INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ANALGESIC AND CYTOTOXIC ACTIVITIES OF THREE REPUTED BANGLADESHI MEDICINAL PLANTS

Md. Taleb Hossain[1, 2]*, Mohammed Rafiquzzaman[2], Md. Saiful Islam[3], Md. Abu Sufian[1], Sukalyan Kumar Kundu[2]
[1]Department of Pharmacy, Northern University Bangladesh, Dhaka-1205, Bangladesh
[2]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
[3]Department of Quality Control, The Ibn Sina Pharmaceutical Industries Ltd. Bangladesh
*Corresponding Author Email: talimmsavar@yahoo.com

Article Received on: 11/07/15 Revised on: 03/08/15 Approved for publication: 18/08/15

DOI: 10.7897/2230-8407.069118

ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand in the present world for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. Pain is a discomfort and its relief must be tackled. Currently available synthetic pain killers possess many known adverse and toxic effects but those do not appear in the treatment with natural products. Again various forms of cancers are rising all over the world, requiring newer therapy with minimum toxicity. So the quest of analgesics and anticancer drugs from natural sources is the demand of time. Therefore, in the present study, three medicinal plants of Bangladesh namely *A. squamosa, C. procera* and *P. betel* were chosen and crude ethanolic extracts of their leaves were prepared and investigated for possible *in vivo* analgesic and cytotoxic properties. The extracts produced significant writhing inhibition in acetic acid induced writhing in mice at the oral doses of 250 mg/kg and 500 mg/kg body wt. ($p < 0.001$) and their effects were comparable to that of the standard drug diclofenac sodium at the dose of 25 mg/kg body weight. The crude extracts also produced prominent cytotoxic activities in brine shrimp (*Artemia salina)*.

Key words: *A. squamosa*, *C. procera*, *P. betel*, Analgesic, Cytotoxic.

INTRODUCTION

Anonna squamosa Linn. (Fam. Anonnaceae) is a small tree, about 3 to 6 meters high, with oblong to lanceolate leaves, having greenish flowers and warty skinned segmented sweet fruits, and planted as a fruit plant in different areas of Bangladesh. Its leaves and tender stems contain alkaloids, anonaine, roemerine, norcorydine, corydine, isocorydine, nor-isocorydine, norlaurelinen glaucine, xylopine and lanuginosine. Its seed oil is rich in the unsaturated fatty acids: oleic and linoleic acids[1]. Leaves and fruits of this plant are used for treating tumors and their extracts possess spasmolytic and oxytocic properties. They also show significant anti-cancer activities. Leaf, bark and unripe fruits of the plant are used to treat diarrhea and dysentery[2]. Ripe fruit can be used as tonic, laxative and anthelmintic. Root is considered as a drastic purgative. Seeds are abortifacient. Leaves, fruits and seeds are also used as insecticides. The aporphines and oxoaporpines isolated from the plant possess strong antiplatelet and vasorelaxing actions[3].

Calotropis procera R. Br. (Fam. Asclepiadaceae) is a small plant with broad obovate fleshy leaves and white flowers. It is a perennial shrub abounding in milky latex which grows commonly in wastelands and graveyards in all over Bangladesh. Root bark of the plant is useful in treating chronic cases of dyspepsia, flatulence, constipation, loss of appetite, indigestion and mucous in stool. Extracts of its leaves and roots stimulate respiration and blood pressure in dog, and are used in cardiac arrhythmia, rheumatism and cancer. Flowers of the plant are useful in asthma. Calotropin isolated from this plant has digitalis-like actions[4].

Piper betel Linn. (Fam. Piperaceae) is a stout twining climber with broadly ovate oblong or ovate cordate leaves, tiny yellow-green flowers and small spherical fruits. It is extensively cultivated as a cash crop throughout Bangladesh. Leaves of the plant are popularly used as carminative, astringent, stimulant and antiseptic. They are also used in headache and cough of children. Leaf stalk of this plant is used as a suppository for rectal evacuation in children while leaf, mixed with honey, is a remedy for coughs. Juice of leaves is used as an eye drop in painful infections and night blindness, and also as a relief in cerebral congestion. Roots of the plant induce permanent sterility in women. Extract of its leaves exerts anti-tumor activity and suppresses mutagenic actions of tobacco specific nitrosamines[5].

We also studied the ethanolic extracts of leaves of these three locally reputed plants for their analgesic and cytotoxic effects and, herein, report the results of our investigation.

MATERIALS AND METHODS

Collection and preparation of the plant materials

A. squamosa and *C. procera* leaves were collected from Jahangirnagar University campus, Dhaka, Bangladesh and *P. betel* leaves were collected from the local market of Dhaka, Bangladesh in February 2011. Their voucher specimens (nos. 39316, 37537 and 37536, respectively) have been deposited in the Bangladesh National Herbarium. Leaves of the above mentioned plants were dried under shed for several days after washing. The plant materials were then ground to coarse powder using Noka Super Blender, Japan. Each of the powdered materials (300 gm) was then soaked in ethanol (1.5 liter) separately and kept for 14 days at room temperature with occasional shaking. The crude extracts were then filtered through cotton plug followed by Whatman number-1 filter paper (Bibby RE200, UK) individually and the extracts were finally concentrated, one by one, with a vacuum rotary evaporator (Buchi, Switzerland).

Animals

Young Swiss-albino mice of both sex, weighing 20-25 gm, were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were kept at animal house (Department of Pharmacy, Northern University Bangladesh) for adaptation under standard laboratory conditions (Relative humidity 55-65%, Room temperature 25.0±2.0°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. All animal experiments were undertaken in a calm and isolated area in accordance with guidelines of the Animal Ethics Committee, Northern University Bangladesh, Dhaka, Bangladesh (112/B/NUB/2012).

Analgesic activity

Analgesic activities of the extracts were studied using acetic acid induced writhing model in mice[6]. The experimental animals were randomly divided into four groups and each of which consisted of five (5) mice. Group I was denoted as 'control group' which received 1% (v/v) Tween 80 in water at the dose of 10 mL/kg body weight; Group II was denoted as 'positive control' and was administered with diclofenac sodium (standard drug) at the dose of 25 mg/kg body weight; Group III and Group IV were test groups and were treated with the plant extracts at dose of 250 mg/kg and 500 mg/kg body weight, respectively. Test samples, standard drug and control vehicle were administered orally 30 min before the intra peritoneal administration of 0.7% acetic acid. After 15 min of injection of acetic acid, the mice were observed for writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

Hatching of shrimp

Artificial sea water was prepared by dissolving 40 g of reagent grade NaCl and 36 g of table salt in two liters of distilled water and was filtered off to get a clear solution. The prepared saline solution was taken into a medium sized rectangular tank which was divided into two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. The eggs of *Artemia salina* were hatched[7] at room temperature (25-30 ºC) for 18-24 h in the tank containing the brine. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. They were then collected by a Pasteur pipette for brine shrimp lethality bioassay.

Brine shrimp lethality bioassay

General toxicity of the extracts was studied with established protocol[8, 9]. Each of the samples was dissolved separately in DMSO and then transferred to test tubes to get concentrations of the samples as 160, 80, 40, 20, 10 and 5µg/mL in 5 mL artificial sea water including ten nauplii in each test tube. The concentration of DMSO did not exceed 0.01% in any of the test tubes. Negative control test tubes contained only DMSO (≤ 0.01%) in artificial sea water while the positive control test tubes contained anticancer drug 5-flurouracil of concentration same as that of the sample concentrations in DMSO (≤ 0.01%) containing sea water. After 24 hrs of incubation at room temperature (25-30ºC), the number of viable naupliis were counted using a magnifying glass.

Statistical analysis

All the experimental results were given as mean±SEM of three parallel measurements and data were evaluated by using student's t test. Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The significant difference between the control group and experimental groups was determined. The results obtained from samples and control group were plotted in standard diagrams and level of significance were found. P values < 0.001 were regarded as significant.

RESULTS

Analgesic activity

Table 1 shows the results of analgesic activities of the extracts on acetic acid induced writhing in mice. At the dose of 250 mg/kg and 500 mg/kg of body weight, writhing inhibition of 51.56% and 56.25% for *A. squamosa*; 35.94% and 43.75% for *C. procera* and 43.75% and 56.25% for *P. betel* were produced, respectively. The results were statistically significant (P <0.001) and comparable to that of the standard drug Diclofenac sodium (71.88 % writhing inhibition at a dose of 25 mg/kg body weight) (Table 1).

Brine shrimp lethality bioassay

In brine shrimp lethality bioassay (Table 2), the extracts showed significant lethality against the brine shrimp nauplii. For the extracts, the number of nauplii died and percent mortality was counted. All the extracts showed different mortality at different concentrations. From the plot of percent mortality versus log concentration (Figure 1, 2, 3), LC_{50} and LC_{90} were deduced. The values found were LC_{50}: 20 µg/mL; LC_{90}: 160 µg/mL for *A. squamosa* (Fig.1), LC_{50}: 40 µg/mL; LC_{90}: 80 µg/mL for *C. procera* (Fig.2) and LC_{50}: 20 µg/mL; LC_{90}: 160 µg/mL for *P. betel* (Fig.3) while the LC_{50} and LC_{90} of the standard anticancer drug 5-flurouracil were 5 µg/mL and 10 µg/mL (Any of the figures 1, 2, 3), respectively.

DISCUSSION

Analgesic activities of the extracts were tested by acetic acid induced writhing model in mice. Acetic acid causes analgesia by liberation of endogenous substances which then excite the nerve endings[10]. Table 1 summarizes the findings of analgesic activity test as the similar and highest writhing inhibition (56.25%) by *A. squamosa* and *P. betel* extracts followed by (43.75%) of *C. procera* extracts at 500 mg/kg body weights. It also reveals writhing inhibition at 250 mg/kg body weight in a descending order of 51.56% > 43.75% > 35.94% produced by *A. quamosa, P. betel* and *C. procera*, respectively. The extracts produced significant writhing inhibition (P < 0.001). On the basis of the results of acetic acid induced writhing test, it can be said that the ethanolic extracts of leaves of the studied plants possess analgesic activities. The peripheral analgesic effect of the plants' extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the extracts may be mediated through inhibition of central pain receptors[11]. This hypothesis is in consonance with those of Koster et al. and Williamson et al. who postulated that acetic acid-induced writhing method is useful technique for the evaluation of peripherally acting analgesic drugs[12, 13]. With respect to the writhing test, the research group of Derardt et al. described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intra-peritoneal injection of acetic acid[14]. These authors found high levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. Therefore the analgesic activities of the plant extracts of the present study might be for the reduction of prostaglandins as well as for the inhibition of pain receptors [10, 11].

Brine shrimp lethality bioassay is an easy and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity[10, 15, 16]. In the brine

shrimp lethality bioassay, brine with DMSO (≤ 0.01%) was used as solvent. Negative control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality of brine shrimps. In the present study, the extracts showed LC_{50} at a low to medium concentration range of the extracts used viz; LC_{50}: 20 µg/mL for *A. squamosa* (Figure 1), LC_{50}: 40 µg/mL for *C. procera* (Figure 2) and LC_{50}: 20 µg/mL for *P.betel* (Figure 3). It can be mentioned here that the LC_{50} of the standard anticancer drug (5-flurouracil) was found as 5 µg/mL during the study. Now comparing the LC_{50} of the extracts to the standard drug, it can be said that though the extracts are not active as that of the standard drug but the extracts are significantly potent cytotoxic agents. The order of cytotoxic potency of the samples is *A. squamosa* = *P. betel* > *C. procera* . It is important to mention here that DMSO (≤ 0.01%) did not show any effect on the mortality of brine shrimp in the present study. Rahman et al. also reported the same observation with DMSO[17]. So, the mortality of brine shrimps was due to the effect of the only extracts used in the study. Moreover, the cytotoxic effect of the extracts could be for the presence of cytotoxic phytochemicals in them. Further study is needed to ascertain those active phytochemicals. In addition, it is necessary to test these extracts against various cancer cell lines to justify their anticancer potential more convincingly and to check their toxic effects to the normal cells which is almost inherent to anticancer drugs[18].

Table 1: Effects of the ethanolic extracts of the leaves of *A. squamosa*, *C. procera* and *P.betel* on acetic acid induced writhing of mice (n = 5 in each case)

Groups		Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Controls	Control: Group-I	1% tween 80 solution 10 ml/kg, p.o.	12.08 (100)	-
	Positive Control: Group-II	Diclofenac sodium 25 mg/kg, p.o.	3.6 (28.13)	71.88
Experimental	Test Group- IIIAS 250	Extract of *A. squamosa* , 250 mg/kg, p.o.	6.20 (48.44)	51.56*
	Test group- IVAS 500	Extract of *A. squamosa,* 500 mg/kg, p.o.	5.6 (43.75)	56.25*
	Test Group- IIICP 250	Extract of *C. procera,* 250 mg/kg, p.o.	8.20 (64.06)	35.94*
	Test group- IVCP 500	Extract of *C. procera,* 500 mg/kg, p.o.	5.8 (56.25)	43.75*
	Test Group- IIIPB 250	Extract of *P. betel,* 250 mg/kg, p.o.	7.20 (56.25)	43.75*
	Test group- IV PB 500	Extract of *P. betel,* 500 mg/kg, p.o.	5.6 (43.75)	56.25*

Values are expressed as mean±SEM (Standard Error of Mean); * indicates P < 0.001; one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral, AS = *A. squamosa,* CP = *C. procera* and PB = *P. betel*.

Table 2: Results of brine shrimp lethality bioassay of the ethanolic extracts of leaves of *A. squamosa*, *C. procera* and *P. betel*

Test sample	Conc. (µg/mL)	Log (Conc.)	No. of alive shrimp	% mortality	LC_{50} (µg/ mL)	LC_{90} (µg/ mL)
Positive control (5- flurouracil)	5	0.69	3	70	**5**	**10**
	10	1	1	90		
	20	1.3	0	100		
	40	1.7	0	100		
	80	1.9	0	100		
	160	2.2	0	100		
Ethanolic extract of the leaves of *A.squamosa*	5	0.69	8	20	20	160
	10	1	7	30		
	20	1.3	5	50		
	40	1.7	3	70		
	80	1.9	2	80		
	160	2.2	1	90		
Ethanolic extract of the leaves of *C. procera*	5	0.69	8	20	40	80
	10	1	7	30		
	20	1.3	6	40		
	40	1.7	5	50		
	80	1.9	1	90		
	160	2.2	0	100		
Ethanolic extract of the leaves of *P. betel*	5	0.69	8	20	20	160
	10	1	7	30		
	20	1.3	6	50		
	40	1.7	3	60		
	80	1.9	2	70		
	160	2.2	0	100		

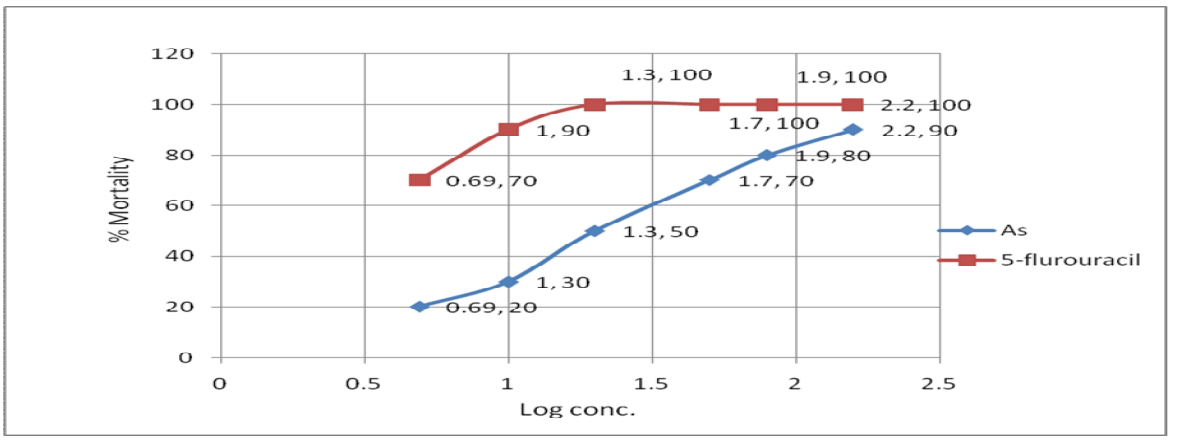

Figure 1: Results of brine shrimp lethality test of ethanolic extracts of the leaves of *A. squamosa* and standard (5-flurouracil)

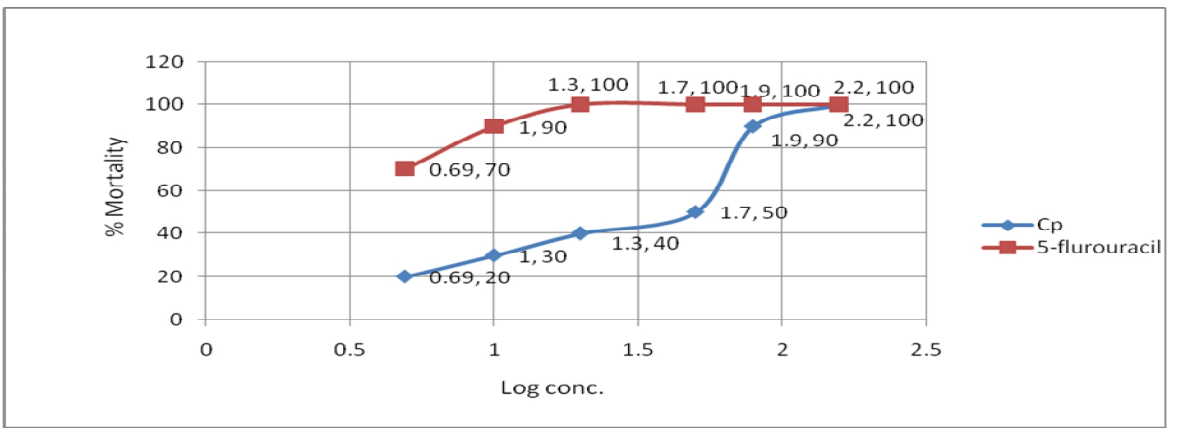

Figure 2: Results of brine shrimp lethality test of ethanolic extracts of the leaves of *C. procera* and standard (5-flurouracil)

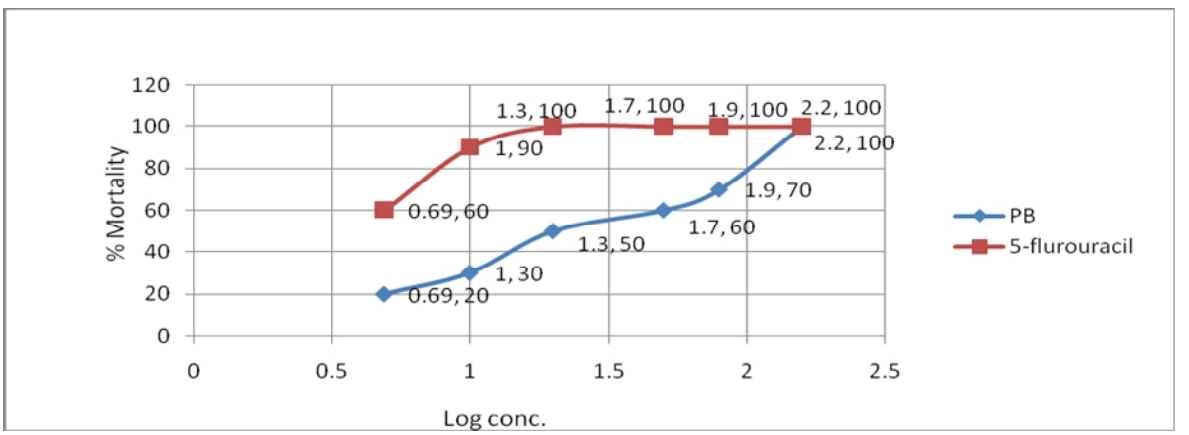

Figure 3: Results of brine shrimp lethality test of ethanolic extracts of the leaves of *P. betel* and standard (5-flurouracil)

CONCLUSION

A. squamosa, *C. procera* and *P. betel* are medicinally important plants and are used in the treatment of various diseases in traditional systems of medicine. This report provides valuable analgesic and cytotoxic information about the plants. Such information may serve as a basis for new pharmacological, toxicological and clinical research.

ACKNOWLEDGEMENTS

We are grateful to the Department of Pharmacy, Northern University Bangladesh and Biopharma Ltd., Tongi, Gazipur, Bangladesh for their materialistic supports.

REFERENCES

1. Ghani A. Medicinal Plants of Bangladesh. 1st ed. Dhaka (BD): Asiatic Society of Bangladesh; 1998.
2. Chevallier A. The encyclopedia of medicinal plants. 1st ed. New York (USA): DK Publishing Inc; 1996.
3. Hopp DC, Zeng L, Gu Z, McLaughlin JL. Squamotacin: an annonaceous acetogenin with cytotoxic selectivity for the human prostate tumor cell line (PC-3). J Nat Prod 1996; 59 Suppl 2: 97-9. http://dx.doi.org/10.1021/np960124i
4. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd ed. Dhaka (BD): Asiatic Society of Bangladesh; 2003.
5. Lalitha MK. Manual on antimicrobial susceptibility testing (under the auspices of Indian association of medical microbiologists). 1st ed. Vellor (India): Christian Medical College; 1996.
6. Nayeem AA, Khatun A, Rahman MS, Rahman M. Evaluation of phytochemical and pharmacological properties of Mikania cordata (Asteraceae) leaves. J Pharmacognosy Phytother 2011; 3: 118-23.
7. Arif A, Zubair K, Shubhra D, Arpona H, Mohamed H, Mohamed H et al. Phytochemical screening, antibacterial and cytotoxic activity of different fractions of Xylocarpus mekongensis. Ibnosina J Med Biomed Sci 2013; 5 Suppl 4: 206-13.
8. Meyer BN, Ferrigni RR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982; 45 Suppl 5: 31-4. http://dx.doi.org/10.1055/s-2007-971236
9. Sufian MA, Mehedi MSA, Rashid MA, Haque MR. Biological investigation of Entada rheedii Spreng. and isolation of entadamide A from its seed. Int Res J Pharm 2015; 6(7): 411-14. http://dx.doi.org/10.7897/2230-8407.06785
10. Sajeesh T, Parimelazhagan T. Analgesic, anti-inflammatory and GC-MS studies on Castanospermum australe A. Cunn. & C. Fraser ex Hook. ScientificWorldJournal 2014; 2014: 9.
11. Ramirez MR, Guterres L, Dickel OE, de Castro MR, Henriques AT, de Souza MM et al. Preliminary studies on the antinociceptive activity of Vaccinium ashei berry in experimental animal models. J Med Food 2010; 13 Suppl 2: 336–42. http://dx.doi.org/10.1089/jmf.2009.0079
12. Koster R, Anderson M, DeBeer EJ . Acetic acid for analgesic screening. Fed Proc 1959; 18:412.
13. Williamson G, Faulkner K, Plumb GW. Glucosinolates and phenolics as antioxidants from plant foods. Eur J Cancer Prev 1998; 7 Suppl 1: 17-21.
14. Derardt R, Jongney S, Delvalcee F, Falhout M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol 1980; 51:17-24. http://dx.doi.org /10.1016/0014-2999(80)90377-5
15. Anderson J, Capper BS, Bromage NR. Measurement and prediction of digestible energy values in feedstuffs for the herbivorous fish tilapia (Oreochromis niloticus Linn.). Br J Nutr 1991; 66 Suppl 1: 37-48. http://dx.doi.org/ 10.1079/BJN19910007
16. Rahman M, Khatun A, Khan S, Hossain F, Khan AA. Phytochemical, cytotoxic and antibacterial activity of two medicinal plants of Bangladesh. Pharmacologyonline 2014; 1: 3-10.
17. Rahman M, Khatun A, Islam M, Akter N, Chowdhury SA, Khan MA. Evaluation of antimicrobial, cytotoxic, thrombolytic, diuretic properties and total phenolic content of Cinnamomum tamala. Int J Green Pharm 2013; 7 Suppl 3: 236-43. http://dx.doi.org/10.4103/0973-8258.120242
18. Priestman T. Cancer chemotherapy in clinical practice. London (UK): Springer-Verlag; 2008.

Cite this article as:

Md. Taleb Hossain, Mohammed Rafiquzzaman, Md. Saiful Islam, Md. Abu Sufian, Sukalyan Kumar Kundu. Analgesic and cytotoxic activities of three reputed Bangladeshi medicinal plants. Int. Res. J. Pharm. 2015;6(9):605-609 http://dx.doi.org/10.7897/2230-8407.069118

Source of support: Nil, Conflict of interest: None Declared