jatrophone, jatropholones A and B, jatrophatrione, apigenin, cyclogossine A (Khumrungsee *et al*., 2009). *J. gossypifolia* is traditionally being used for the treatment of various ailments. In Latin America and the Caribbean, the leaves are traditionally being used in boils, carbuncles, eczema, itches and veneral diseases (Parvathi *et al*., 2012). The leaves are also used as febrifuge (Dhale and Birari, 2010). The bark is used as emmenagogue (Dhale and Birari, 2010) while seeds are used as emetic, purgative and also used for cancer and body pain (Dhale and Birari, 2010). The use of roots of the shrub to treat leprosy is well known to date (Dhale and Birari, 2010). *J. gossypifolia* has also been reported for its antialergic, molluscicidal, antimicrobial, insect repellent (Khumrungsee *et al*., 2009), larvicidal (Bullangpoti *et al*., 2012), coagulating and anti-coagulating (Parvathi *et al*., 2012) activities by various researchers.

The present investigations were carried out to identify the possible analgesic, neuropharmacological and anti-diarrheal activities of methanol extract of *J. gossypifolia* leaves available in Bangladesh.

MATERIALS AND METHODS

Plant collection and identification: The leaves of *J. gossypifolia* were collected in August, 2011 from Sadhuhati, Bangladesh. The collected plant part was identified by a taxonomist (Dr. Bushra Khan, Principal Scientific Officer) of Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen of the plant (DACB Accession No. 35937) has been deposited for future reference.

Preparation of methanol extract: The collected leaves were thoroughly washed with water to remove the adhering dirt and sun dried for 15 days. The dried, coarsely powdered material (1 kg) was extracted by cold extraction process using methanol (2.5 L) as the solvent at room temperature. The extract was then filtered and concentrated with a rotary evaporator (IKA, Germany) at low temperature (45°C) and reduced pressure to get dry extract (12.6% w/w). The dry extract was stored at 4°C until use.

Experimental animals: Twenty Swiss albino mice of either sex, purchased from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B), were taken for the tests. Mice were housed under standard laboratory conditions (room temperature 25.0±2.0°C, relative humidity 55-65% and 12 h light: dark cycle). They were fed with standard food (ICDDR, B formulated) and had free access to tap water. The experiments were performed on an isolated and noiseless room. The time interval between the tests was two weeks. The experimental protocols were in accordance with the principles and guidelines adopted by the Animal Experimentation Ethics Committee (AEEC) of East West University.

Analgesic activity: Acetic acid induced writhing inhibition test was used to assess the analgesic activity of methanol leaves extract of *J. gossypifolia*. The test was performed according to the method described by Al-Amin *et al*. (2011). According to the method, mice were fasted overnight with water *ad libitum* and randomly divided into four groups comprising of five mice each. Each group received a particular treatment i.e., control group received 1% (v/v) Tween-80 (Merck, Germany) in normal saline (Beximco Infusion Ltd., Bangladesh) at a dose of 0.5 mL mice^{-1}, positive control received diclofenac sodium (Square Pharmaceutical Ltd., Bangladesh) at dose of 10 mg kg^{-1} b.wt. and test groups received methanol extract at the dose of 200 and 400 mg kg^{-1} b.wt., respectively. A 0.7% v/v acetic acid (Merck, Germany) solution was administered (0.1 mL/10 g) intraperitoneally to each mouse to generate pain, 30 min after oral administration of control and test groups. The positive control was administered orally 15 min prior to administration of acetic acid. Just 5 min after the administration of acetic acid solution, the number of writhing by each mouse was counted individually for a period of 20 min. Mice did not always accomplish full writhing. This incomplete writhing was taken as a half-writhing and two half-writhing were counted as one full writhing (Al-Amin *et al*., 2011; Zulfiker *et al*., 2010). Analgesic activity was expressed as writhing inhibition (%) and was calculated by using the following formula:

$$\text{Writhing inhibition (\%)} = \frac{W_c - W_s}{W_c} \times 100$$

where, W_c is the mean number of writhing of control and W_s is the mean number of writhing of the test sample.

Neuropharmacological activities: The neuropharmacological activities of *J. gossypifolia* leaves was evaluated by performing hole cross, hole board and elevated plus-maze tests. Mice were divided into four groups each comprising of 5 mice during each experiment. The groups received particular treatments:

Group 1: Control (1% v/v tween-80 in normal saline, 0.5 mL mice^{-1})
Group 2: Positive control (diazepam, Square Pharmaceuticals Ltd., Bangladesh, 1 mg kg^{-1} b.wt.)
Group 3: Test sample I (methanol extract at the dose of 200 mg kg^{-1} b.wt.)
Group 4: Test sample II (methanol extract at the dose of 400 mg kg^{-1} b.wt.)

Hole cross test: The sedative activity of the extract was assessed by using hole cross test, as described by Subhan *et al.* (2008). A wooden box partitioned in the middle and having a dimension of 30×20×14 cm was taken for the test. A hole of 3 cm diameter was made in the center partition of the box at a height of 7.5 cm. The spontaneous movement of the mice from one chamber to other through the hole was observed immediately after oral administration of test drugs. The observation was carried out for 3 min at 30, 60, 90 and 120 min.

Hole board test: The hole board test is the widely used for evaluating anxiolytic and/or anxiogenic activity of mice (Takeda *et al*., 1998). The test was done according to the method described by Somani *et al.* (2010). A wooden hole board apparatus (20×40 cm) with sixteen evenly spaced holes and elevated to a height of 25 cm was used for the test. The diameter of each hole was 3 cm. Each mouse was placed on the center of the board after 30 min of the oral administration of particular treatment and the number of head dipping and the latency until the first entry was calculated during a period of 5 min.

Elevated plus-maze test: Elevated plus Maze (EPM) test was performed according to the method described by Thippeswamy *et al.* (2011). The EPM apparatus was consisted of two open arms (16×5 cm) and two closed arms (16×5×12 cm) which emerged from a central platform (5×5 cm) and elevated to a height of 40 cm. The apparatus was set up in a dimly illuminated and noiseless room. Mice were placed on the central platform of the maze facing towards one of the open arms after 30 min of oral administration of treatments. For each mouse both the total exploratory activity (number of entries in both arms) and other ethologically derived measures (such as grooming, rearing and stretch-attend postures) were recorded by using digitized video camera for a period of 5 min.

Anti-diarrheal activity: Castor oil induced diarrhea inhibition test, described by Shoba and Thomas (2011), was used to evaluate the anti-diarrheal activity of the methanol extract. In this method, mice were fasted overnight and randomly divided into four groups having five mice in each. Each group received a particular treatment i.e., control (1% v/v tween-80 in normal saline, 0.5 mL mice^{-1}), positive control (loperamide, Square Pharmaceuticals Ltd., Bangladesh, 2 mg kg^{-1} b.wt.) and test samples (200 and 400 mg kg^{-1} of b.wt.). A 0.2 mL castor oil (BDH Chemicals Ltd., UK) was fed to each mouse to induce diarrhea, 30 min after the treatments. After that mice were placed in separate beakers lined with filter papers for observation. During an observation period of 2 h, a numbers of parameters were recorded: (1) Onset of dry stool (2) No. of wet stool (3) Weight of wet stool (4) Total weight of fecal output and (5) Onset of wet stool.

Statistical analysis: All the data were presented as Mean±SEM. SPSS for WINDOWS™ (version 12.0) was applied for the data analysis and statistically analyzed by one-way ANOVA followed by Dunnett t-test (2-sided). $p<0.05$ was taken to be the level of significance, $p<0.001$ was taken to be the level of highly significance.

RESULTS

Analgesic activity: In the acetic acid-induced writhing inhibition test, the extract at both doses (200 and 400 mg kg^{-1}) demonstrated highly significant ($p<0.001$) inhibition of writhing response induced by the acetic acid in a dose dependant manner. The percent inhibitions of the writhing response at the doses 200 and 400 mg kg^{-1} were found to be 67.56 and 65.14%, respectively (Table 1).

Table 1: Effect of methanol extract of *J. gossypifolia* leaves (JGL) and diclofenac sodium on acetic acid induced writhing response in mice

Treatment	Dose (p.o.)	No. of writhing	Inhibition (%)
Control	0.5 mL mice^{-1}	78.6±0.29	-
Positive control	10 mg kg^{-1}	1.5±0.15**	98.09
JGL200	200 mg kg^{-1}	25.5±0.70**	67.56
JGL400	400 mg kg^{-1}	27.4±0.33**	65.14

Values are expressed as Mean±SEM (n = 5), **p<0.001 compared to control, JGL200 and JGL400 indicate dose of 200 and 400 mg kg^{-1} b.wt., respectively, Positive control: Diclofenac sodium

Neuropharmacological activities

Hole cross test: In the hole cross test, the extract at both doses of 200 and 400 mg kg^{-1} b.wt. showed highly significant ($p<0.001$) decrease in locomotion activity in the test animals at the observation periods compare to the control group (Table 2). The positive control, diazepam showed decrease in locomotion activity at all the observation periods (Table 2).

Hole board test: In the hole board test, the extract at dose 200 mg kg^{-1} b.wt. showed an increase number in head dipping (50.6±0.87) behavior compared to the control group which was statistically highly significant ($p<0.001$) (Table 3). But the extract at dose 400 mg kg^{-1} showed highly significant ($p<0.001$) decrease in head dipping (27.8±0.58) behavior and latency until the first head dipping (6.4±0.51) behavior compared with the control group (Table 3).

Elevated plus-maze test: In Elevated Plus-maze (EPM) test, the extract at dose 200 mg kg^{-1} b.wt. showed decrease in time spent in open arm compare to control. But the extract at dose 400 mg kg^{-1} b.wt. showed increase in the time spent in the open arm compare to control (Table 4). The effects of extract on mice ethologically derived measures after oral administration were shown in Fig. 1. A significant ($p<0.05$) decrease in rearing behavior was observed by treatment with the extract at dose of 400 mg kg^{-1} b.wt. (Fig. 1).

Table 2: Effect of methanol extract of *J. gossypifolia* leaves (JGL) and diazepam on number of movements in hole cross test

		No. of movements (min)			
Treatment	Dose (p.o.)	30	60	90	120
Control	0.5 mL $mice^{-1}$	8.6±0.60	7.2±0.58	7.0±0.55	6.2±0.37
Positive control	1 mg kg^{-1}	6.2±0.80*	5.8±0.73	4.2±1.16*	3.6±0.51**
JGL200	200 mg kg^{-1}	4.2±0.20**	2.6±0.24**	1.2±0.37**	1.6±0.24**
JGL400	400 mg kg^{-1}	2.2±0.37**	1.0±0.45**	1.0±0.32**	2.2±0.37**

Values are expressed as Mean±SEM (n = 5), *p<0.05, **p<0.001 compared to control, JGL200 and JGL400 indicate dose of 200 and 400 mg kg^{-1} b.wt., respectively, Positive control: Diazepam

Table 3: Effect of methanol extract of *J. gossypifolia* leaves (JGL) and diazepam in hole board test in mice

Treatment	Dose (p.o.)	No. of head dipping	Latency until the first head dipping (sec)
Control	0.5mL $mice^{-1}$	44.8±1.24	14.8±0.37
Positive control	1 mg kg^{-1}	29.6±0.93**	2.0±0.45**
JGL200	200 mg kg^{-1}	50.6±0.87**	6.0±0.32**
JGL400	400 mg kg^{-1}	27.8±0.58**	6.4±0.51**

Values are expressed as Mean±SEM (n = 5), **p<0.001 compared to control, JGL200 and JGL400 indicate dose of 200 and 400 mg kg^{-1} b.wt., respectively, Positive control: Diazepam

Anti-diarrheal activity: In castor oil-induced diarrhea inhibition test, the extract at both doses of 200 and 400 mg kg^{-1} b.wt. showed highly significant ($p<0.001$) decrease in mean number of stool and total weight of fecal output compare to control group (Table 5).

DISCUSSION

Analgesic activity: In the acetic acid induced writhing inhibition test in mice, the extract showed highly significant ($p<0.001$) inhibition of writhing response in a dose dependent manner (Table 1). The highly significant inhibition of writhing response by the extract may be due to the presence of analgesic principals in the plant such as alkaloids, flavonoids, saponins, resins and tannins which were reported by other researchers (Oduola *et al*., 2005b; Khumrungsee *et al*., 2009). These phytochemicals were reported to inhibit pain sensation primarily by acting

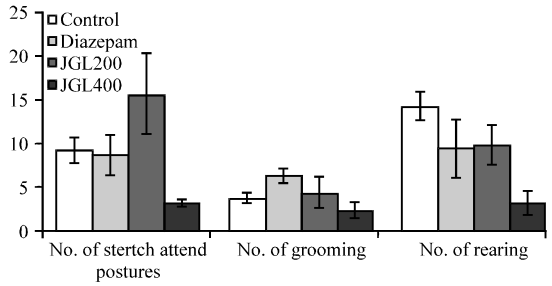

Fig. 1: Effect of diazepam and methanol extract of *J. gossypifolia* leaves (JGL) on ethologically derived measures in mice tested on the elevated plus-maze. Results are shown as mean±SEM, n = 5, *p<0.05, compared to control; JGL200 and JGL400 indicate dose of 200 and 400 mg kg^{-1} b.wt., respectively. diazepam: Positive control

Table 4: Effect of methanol extract of *J. gossypifolia* leaves (JGL) and diazepam on elevated plus-maze model test in mice

		Time spent (sec)		No. of entry		Total time spent (sec) (%)		Entries (%)	
Treatment	Dose (p.o.)	Open arm	Close arm	Open arm	Close arm	Open arm	Close arm	Open arm	Close arm
Control	0.5 mL $mice^{-1}$	7.6±0.51	215±1.52	1.2±0.49	9.4±0.51	3.41±0.51	96.59±1.52	11.32±0.49	88.68±0.51
Positive control	1 mg kg^{-1}	6.8±0.58	260±1.87	1.6±0.81	12.0±0.55	2.55±0.58	97.45±1.87	11.76±0.81	88.24±0.55
JGL200	200 mg kg^{-1}	3.0±.32	267.20±1.71*	0.2±0.20	8.80±2.56	1.11±0.32	98.89±1.71	2.22±0.20	97.78±2.56
JGL400	400 mg kg^{-1}	10±0.55	275.20±1.16*	0.60±0.24	6.40±1.57	3.51±0.55	96.49±1.16	8.57±0.24	91.43±1.57

Values are expressed as Mean±SEM (n = 5), *p<0.05 compared to control, JGL200 and JGL400 indicate dose of 200 and 400 mg kg^{-1} b.wt., respectively. Positive control: Diazepam

Table 5: Effect of methanol extract of *J. gossypifolia* leaves (JGL) and loperamide on castor oil induced diarrhea in mice

Treatment (dose)	Dose (p.o.)	Latent period (min)	No. of stool	Total weight of fecal output
Control	0.5mL mice^{-1}	42.8±1.16	14.4±1.75	0.94±0.02
Positive control	2 mg kg^{-1}	95.2±1.71**	8.6±0.75*	0.73±0.03**
JGL200	200 mg kg^{-1}	7.2±0.66**	7.6±0.51**	0.48±0.03**
JGL400	400 mg kg^{-1}	53.6±1.36**	9.2±0.66**	0.30±0.02**

Values are expressed as Mean±SEM (n = 5), *p<0.05, **p<0.001 compared to control; JGL200 and JGL400 indicate dose of 200 and 400 mg kg^{-1} b.wt., respectively. Positive control: Loperamide

with prostaglandin pathways (Rahman, 2012). Further detailed investigations are needed to determine the actual mechanism by which the plant shows its non-narcotic analgesic activity.

Neuropharmacological activities

Hole cross test: The extract at both doses showed decrease in spontaneous motor activity in the hole cross test (Table 2). The decrease in number of hole cross indicates the sedative activity of the plant. Drug acting on CNS mainly exert their action through $GABA_A$ receptor as Gamma-amino-butyric Acid (GABA) is the major inhibitory neurotransmitter in the CNS (Dolai *et al*., 2012). The observed sedative effect of the extract may be due to CNS hyperpolarization via interaction with $GABA_A$ receptor. The positive control showed decrease in number of hole cross compare to control which may be due to the dose (1 mg kg^{-1}) used in the test that can produce sedation in mice (Apu *et al*., 2013).

Hole board test: The hole board experiment is a widely accepted test to measure the anxiolytic and anxiogenic behiviour of mice (Takeda *et al*., 1998). The extract showed highly significant (p<0.001) anxiolytic and anxiogenic behavior at dose 200 and 400 mg kg^{-1} b.wt. respectively (Table 3). Preliminary qualitative phytochemical screening reveals the presence of alkaloids flavonoids, saponins and tannins (Oduola *et al*., 2005a; Khumrungsee *et al*., 2009) in *J. gossypifolia* which are well known to have activity against many CNS disorders (Adeyemi *et al*., 2006). So the observed anxiolytic activity at lower doses (200 mg kg^{-1} b.wt.) may be due to the binding of any of the phytochemicals to the $GABA_A$-BZDs complex; however further studies are needed to assurance this. The highly significant (p<0.001) decrease in head-dipping behavior observed in diazepam treated mice compare to control may be due to the sedative dose (1 mg kg^{-1}) used in the test (Apu *et al*., 2013).

Elevated plus-maze test: The elevated plus maze test is considered to be an etiologically valid animal model of testing psychomotor performance and emotional aspects of mice. In the elevated plus-maze test (Table 4, Fig. 1), the extract at higher dose (400 mg kg^{-1} b.wt.) showed increased in the time spent in open arm which indicates the anxiolytic activity of the plant, whereas anxiogenic effect was observed at dose (200 mg kg^{-1} b.wt.) as described by Apu *et al*. (2013). The observed anxiolytic activity of the extract may be due to the presence of various phytoconstituents such as sterols, flavonoids, saponins, tannins in the plant (Gadekar *et al*., 2011).

Anti-diarrheal activity: Castor oil develops diarrhea upon oral administration in mice due to the presence of recinoleic acid which can cause intestinal stimulation of motility and secretion (Apu *et al*., 2013). It is well evident that recinoleic acid showed the effects by irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins (Ezeonwumelu *et al*., 2012). Di Carlo *et al*. (1993) reported the activity of flavonoids in diarrhea by inhibition of release of autacoids and prostaglandins, which result in inhibition of motility and secretion induced by castor oil. Tannins are also reported to have anti-diarrheal property since these phytoconstituents may precipitate proteins of the enterocytes; reduce peristaltic movement and intestinal secretions (Okudo *et al*., 1989). So, the observed anti-diarrheal activity (Table 5) of the methanol extract may be due to the presence of these phytochemicals.

CONCLUSION

Finally, it can be concluded from the results of the study that methanol leaves extract of *J. gossypifolia* has significant analgesic, neuropharmacological and anti-diarrheal activities at the investigated doses. However extensive studies can be carried out to identify the phytoconstituent(s) with their mechanism of actions responsible for the observed pharmacological activities.

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REFERENCES

Adeyemi, O.O., O.K. Yetmitan and A.E. Taiwo, 2006. Neurosedative and muscle relaxant activities of ethyl acetate extract of *Baphia nitida* AFZEL. J. Ethnopharmacol., 106: 312-316.

Al-Amin, M., G.N.N. Sultana and C.F. Hossain, 2011. Analgesic and anti-inflammatory activities of *Rhynchostylis retusa*. Biol. Med. J., 3: 55-59.

Apu, A.S., M. Matin, S.H. Bhuyan, M.F. Hossain and K. Ireen, 2013. Study of analgesic, neuropharmacological and anti-diarrheal activities of ethanol extract of *Solanum sisymbriifolium* fruits. Pharmacologia, 4: 164-169.

Bullangpoti, V., E. Wajnberg, P. Audantb and R. Feyereisenb, 2012. Antifeedant activity of *Jatropha gossypifolia* and *Melia azedarach* senescent leaf extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use as synergists. Pest Manage. Sci., 68: 1255-1264.

Dhale, D.A. and A.R. Birari, 2010. Preliminary screening of antimicrobial and phytochemical studies of *Jatropha gossypifolialinn*. Recent Res. Sci. Technol., 2: 24-28.

Di Carlo, G., G. Autore, A.A. Izzo, P. Maiolino and N. Mascolo *et al*., 1993. Inhibition of intestinal motility and secretion by flavonoids in mice and rats: Structure activity relationships. J. Pharm. Pharmacol., 45: 1054-1059.

Dolai, N., N. Karmakar, R.B.S. Kumar and P.K. Haldar, 2012. CNS depressant activity of *Castanopsis indica* leaves. Orient. Pharm. Exp. Med., 12: 135-140.

Ezeonwumelu, J.O., R.G. Omolo, A.M. Ajayi, E. Agwu, J.K. Tanayen and C.P. Adiukwu, 2012. Studies of phytochemical screening, acute toxicity and anti-diarrhoeal effect of aqueous extract of kenyan *Tithonia diversifolia* leaves in rats. Br. J. Pharmacol., 3: 127-134.

Gadekar, D.H., J. Sourabh and M.K. Jitender, 2011. Evaluation of anxiolytic activity of *Boerhaavia diffusa* hydro-alcoholic extract of leaves in rats. Int. Res. J. Pharm., 2: 90-92.

Khumrungsee, N., V. Bullangpoti and W. Pluempanupat, 2009. Efficiency of *Jatropha gossypifolia* L. (Euphorbiaceae) *Against spodoptera* exigua hubner (Lepidoptera: Noctuidae): Toxicity and its detoxifying enzyme activities. KKU Sci. J., 37: 50-55.

Oduola, T., G.O. Adeosun, T.A. Oduola, G.O. Avwioro and M.A. Oyeniyi, 2005a. Mechanism of action of *Jatropha gossypifolia* stems latex as a haemostatic agent. Eur. J. Gen. Med., 2: 140-143.

Oduola, T., O.G. Avwioro and T.B. Ayanniyi, 2005b. Suitability of the leaf extract of *Jatropha gossypifolia* as an anticoagulant for biochemical and haematological analysis. Afr. J. Biotechnol., 4: 679-681.

Okudo, T., T. Yoshoda and T. Hatano, 1989. New methods of analyzing tannins. J. Nat. Prod., 52: 1-31.

Parvathi, V.S., B.S. Jyothi, T. Lakshmi, P.S. Babu and R. Karthikeyan, 2012. Morpho-anatomical and physicochemical studies of *Jatropha gossypifolia* (L.). Der Pharm. Lett., 4: 256-262.

Rahman, S., 2012. Antioxidant, analgesic, cytotoxic and antidiarrheal activities of ethanolic *Zizyphus mauritiana* bark extract. Orient Pharm. Exp. Med., 12: 67-73.

Shoba, F.G. and M. Thomas, 2001. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhea. J. Ethnopharmacol., 76: 73-76.

Somani, R.R., G. Kadam, R. Vohra, S. Vijayaraghavan and P.Y. Shirodkar, 2010. Studies of cns activities of some mannich bases of 1,3,4-oxadiazole. Int. J. Pharmacol., 6: 696-704.

Subhan, N., M.A. Alam, F. Ahmed, I.J. Shahid, L. Nahar and S.D. Sarker, 2008. Bioactivity of *Excoecaria agallocha*. Braz. J. Pharmacognosy, 18: 521-526.

Takeda, H., M. Tsuji and T. Matsumiya, 1998. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur. J. Pharmacol., 350: 21-29.

Thippeswamy, B.S., B. Mishra, V.P. Veerapur and G. Gupta, 2011. Anxiolytic activity of *Nymphaea alba* Linn. in mice as experimental models of anxiety. Indian J. Pharmacol., 43: 50-55.

Uddin, S.N., 2006. Traditional Uses of Ethnomedicinal Plants of the Chittagong Hill Tracts. 1st Edn., Bangladesh National Herbarium, Dhaka, pp: 317-880.

Zulfiker, A.H.M., M.M. Rahman, M.K. Hossain, K. Hamid, M.E.H. Mazumder and M.S. Rana, 2010. *In vivo* analgesic activity of ethanolic extracts of two medicinal plants-*Scoparia dulcis* L. and *Ficus racemosa* Linn. Biol. Med., 2: 42-48.