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Research Article

Evaluation of Analgesic and Central Nervous System Depressant Effects of *Microcos paniculata* Leaves Extracts on Swiss Albino Mice

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Abstract: The main objective of the study was to evaluate the possible analgesic and central nervous system (CNS) depressant effects of methanol (MMPL), petroleum ether (PMPL), chloroform (CMPL), dichloromethane (DMPL) and aqueous (AMPL) extracts of the *M. paniculata* leaves. Analgesic effect was evaluated by acetic acid induced writhing and hot plate methods at 50 and 100 mg/kg dose. The CNS-depressant effect was assessed by using open field, hole cross and head deep tests at 100 and 200 mg/kg dose. All the extracts had exhibited significant ($P^b < 0.01$, $P^a < 0.001$) analgesic and CNS depressant effects at dose dependant manner. AMPL showed maximum analgesic effect with 72.92% inhibition of abdominal writhing and 33.03% maximal possible effect (MPE) of paw licking time at 100mg/kg dose. On the other hand, CMPL had shown highest CNS-depressant effect with 93.80% inhibition in open field, 84.63% inhibition in hole cross and 81.54% inhibition (head deeping) in head deep test at 200mg/kg dose. Among the five extracts AMPL is a potent analgesics and CMPL is a potent CNS-depressant agents. These findings may unveil the efficiency of these extracts as analgesics and CNS depressant drug which may lead to develop a new phyto-medicine.
Keywords: *Microcos paniculata*; Traditional medicine; Analgesic effect; CNS-depressant effect; Writhing

INTRODUCTION

Microcos paniculata L. is a herbaceous plant or small tree which belongs to the tiliacece family. It is widely distributed throughout Bangladesh and also grown in India, Sri Lanka, China, Cambodia, Myanmar, Thailand, Vietnam, Indonesia and Malaysia. In Bangladesh, its local name is Kathgua or Fattashi [1]. Traditionally it is used to treat hepatitis, diarrhea, dyspepsia, typhoid fever, small pox, eczema and itches. Important bioactive phytochemicals like alkaloids, flavonoids, resins, saponons, steroids and carbohydrates have been reported in it [2].

Nociception, mediates through neural path, and the mechanical, thermal, or chemical stimuli excite primary afferent nociceptors in the peripheral and central nervous system. If a stimuli is induced then the nociceptor transmits signal to the brain and causes pain [3]. Anxiety is associated with psychological and physiological state marked by cognitive, somatic, emotional and behavioral elements that leads fear, worry as well as restlessness [4]. So, there is a need to develop new analgesic and CNS-depressant drugs as the currently available drugs are associated with severe side effect and many patients are resistance to these drugs. Medicinal plants are cost-effective and harmless source of bioactive having strong therapeutic effect [5]. Therefore, the present study was undertaken to investigate analgesic and CNS depressant activities of the *M. paniculata* leaves extracts that may unveil safe and cost effect analgesic and CNS depressant drugs.

MATERIALS AND METHODS

Plant materials

Microcos paniculata leaves were collected from Comilla Hill tract, Bangladesh in September, 2014 and identified by an expert of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained with accession no DACB-40638. The collected leaves were cleaned, dried for one week, and then pulverized into a coarse powder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis was taken.

Extract preparation

Approximately 500g of each powdered material was soaked in methanol, petroleum ether, chloroform, dichloromethane and water, and kept for 7 days. Then extraction was carried out using Ultrasonic Sound Bath accompanied by sonication. Then the mixture was firstly filtered by a piece of clean cotton material. The filtrate was again filtered through filter paper and was dried to obtain the methanol (12.5g), chloroform (6.5g), dichloromethane (8.35g),

and aqueous (10.25g) extracts. The gummy extracts were transferred to a closed container for further use and storage.

Drugs and chemicals

Methanol, petroleum ether, dichloromethane, chloroform and were purchased from Active Fines, Bangladesh. Acetic acid and Tween-80 were purchased from Merck, Germany. Diazepam and dichlofenac sodium (DS) were collected from Square Pharmaceuticals Ltd. Bangladesh.

Animals

Swiss albino mice of either sex weighing approximately 25-30g were used for this experiment. The mice were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh. The set of rules for animal experiment were followed according to international guidelines [6].

Evaluation of analgesic effect:

The analgesic effect was evaluated by using acetic acid-induced writhing and hot-plate tests that were previously described by Koster et al. [7] as well as Eddy and Leimbach [8], respectively. Briefly, 72 mice (Swiss albino) were divided into 12 groups. Then, mice of specific group were feed with vehicle (2% acacia), diclofenac sodium (10mg/kg), and the extracts (50 and 100mg/kg, b.w.). 30 minutes later, acetic acid induced writhing response was counted for 20minutes and heat induced reaction time in hot plate (licking or jumping of mice) was measured at 0, 30, 60, 90 and 120 minutes of the experiment. A cut off period of 20s was maintained to avoid paw tissue damage. Percent reduction of writhing, an index of analgesia, was calculated as:

$[(N_c - N_t)/ N_c] \times 100$,

where, N_c = writhing number of control group, N_t = writhing number of treated group. In the hot plate test, percentage of the maximal possible effect (%MPE) was calculated as: %MPE = [(Post drug latency−pre drug latency) / (Cut off period−pre drug latency)] × 100.

Evaluation of CNS depressant effect:

This effect was evaluated by applying open field [9], hole cross [10] and head deep [11] tests. Shortly, 72 mice were divided into 12 groups and feed with vehicle (2% acacia), diazepam (2mg/kg), and the extracts (100 and 200mg/kg, b.w.). 30 minutes later, each mice was placed in a open field box, a hole cross box, and a head deep box where number of mice movement (open field), passes through hole (hole cross) and head deeping were counted for 5 minutes at 0, 30, 60, 90, and 120 minutes of the experiment. Reduction of mice locomotion indicates CNS-depressant potential of the extracts. Percentage inhibition of locomotion was calculated at 120 min by the following formula: % Inhibition = $[(N_0-N_S)/N_0] \times 100$, where, N_0 is the average number of movements or passes or head deeping in control group and N_S is the average number of movements or passes or head deeping in treated group (extract or standard drug).

Statistical analysis

All the values were expressed as the mean ± SEM (Standard Error Mean) of triplicate experiment (n = 6 mice per group). The analysis was done in SPSS statistical package, version 15.0. $P^b<0.01$, $P^a<0.001$ were considered to be statistically significant compared to vehicle control group. ANOVA followed by Dunnett ´s test was done by the SPSS software.

RESULTS

Analgesic effect

All the extracts were significantly ($P^b < 0.01$, $P^a < 0.001$) effective to reduce the writing response, and to increase reaction time (licking or jumping of mice) as dose dependant manner. In writing test, DS (10mg/kg) showed 77.41% inhibition. AMPL, among the extracts had shown the highest effect with 64.47% (50mg/kg) as well as 72.92% (100mg/kg) inhibition whereas MMPL had shown the lowest effect with 35.05% (50mg/kg) as well as 51.74% (100mg/kg) inhibition (Table-1). In hot plate test, DS had shown 30.34%, 46.93%, 70.74% and 82.99% MPE at 30, 60, 90, 120 minute. Among the five extracts, AMPL had shown the highest effect with 9.83%, 15.14%, 28.23% and 33.03% MPE at 30, 60, 90, 120 minute (Table-2). The order of writhing inhibition and MPE is DS> AMPL> CMPL > DMPL > PMPL>MMPL (Table-1 and Table-2).

CNS depression effect

All the extracts had shown significant ($P^b<0.01$, $P^a<0.001$) reduction of mice locomotion dose dependently. The standard drug diazepam (2mg/kg) had shown 94.72% (open field test), 86.36% (hole cross test) and 85.02% (head deep test) inhibition of mice locomotion at the fifth observation. CMPL, among the four extracts, had shown maximum effect in all the three experiments, and PMPL had shown the lowest effect. After 120min of treatment, the CMPL had shown 88.49% and 93.80% inhibition of movement in open field, 76.75% and 84.63% inhibition in hole cross as well as 72.29% and 81.54% inhibition in head deep test at 100 and 200 mg/kg dose, respectively. The order of CNS depressant effect of the extracts in all the experiments was CMPL > DMPL > MMPL > AMPL >PMPL (Table-3, Table-4, Table-5)

Table-1: **Analgesic effect of *M. paniculata* leaves extracts in acetic acid-induced writhing test.**

Sample	Dose mg/kg,b.w.	Writhing number	Percent inhibition of writhing
Control(vehicle)	0.1ml/mice	42.50±3.21	00
DS	10mg/kg	9.60±1.12[a]	77.41
MMPL	100	27.60±2.40[a]	35.05
	200	20.51±3.21[a]	51.74
PMPL	100	25.64±2.47[a]	39.76
	200	18.14±3.11[a]	57.32
DMPL	100	23.34±2.50[a]	45.08
	200	16.20±2.20[a]	61.88
CMPL	100	18.60±2.28[a]	56.23
	200	13.52±2.40[a]	68.18
AMPL	100	15.10±3.33[a]	64.47
	200	11.50±2.11[a]	72.92

Each value is presented as mean ± SEM (n = 6); $p^a < 0.001$ compared with the control group. ANOVA followed by Dunnett's test is done in SPSS version 15.

Table-2: Analgesic effect of *M. paniculata* leaves extracts in hot plate test.

Sample	Dose mg/kg, b.w.	Response time (s) (%MPE)				
		0 min	30 min	60 min	90 min	120 min
Vehicle	0.1ml/mice	2.60±0.18	2.62±0.30	2.74 ±0.45	3.25±0.55	3.45±0.25
DS	10	2.65±0.20	4.88±0.35[a] (12.85)	6.10±0.32[a] (19.88)	7.85±0.56[a] (29.97)	8.75 ± 0.65[a] (35.16)
MMPL	100	2.54±0.75	2.89±0.18 (2.00)	3.30 ± 0.10[b] (4.35)	4.55±0.65[a] (11.51)	5.24±0.85[a] (15.46)
	200	2.49±0.18	3.57±0.20[b] (6.17)	4.12±0.48[a] (9.31)	5.19±0.12[a] (15.42)	6.18±0.45[a] (21.07)
PMPL	100	2.58±0.70	2.92±0.15 (1.95)	3.42 ± 0.18[b] (4.82)	4.67±0.68[a] (12.00)	5.52±0.45[a] (16.88)
	200	2.62±0.20	3.64±0.22[b] (5.87)	4.22±0.28[a] (9.21)	5.36±0.37[a] (15.77)	6.63±0.41[a] (23.07)
DMPL	100	2.78±0.26	3.23±0.16 (2.61)	3.82±0.23[a] (6.04)	4.89±0.21[a] (12.25)	6.12±0.38[a] (19.40)
	200	2.56±0.38	3.80±0.36[b] (7.11)	4.45±0.26[a] (10.84)	5.82±0.20[a] (18.69)	6.97±0.57[a] (25.29)
CMPL	100	2.64±0.27	3.87±0.53[b] (7.09)	4.10±0.46[a] (8.41)	5.41±0.75[a] (15.96)	6.67±0.86[a] (23.21)
	200	2.71±0.42	4.27±0.28[a] (9.02)	4.82±0.42[a] (12.20)	6.53± 0.48[a] (22.09)	7.48±1.82[a] (27.59)
AMPL	100	2.62±0.38	3.94±0.20[b] (7.59)	4.67 ±0.26[a] (11.80)	6.23± 0.48[a] (20.77)	7.17±0.25[a] (26.18)
	200	2.50±0.40	4.22±0.12[a] (9.83)	5.15±0.32[a] (15.14)	7.44 ±0.35[a] (28.23)	8.28±0.62[a] (33.03)

Each value is presented as mean ± SEM (n = 6); $p^b < 0.01$, $p^a < 0.001$ compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

Table-3: CNS depressant effect of different extracts of *M. paniculata* at open field test.

Sample	Dose mg/kg, b.w.	Number of movements					% Inhibition of movements after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	215.23±12.85	210.39±10.47	203.72±14.20	212.92±9.13	205.80±11.	--
Diazepam	2	212.32 ±10.62	115.20±7.15[a]	40.58±3.25[a]	16.45±2.10[a]	10.85±1.21[a]	94.72
MMPL	100	222.10±12.78	169.45±9.15[b]	88.36±7.70[a]	64.36±5.14[a]	52.36±3.90[a]	74.55
	200	215.45±10.42	143.80±8.27[a]	61.30±5.41[a]	50.10±3.75[a]	41.23±2.50[a]	79.96
PMPL	100	210.78±9.75	190.23±12.28	112.78±11.15[a]	80.10±7.47[a]	82.37±6.20[a]	59.97
	200	218.20±15.50	176.12±10.20[b]	85.69±9.18[a]	62.80±5.16[a]	73.12±5.20[a]	64.46
CMPL	100	212.55±8.20	124.30±9.28[a]	62.30±6.43[a]	31.80±3.92[a]	23.67±2.28[a]	88.49
	200	217.98±12.23	110.58±7.53[a]	45.05±8.22[a]	20.15±3.22[a]	12.74±1.50[a]	93.80
DMPL	100	225.45±14.69	161.78±8.21[b]	74.69±6.22[a]	42.70±4.30[a]	32.72±2.28[a]	84.10
	200	227.56±9.78	132.27±7.45[a]	53.47±5.12[a]	28.41±2.12[a]	22.50±2.12[a]	89.06
AMPL	100	210.30±11.34	172.56±10.20[b]	92.57±5.15[a]	79.64±5.37[a]	68.28±6.90[a]	66.82
	200	215.77±14.73	160.39±12.35[b]	72.75±6.20[a]	68.20±4.20[a]	57.36±3.85[a]	72.12

Table-4: CNS depressant effect of different extracts of *M. paniculata* at hole cross test.

Sample	Dose mg/kg, b.w.	Number of movements					% Inhibition of movements after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	16.38±3.10	15.70±2.25	17.56±1.69	14.22±2.11	12.69±1.80	- -
Diazepam	2	18.23±2.32	4.89±0.35[a]	2.95±0.25	1.88±0.20[a]	1.73±0.02[a]	86.36
MMPL	100	20.38±3.05	10.90±1.10[a]	8.15±0.22[a]	6.26±0.54[a]	5.36±0.06[a]	57.76
	200	17.95±2.74	7.30±0.15[a]	5.12±0.37[a]	4.87±0.25[a]	4.85±0.09[a]	61.78
PMPL	100	19.36±3.10	12.47±2.11[a]	10.49±0.40[a]	9.10±0.75[a]	8.25±0.08[a]	34.98
	200	17.68±2.63	8.90±1.05[a]	7.18±0.65[a]	7.05±0.43[a]	6.18±0.05[a]	51.30
CMPL	100	18.23±2.20	6.60±0.40[a]	4.50±0.13[a]	3.28±0.25[a]	2.95±0.20[a]	76.75
	200	15.36±2.38	4.90±0.55[a]	3.59±0.65[a]	2.14±0.10[a]	1.95±0.10[a]	84.63
DMPL	100	20.30±1.47	6.83±1.10[a]	4.89±0.27[a]	3.70±0.08[a]	3.53±0.40[a]	72.18
	200	17.45±2.10	5.25±0.25[a]	3.10±0.15[a]	2.95±0.05[a]	2.43±0.10[a]	80.85
AMPL	100	15.36±2.45	10.12±1.36[a]	7.50±0.21[a]	7.10±0.40[a]	6.95±0.27[a]	45.23
	200	16.97±2.05	8.77±1.20[a]	6.25±0.85[a]	5.94 ±0.30[a]	5.20±0.45[a]	59.02

Each value is presented as mean ± SEM (n = 6); $p^a < 0.001$ compared with the control group. ANOVA followed by Dunnett's test is done in SPSS version 15.

Table-5: CNS depressant effect of different extracts of *M. paniculata* at head deep test.

Sample	Dose mg/kg, b.w.	Number of movements					% Inhibition of movements after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	90.45±7.58	91.58±6.12	85.69±7.12	83.10±6.14	80.48±5.74	- -
Diazepam	2	93.56±6.71	29.33±2.45[a]	20.36±3.22[a]	18.54±0.43[a]	12.05±2.25[a]	85.02
MMPL	100	91.36±7.12	58.34±5.50[a]	48.20±3.10[a]	39.20±3.20[a]	34.14±3.82[a]	57.57
	200	90.89±6.50	45.25±4.15[a]	40.17±3.18[a]	35.46±3.18[a]	28.60±2.25[a]	64.46
PMPL	100	89.14±8.53	65.10±6.10[a]	57.20±5.96[a]	52.30±5.25[a]	46.28±4.56[a]	42.49
	200	86.36±6.14	57.28±5.28[a]	51.28±5.73[a]	47.33±4.10[a]	37.15±3.38[a]	53.83
CMPL	100	88.23±7.20	45.15±4.58[a]	32.22±2.17[a]	26.63±2.57[a]	22.30±1.09[a]	72.29
	200	83.64±8.12	31.41±3.18[a]	26.25±2.32[a]	20.58±2.68[a]	14.85±1.10[a]	81.54
DMPL	100	90.30±7.69	50.43±5.40[a]	41.35±3.18[a]	36.45±2.85[a]	29.78±2.65[a]	62.99
	200	85.12±6.20	42.30±3.84[a]	34.60±2.86[a]	26.35±3.12[a]	22.56±2.87[a]	71.96
AMPL	100	86.10±5.80	60.23±6.25[a]	51.36±5.20[a]	46.32±3.24[a]	40.23±4.60[a]	50.01
	200	84.78±8.12	46.80±5.34[a]	45.36±3.24[a]	42.42±4.29[a]	32.12±3.14[a]	60.08

Each value is presented as mean ± SEM (n = 6); $p^a < 0.001$ compared with the control group. ANOVA followed by Dunnett's test is done in SPSS version 15.

DISCUSSION

Pain is associated with many diseases and several natural products are used to relieve pain and inflammation. Abdominal writhing mediates via local peritoneal receptor. Intraperitonial injection of acetic acid induces capillary permeability that promotes generation of pain sensitive prostaglandin specifically PGE2 and PGF2α, and release of free arachidonic acid, an endogenous inflammatory substance, from tissue phospholipids by the action of cyclo-oxygenase (COX) enzyme [12]. Substance(s) inhibiting the writhing response may have analgesic effect preferably by inhibition of prostaglandin synthesis **[**13]**.** Non steroidal anti-inflammatory drugs (NSAIDs) inhibit COX in peripheral tissues, and therefore interfere with the pain sensation. The extracts could block the pain inducing endogenous substances similar to that of NSAIDs [12]. The hot plate method can evaluate centrally acting analgesic properties of a drugs or chemicals. Heat induces pain sensation through opioid receptors. So, the agent that elongates reaction time in the hot plate test, acts through the opioid receptors and called centrally acting analgesics [14]. Thus, the significant ($p^a < 0.001$) reduction of acetic acid-induced writhing (Table 1), and elongation of reaction time in hotplate test (Table-2) indicates the peripherally and centrally acting analgesic effect of the extracts, respectively.

Open field, hole cross and head deep tests (locomotor test) are widely applied to evaluate CNS depressant potential of an agent. These tests evaluates index of alertness and a reduction of it is an indicative of sedative or CNS depressant activity [15]. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS [16]. Various drugs such as anxiolytic, muscle relaxant and sedative-hypnotic acts via the GABA. Sedative agents may give the CNS depressant effect through modification of the GABA system where modification may take place by potentiating postsynaptic inhibition of the GABA receptors and elevating the GABA-induced chloride conductance with simultaneous depression of voltage activated Ca^{++} currents [17]. Therefore, it is predictable that the extracts may act by potentiating GABAergic inhibition in the CNS via membrane hyper-polarization leading to a reduction in the firing rate of critical neurons in the brain [16]. The locomotor testes have shown that all doses of the extracts significantly ($p^b < 0.01$, $p^a < 0.001$) reduced the frequency and the amplitude of movements in dose dependant manner from the second observation (30 min) and continued up to the fifth observation (120 min) period (Table 3, Table 4 and Table 5) which may be due to the presence of compound(s) having CNS depressant potential.

Natural products like alkaloids, flavonoids and tannins are potent analgesic compounds. Flavanoids exert their effect through inhibition of prostaglandin synthatase [18]. Various flavonoid derivatives including quercetin have inhibitory effect of arachidonic acid metabolizing enzymes (phospholipase A_2, cycloxygenase and lipoxygenase). Several phytochemicals like flavonoids, saponins and tannins etc have CNS depressant effect. Many flavonoids and neuroactive steroids are ligands for $GABA_A$ receptors in the central nervous system which suggests that they can act as benzodiazepine-like agents [15].

Literature review of the plant has revealed alkaloids, flavonoids, resins, saponons, steroids, stigmasterol, triterpene, and epicatechin in it. These agents may be responsible for the analgesic and CNS-depressant effects of the plant [2].

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REFERENCES

1. Abdullah, A.M., Mohammad Mahfuz, A.K.S., Shahariar, R., Tariqul, I., Mohoni, M., Abdullah, F. & sohel, R.M. (2013). Secondary metabolites, antimicrobial, brine shrimp lethality & 4th instar *Culex quinquefasciatus* mosquito larvicidal screening of organic & inorganic root extracts of *Microcos paniculata.* IOSR Journal of Pharmacy and Biological Sciences, 8(5), 58-65.
2. Arun, J., Maya, B. & Ashma, S. (2013). Phytochemical investigation of the roots of*Grewiamicrocos* Linn. Journal of Chemical and Pharmaceutical Research, 5(7):80-87.
3. Yogesh, A.K., Sneha, A. & Mayuresh SG. (2015). Effect of Jyotishmati (*Celastrus paniculatus*) seeds in animal models of pain and inflammation. Journal of Ayurveda and Integrative Medicine, 6(2), 82–88.
4. Xueli, Z., Yang, Y., Zhou, C., Shan, L., Yunguo, Z. & Xiaoling Y. (2014). Anti-depressant effect of *Chimonanthus salicifolicus* essential oil in chronic stressed rats. Journal of Medicinal Plant Research, 8(10), 430-435.
5. Hammad, I. & Bushra M. (2015). Evaluation of analgesic, anti-inflammatory, anti-depressant and anti-coagulant properties of *Lactuca sativa* (CV. Grand Rapids) plant tissues and cell suspension in rats. BMC Complementary and Alternative Medicine, 15, 199.
6. Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in concious animals. *Pain*, 16, 109-110.

7. Koster, R., Anderson, M. & Debeer, E.J. (1959). Acetic acid for analgesic screening. Federation Proceedings, 18, 412-416.
8. Eddy, N.B. & Leimbach, D. (1953). Synthetic analgesics: II. Dithienylbutenyl and Dithienylbutylamines. Journal of Pharmacology and experimental Therapeutics, 107, 385–393.
9. Gupta, B.D., Dandiya, P.C. & Gupta, M.L. (1971). A psycho-pharmacological analysis of behaviour in rats. The Japanese Journal of Pharmacology, 21, 293–298.
10. Takagi, K., Watanabe, M. & Saito, H. (1971). Studies of the spontaneous movement of animals by the hole cross test; effect of 2-dimethyl-aminoethanol and its acyl esters on the central nervous system. The Japanese Journal of Pharmacology, 21, 797–810.
11. Dorr, M., Stienberg, H., Tomkiewiez, M., Joyee, D., Porosolt, R.D. & Summerfield, A. (1971). Persistence of dose related behavior in mice. Nature, 231, 121–123.
12. Chandana, C.B., Jayanti, D.R., Bhaben, B., Acheenta, G.B., Prabodh, B. & Mangala, L. (2011). Analgesic and anti-nociceptive activity of hydroethanolic extract of *Drymaria cordata* willd. Indian Journal of Pharmacology, 43(2), 121-125.
13. Luiz, H.A. C., Brito, M.C.B, Araújo, M.V., Barbosa-Filho, J.M., Lira, D.P. & Oliveira, S.B. V. (2012). Antinociceptive and anti-inflammatory activities of crude methanolic extract of red alga *Bryothamnion triquetrum.* Marine Drugs, 10, 1977-1992.
14. Moniruzzaman, M. & Mohammad, Z.I. (2014). Evaluation of antinociceptive effect of methanolic extract of leaves of *Crataeva nurvala* Buch.-Ham. BMC Complementary and Alternative Medicine, 14, 354.
15. Protapaditya, D., Sangita, C., Priyanka, C. & Sanjib, B. (2011). Neuropharmacological properties of *Mikania scandens* (L.) Willd. (Asteraceae). Journal of Advanced Pharmaceutical Technology and Research, 2(4), 255–259.
16. Kavita, G., Vijay, K.L. & Shivesh, J. (2013). Anticonvulsant potential of ethanol extracts and their solvent partitioned fractions from *Flemingia strobilifera* root. Pharmacognosy Research, 5(4), 265–270.
17. Uma, A.B., Radha, Y., Prachi, D.P., Mandar, R.Z. & Raul, S.S. (2011). Study of central nervous system depressant and behavioral activity of an ethanol extract of *Achyranthes aspera* (Agadha) in different animal models. International Journal of Applied and Basic Medical Research, 1(2), 104–108.
18. Mojahid-Ul, I. & Sanadel, A.E. Phytrochemical investigation and evaluation of analgesic activity of ethanolic extract of delbergia sisso (Roxb) bark. Journal of natural product and plant resources, 2012; 2(6) : 701-704