Advances in Natural and Applied Sciences, 8(2) February 2014, Pages: 96-102

AENSI Journals

Advances in Natural and Applied Sciences

ISSN:1995-0772 EISSN: 1998-1090

Journal home page: www.aensiweb.com/anas/index.html

Antinociceptive Activity Evaluation of Leaves of *Centella asiatica* and *Zizyphus mauritiana*

Sanjoy Sarker, Sk. Mizanur Rahman, Md. Zahirul Kabir, Md. Rashedul Islam, Md. Shamim Al Azad, Shahnaz Rahman, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh.

ARTICLE INFO

Article history:
Received 19 January 2014
Received in revised form 16 March 2014
Accepted 23 March 2013
Available online 10 April 2014

Key words:
Centella asiatica, Zizyphus mauritiana, antinociceptive

ABSTRACT

The antinociceptive effects of methanolic extracts of *Zizyphus mauritiana* and *Centella asiatica* leaves were studied through the intraperitoneally administered acetic acid-induced pain model in Swiss albino mice. At doses of 50, 100, 200, and 400 mg methanolic extract of *Zizyphus mauritiana* leaves (MEZM) per kg body weight, MEZM caused dose-dependent and significant reductions in the number of abdominal constrictions induced in mice by intraperitoneal administration of acetic acid. At the afore-mentioned four doses, the number of abdominal constrictions (writhings) was reduced, respectively, by 31.2, 46.9, 56.2, and 62.5%. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 34.4 and 46.9%, respectively. Thus MEZM produced antinociceptive effects and caused alleviation of pain, which was greater than that of the pain-alleviating drug, aspirin, even when the latter was used at a high dose of 400 mg per kg body weight. Methanolic extract of *Centella asiatica* leaves (MECA), at doses of 50, 100, 200, and 400 mg per kg body weight, caused reductions in the number of writhings, respectively, by 46.9, 50.0, 53.1, and 71.9%. Dose for dose, MECA was more effective than MEZM in alleviating pain, and both extracts demonstrated stronger potency than aspirin. Since pain is a universal problem afflicting human beings of all age groups throughout the world, the extracts even in the crude form can serve as a readily available and cheaper alternative in alleviating pain. The extracts deserve further studies for isolation and identification of the responsible bioactive components responsible for the observed antinociceptive effects.

© 2014 AENSI Publisher All rights reserved.

To Cite This Article: Sanjoy Sarker, Sk. Mizanur Rahman, Md. Zahirul Kabir, Md. Rashedul Islam, Md. Shamim Al Azad, Shahnaz Rahman, Mohammed Rahmatullah., Antinociceptive Activity Evaluation of Leaves of *Centella asiatica* and *Zizyphus mauritiana*. **Adv. in Nat. Appl. Sci.,** *8(2): 96-102, 2014*

INTRODUCTION

Ziziphus mauritiana Lam., also known as Ber, Chinese Apple, Jujube, or Indian plum (local name: Boroi) is a tropical fruit tree species belonging to the family Rhamnaceae. The tree is widely distributed in Bangladesh, and the seeds obtained from fruits are used by folk medicinal practitioners for treatment of diabetes. The plant or plant parts reportedly has a number of pharmacological activities. Leaf extract has been shown to demonstrate hepatoprotective effects against chronic ethanol-induced hepatotoxicity in rat liver [9]. Aqueous ethanol extract of seed reportedly showed hypoglycemic activity in alloxan-induced diabetic mice [8]. Anticancer potential of aqueous ethanol seed extract has been shown against cancer cell lines and Ehrlich Ascites Carcinoma [23]. Antiplasmodial and antimycobacterial cyclopeptide alkaloids have been reported from roots of the plant [24]. Hypnotic effect of seed extract in mice has also been described [49].

Centella asiatica (L.) Urb. (local name: Thankuni) is a small, herbaceous, annual plant of the family Mackinlayaceae or subfamily Mackinlayoideae of family Apiaceae, and is native to Bangladesh, India, Sri Lanka, northern Australia, Indonesia, Iran, Malaysia, Melanesia, Philippines, Papua New Guinea, and other parts of Asia. *Centella asiatica* is an important medicinal herb that is widely used in south Asia. Triterpenoids and saponins, the primary constituents of *Centella asiatica* are mainly believed to be responsible for its wide therapeutic actions. Apart from wound healing, the herb is recommended for the treatment of various skin conditions such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhea, fever, amenorrhea, and diseases of the female genitourinary tract, and also for relieving anxiety and improving cognition [14].

Corresponding Author: Professor Dr. Mohammed Rahmatullah, Pro-Vice Chancellor and Dean, Faculty of Life Sciences, University of Development Alternative, House No. 78, Road No. 11A (new), Dhanmondi, Dhaka-1205, Bangladesh
Tele: +88-01715032621 Fax: +88-02-815739 E-mail: rahamatm@hotmail.com

Protective action of the plant against adriamycin-induced cardiomyopathy in rats has been reported [13]. The beneficial effect of aqueous extract of the plant against arsenic-induced oxidative stress has been reported [12]. Treatment with fresh leaf extract reportedly enhanced learning ability and memory retention power in rats [46]. Neuroprotective property of the plant has been shown against 3-nitropropionic acid-induced oxidative stress and mitochondrial dysfunctions in brain regions of prepubertal mice [52]. Neuroprotective effect of the plant has also been seen against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress [22], and experimentally induced Parkinsonism in aged Sprague-Dawley rats [15]. Extract of the plant has been shown to selectively decrease amyloid beta levels in hippocampus of Alzheimer's disease animal model [11]. The anticonvulsant effect of the plant has been shown in pentylenetetrazol-induced seizures with respect to cholinergic neurotransmission [57].

Antigenotoxic effect of the plant extract has been seen against cyproterone acetate-induced genotoxic damage in cultured human lymphocytes [54]. The plant extract has been observed to induce apoptosis in human breast cancer cells [6].

The efficacy of the plant extract has been shown for wound healing in diabetic wound patients [25]. The plant has been seen to exert a protective role on lead-induced oxidative stress and suppressed reproductive health in male rats [48]. The plant has been shown to protect against UVB-induced HaCaT keratinocyte damage [3].

Studies conducted by our research group have centered on ethnomedicinal surveys [28-37,39-44], followed by screening of the plants obtained for antihyperglycemic, antinociceptive and cytotoxic activities [4,19,26,38,53,2,7,17,20,27,56,1,5,16,50]. As part of the screening process to locate plants with antinociceptive properties, this study was conducted to evaluate the antinociceptive potential of methanol extract of leaves of *Centella asiatica*, and *Zizyphus mauritiana* in intraperitoneally administered gastric pain model mice.

MATERIALS AND METHODS

Leaves of *Ziziphus mauritiana* were collected from Mirpur in Dhaka district, Bangladesh during June 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession Number 38,369). The sliced and air-dried leaves of *Ziziphus mauritiana* were grounded into a fine powder and 100g of the powder was extracted with methanol (1:8.5, w/v) for 48 hours. The extract was evaporated to dryness at 40°C. The final weight of the extract was 4g.

Leaves of *Centella asiatica* were collected from Dhaka district during May 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession Number 38,359). The sliced and air-dried leaves of *Centella asiatica* were grounded into a fine powder and 57g of the powder was extracted with 300 ml methanol for 48 hours. The extract was evaporated to dryness at 40°C. The final weight of the extract was 4.35g.

Chemicals:

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh.

Animals:

In the present study, Swiss albino mice (male), which weighed between 12-15g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Antinociceptive activity:

Antinociceptive activity of the methanol extracts of *Ziziphus mauritiana* leaves (MEZM) and methanol extracts of *Centella asiatica* leaves (MECA) was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of six mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic whole plant extract of MEZM or MECA at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of abdominal constrictions (writhings) was counted for 10 min.

Statistical analysis:

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

RESULTS AND DISCUSSION

The antinociceptive effects of methanolic extracts of *Zizyphus mauritiana* and *Centella asiatica* leaves were studied through the intraperitoneally administered acetic acid-induced pain model in Swiss albino mice. At doses of 50, 100, 200, and 400 mg methanolic extract of *Zizyphus mauritiana* leaves (MEZM) per kg body weight, MEZM caused dose-dependent and significant reductions in the number of abdominal constrictions induced in mice by intraperitoneal administration of acetic acid. At the afore-mentioned four doses, the number of abdominal constrictions (writhings) was reduced, respectively, by 31.2, 46.9, 56.2, and 62.5%. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 34.4 and 46.9%, respectively. Thus MEZM produced antinociceptive effects and caused alleviation of pain at the two higher doses of 200 and 400 mg per kg body weight, which was greater than that of the pain-alleviating drug, aspirin, even when the latter was used at a high dose of 400 mg per kg body weight. The results suggest that MEZM contains phytochemical component(s) with a high degree of pain relieving activity.

Table 1: Antinociceptive effect of crude methanol extract of *Ziziphus mauritiana* leaves in the acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	6.40 ± 0.40	-
Aspirin (Group 2)	200 mg	4.20 ± 0.58	34.4*
Aspirin (Group 3)	400 mg	3.40 ± 0.24	46.9*
MEZM (Group 4)	50 mg	4.40 ± 0.51	31.2*
MEZM (Group 5)	100 mg	3.40 ± 0.87	46.9*
MEZM (Group 6)	200 mg	2.80 ± 0.58	56.2*
MEZM (Group 7)	400 mg	2.40 ± 0.51	62.5*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$; significant compared to control.

Table 2: Antinociceptive effect of crude methanol extract of *Centella asiatica* leaves in the acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	6.40 ± 0.40	-
Aspirin (Group 2)	200 mg	4.20 ± 0.58	34.4*
Aspirin (Group 3)	400 mg	3.40 ± 0.24	46.9*
MECA (Group 4)	50 mg	3.40 ± 0.81	46.9*
MECA (Group 5)	100 mg	3.20 ± 0.37	50.0*
MECA (Group 6)	200 mg	3.00 ± 0.32	53.1*
MECA (Group 7)	400 mg	1.80 ± 0.58	71.9*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$; significant compared to control.

Methanolic extract of *Centella asiatica* leaves (MECA), at doses of 50, 100, 200, and 400 mg per kg body weight, caused reductions in the number of writhings, respectively, by 46.9, 50.0, 53.1, and 71.9%. Dose for dose, MECA was more effective than MEZM in alleviating pain, and MECA demonstrated at the three higher doses of 100, 200 and 400 mg per kg body weight, stronger potency than aspirin. It is to be noted that asiatic acid is present in *Centella asiatica* leaves, and the compound reportedly showed antinociceptive and anti-inflammatory activities in mice [18]. Asiaticoside, another component present in *Centella asiatica*, showed antipyretic and anti-inflammatory effects in lipopolysaccharide-trested rat [58]. Thus these two compounds, asiatic acid and asiaticoside, can account for the observed antinociceptive effects of *Centella asiatica* leaves as observed in the present study.

Sensation of pain has been attributed to any increase in the expression of prostaglandins [mainly prostacyclines (PGI2) and prostaglandin- (PG-E)], and so causing sensation of pain [47,45]. Intraperitoneal administration of acetic acid releases prostaglandins like PGE2 and PGF2alpha and their levels are increased [10]. As such, the antinociceptive activity exhibited by crude methanolic extract of the leaves of *Ziziphus mauritiana* and *Centella asiatica* may be due to the extract's ability to block any further expression of prostaglandins, which may be mediated through inhibition of cyclooxygenase and/or lipooxygenase activities. It is noteworthy that a similar mechanism has been proposed for antinociceptive activity of *Ficus deltoidea* Jack (Moraceae) aqueous extract in acetic acid-induced gastric pain model [55], and this can also be the mechanism operating in the present study.

REFERENCES

1. Ahmed, T., K.M.S.U. Imam, S. Rahman, S.M. Mou, M.S. Choudhury, M.J. Mahal, S. Jahan, M.S. Hossain and M. Rahmatullah, 2012. Antihyperglycemic and antinociceptive activity of Fabaceae family plants - an evaluation of *Mimosa pigra* L. stems. Advances in Natural and Applied Sciences, 6: 1490-1495.
2. Ali, M., K. Nahar, M. Sintaha, H.N. Khaleque, F.I. Jahan, K.R. Biswas, A. Swarna, M.N. Monalisa, R. Jahan and M. Rahmatullah, 2011. An evaluation of antihyperglycemic and antinociceptive effects of methanol extract of *Heritiera fomes* Buch.-Ham. (Sterculiaceae) barks in Swiss albino mice. Advances in Natural and Applied Sciences, 5: 116-121.
3. An, I.S., S. An, T.B. Choe, S.M. Kang, J.H. Lee, I.C. Park, Y.W. Jin, S.J. Lee and S. Bae, 2012. Centella asiatica protects against UVB-induced HaCaT keratinocyte damage through microRNA expression changes. International Journal of Molecular Medicine, 30: 1349-1356.
4. Anwar, M.M., M.A. Kalpana, B. Bhadra, S. Rahman, S. Sarker, M.H. Chowdhury and M. Rahmatullah, 2010. Antihyperglycemic activity and brine shrimp lethality studies on methanol extract of *Cajanus cajan* (L.) Millsp. leaves and roots. Advances in Natural and Applied Sciences, 4: 311-316.
5. Arefin, S.A., S. Rahman, S. Rahman, M. Akter, M. Munmun, M.A. Kalpana, S. Jahan, M.S.A. Bhuiyan and M. Rahmatullah, 2012. Scientific validation of folk medicinal uses in Bangladesh of *Piper betle* leaves to alleviate pain and lower blood sugar. Advances in Natural and Applied Sciences, 6: 1496-1502.
6. Babykutty, S., J. Padikkala, P.P. Sathiadevan, V. Vijayakurup, T.K. Azis, P. Srinivas and S. Gopala, 2008. Apoptosis induction of *Centella asiatica* on human breast cancer cells. African Journal of Traditional, Complementary and Alternative Medicines, 6: 9-16.
7. Barman, M.R., Saleh Uddin, Md., S. Akhter, Md.N. Ahmed, Z. Haque, S. Rahman, F. Mostafa, M. Zaman, F.A. Noor and M. Rahmatullah, 2011. Antinociceptive activity of methanol extract of *Areca catechu* L. (Arecaceae) stems and leaves in mice. Advances in Natural and Applied Sciences, 5: 223-226.
8. Bhatia, A. and T. Mishra, 2010. Hypoglycemic activity of *Ziziphus mauritiana* aqueous ethanol seed extract in alloxan-induced diabetic mice. Pharmaceutical Biology, 48: 604-610.
9. Dahiru, D. and O. Obidoa, 2007. Evaluation of the antioxidant effects of *Ziziphus mauritiana* Lam. leaf extracts against chronic ethanol-induced hepatotoxicity in rat liver. African Journal of Traditional, Complementary and Alternative Medicines, 5: 39-45.
10. Deraedt, R., S. Joughney, F. Delevakee and M. Falhour, 1980. Release of prostaglandin E and F in an alogogenic reaction and its inhibition. European Journal of Pharmacology, 51: 17-24.
11. Dhanasekaran, M., L.A. Holcomb, A.R. Hitt, B. Tharakan, J.W. Porter, K.A. Young and B.V. Manyam, 2009. *Centella asiatica* extract selectively decreases amyloid beta levels in hippocampus of Alzheimer's disease animal model. Phytotherapy Research, 23: 14-19.
12. Flora, S.J. and R. Gupta, 2007. Beneficial effects of *Centella asiatica* aqueous extract against arsenic-induced oxidative stress and essential metal status in rats. Phytotherapy Research, 21: 980-988.
13. Gnanapragasam, A., K.K. Ebenazar, V. Sathish, P. Govindaraju and T. Devaki, 2004. Protective effect of *Centella asiatica* on antioxidant tissue defense system against adriamycin induced cardiomyopathy in rats. Life Sciences, 76: 585-597.
14. Gohil, K.J., J.A. Patel and A.K. Gajjar, 2010. Pharmacological review on *Centella asiatica*: A potential herbal cure-all. Indian Journal of Pharmaceutical Sciences, 72: 546-556.
15. Haleagrahara, N. and K. Ponnusamy, 2010. Neuroprotective effect of *Centella asiatica* extract (CAE) on experimentally induced parkinsonism in aged Sprague-Dawley rats. The Journal of Toxicological Sciences, 35: 41-47.
16. Haque, M.M., M.S. Choudhury, M.S. Hossain, M.A. Haque, K. Debnath, S. Hossain, S.M. Mou, I. Malek, and M. Rahmatullah, 2012. Evaluation of antihyperglycemic and antinociceptive properties of leaves of *Calotropis gigantea* R.Br. (Asclepiadaceae) – a medicinal plant of Bangladesh. Advances in Natural and Applied Sciences, 6: 1508-1514.
17. Hossan, A.N.M.F., F. Zaman, M.R. Barman, S. Khatoon, M. Zaman, F. Khatun, T. Mosaiab, F. Mostafa, M. Sintaha, F. Jamal and M. Rahmatullah, 2011. Antinociceptive activity of *Xanthium indicum* J. Koenig ex Roxb. (Asteraceae) leaves and *Leucas aspera* (Willd.) Link (Lamiaceae) whole plants. Advances in Natural and Applied Sciences, 5: 214-217.
18. Huang, S.S., C.S. Chiu, H.J. Chen, W.C. Hou, M.J. Sheu, Y.C. Lin, P.H. Shie and G.J. Huang, 2011. Antinociceptive activities and the mechanisms of anti-inflammation of asiatic acid in mice. Evidence Based Complementary and Alternative Medicine, 2011: 895857, doi: 10.1155/2011/895857.
19. Jahan, F.I., M.S. Hossain, A.A. Mamun, M.T. Hossain, S. Seraj, A.R. Chowdhury, Z. Khatun, N.Z. Andhi, M.H. Chowdhury and M. Rahmatullah, 2010. An evaluation of antinociceptive effect of methanol extracts of *Desmodium gangeticum* (L.) DC. stems and *Benincasa hispida* (Thunb.) Cogn. leaves on acetic acid-induced gastric pain in mice. Advances in Natural and Applied Sciences, 4: 365-369.

20. Jahan, T., S. Shahreen, J. Banik, F. Islam, A.A. Mamun, R. Das, S. Rahman, S. Seraj, R. Jahan and M. Rahmatullah, 2011. Antinociceptive activity studies with methanol extracts of *Ficus hispida* L.f. leaves and fruits in Swiss albino mice. Advances in Natural and Applied Sciences, 5: 131-135.
21. Jahan, T., S. Shahreen, J. Banik, F. Islam, A.A. Mamun, R. Das, S. Rahman, S. Seraj, R. Jahan and M. Rahmatullah, 2011. Antinociceptive activity studies with methanol extracts of *Ficus hispida* L.f. leaves and fruits in Swiss albino mice. Advances in Natural and Applied Sciences, 5: 131-135.
22. Kumar, A., S. Dogra and A. Prakash, 2009. Neuroprotective effects of *Centella asiatica* against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress. International Journal of Alzheimers Disease, doi: pii: 972178.10.4061/2009/972178.
23. Mishra, T., M. Khullar and A. Bhatia, 2011. Anticancer potential of aqueous ethanol seed extract of *Ziziphus mauritiana* against cancer cell lines and Ehrlich Ascites Carcinoma. Evidence Based Complementary and Alternative Medicine, 2011: 2011, doi: pii: 765029.10.1155/2011/765029.
24. Panseeta, P., K. Lomchoey, S. Prabpai, P. Kongsaeree, A. Suksamrarn, S. Richirawat and S. Suksamrarn, 2011. Antiplasmodial and antimycobacterial cyclopeptide alkaloids from the root of *Ziziphus mauritiana*. Phytochemistry, PMID: 21450320.
25. Paocharoen, V., 2010. The efficacy and side effects of oral *Centella asiatica* extract for wound healing promotion in diabetic wound patients. Journal of the Medical Association of Thailand, 93(7): S166-S170.
26. Rahman, M., A. Siddika, B. Bhadra, S. Rahman, B. Agarwala, M.H. Chowdhury and M. Rahmatullah, 2010. Antihyperglycemic activity studies on methanol extract of *Petrea volubilis* L. (Verbenaceae) leaves and *Excoecaria agallocha* L. (Euphorbiaceae) stems. Advances in Natural and Applied Sciences, 4: 361-364.
27. Rahman, M.M., M.N. Hasan, A.K. Das, M.T. Hossain, R. Jahan, M.A. Khatun and M. Rahmatullah, 2011. Effect of *Delonix regia* leaf extract on glucose tolerance in glucose-induced hyperglycemic mice. African Journal of Traditional, Complementary and Alternative Medicines, 8: 34-36.
28. Rahmatullah, M., D. Ferdausi, M.A.H. Mollik, M.N.K. Azam, M.T. Rahman and R. Jahan, 2009a. Ethnomedicinal Survey of Bheramara Area in Kushtia District, Bangladesh. American Eurasian Journal of Sustainable Agriculture, 3: 534-541.
29. Rahmatullah, M., A. Noman, M.S. Hossan, M.H. Rashid, T. Rahman, M.H. Chowdhury and R. Jahan, 2009b. A survey of medicinal plants in two areas of Dinajpur district, Bangladesh including plants which can be used as functional foods. American Eurasian Journal of Sustainable Agriculture, 3: 862-876.
30. Rahmatullah, M., A.K. Das, M.A.H. Mollik, R. Jahan, M. Khan, T. Rahman and M.H. Chowdhury, 2009c. An Ethnomedicinal Survey of Dhamrai Sub-district in Dhaka District, Bangladesh. American Eurasian Journal of Sustainable Agriculture, 3: 881-888.
31. Rahmatullah, M., D. Ferdausi, M.A.H. Mollik, R. Jahan, M.H. Chowdhury and W.M. Haque, 2010a. A Survey of Medicinal Plants used by Kavirajes of Chalna area, Khulna District, Bangladesh. African Journal of Traditional, Complementary and Alternative Medicines, 7: 91-97.
32. Rahmatullah, M., M.A. Khatun, N. Morshed, P.K. Neogi, S.U.A. Khan, M.S. Hossan, M.J. Mahal and R. Jahan, 2010b. A randomized survey of medicinal plants used by folk medicinal healers of Sylhet Division, Bangladesh. Advances in Natural and Applied Sciences, 4: 52-62.
33. Rahmatullah, M., A.A.B.T. Kabir, M.M. Rahman, M.S. Hossan, Z. Khatun, M.A. Khatun and R. Jahan, 2010c. Ethnomedicinal practices among a minority group of Christians residing in Mirzapur village of Dinajpur District, Bangladesh. Advances in Natural and Applied Sciences, 4: 45-51.
34. Rahmatullah, M., M.A. Momen, M.M. Rahman, D. Nasrin, M.S. Hossain, Z. Khatun, F.I. Jahan, M.A. Khatun and R. Jahan, 2010d. A randomized survey of medicinal plants used by folk medicinal practitioners in Daudkandi sub-district of Comilla district, Bangladesh. Advances in Natural and Applied Sciences, 4: 99-104.
35. Rahmatullah, M., M.A.H. Mollik, M.N. Ahmed, M.Z.A. Bhuiyan, M.M. Hossain, M.N.K. Azam, S. Seraj, M.H. Chowdhury, F. Jamal, S. Ahsan and R. Jahan, 2010e. A survey of medicinal plants used by folk medicinal practitioners in two villages of Tangail district, Bangladesh. American Eurasian Journal of Sustainable Agriculture, 4: 357-362.
36. Rahmatullah, M., M.A.H. Mollik, M.K. Islam, M.R. Islam, F.I. Jahan, Z. Khatun, S. Seraj, M.H. Chowdhury, F. Islam, Z.U.M. Miajee and R. Jahan, 2010f. A survey of medicinal and functional food plants used by the folk medicinal practitioners of three villages in Sreepur Upazilla, Magura district, Bangladesh. American Eurasian Journal of Sustainable Agriculture, 4: 363-373.
37. Rahmatullah, M., R. Jahan, M.A. Khatun, F.I. Jahan, A.K. Azad, A.B.M. Bashar, Z.U.M. Miajee, S. Ahsan, N. Nahar, I. Ahmad and M.H. Chowdhury, 2010g. A pharmacological evaluation of medicinal plants used by folk medicinal practitioners of Station Purbo Para Village of Jamalpur Sadar Upazila in Jamalpur district, Bangladesh. American Eurasian Journal of Sustainable Agriculture, 4: 170-195.

38. Rahmatullah, M., Sk.Md.I. Sadeak, S.C. Bachar, Md.T. Hossain, Abdullah-al-Mamun, Montaha, N. Jahan, M.H. Chowdhury, R. Jahan, D. Nasrin, M. Rahman and S. Rahman, 2010h. Brine shrimp toxicity study of different Bangladeshi medicinal plants. Advances in Natural and Applied Sciences, 4: 163-173.
39. Rahmatullah, M., T. Ishika, M. Rahman, A. Swarna, T. Khan, M.N. Monalisa, S. Seraj, S.M. Mou, M.J. Mahal and K.R. Biswas, 2011a. Plants prescribed for both preventive and therapeutic purposes by the traditional healers of the Bede community residing by the Turag River, Dhaka district. American Eurasian Journal of Sustainable Agriculture, 5: 325-331.
40. Rahmatullah, M., M.N.K. Azam, M.M. Rahman, S. Seraj, M.J. Mahal, S.M. Mou, D. Nasrin, Z. Khatun, F. Islam and M.H. Chowdhury, 2011b. A survey of medicinal plants used by Garo and non-Garo traditional medicinal practitioners in two villages of Tangail district, Bangladesh. American Eurasian Journal of Sustainable Agriculture, 5: 350-357.
41. Rahmatullah, M. and K.R. Biswas, 2012a. Traditional medicinal practices of a Sardar healer of the Sardar (Dhangor) community of Bangladesh. Journal of Alternative and Complementary Medicine, 18: 10-19.
42. Rahmatullah, M., A. Hasan, W. Parvin, M. Moniruzzaman, A. Khatun, Z. Khatun, F.I. Jahan and R. Jahan, 2012b. Medicinal plants and formulations used by the Soren clan of the Santal tribe in Rajshahi district, Bangladesh for treatment of various ailments. African Journal of Traditional, Complementary and Alternative Medicines, 9: 342-349.
43. Rahmatullah, M., Z. Khatun, A. Hasan, W. Parvin, M. Moniruzzaman, A. Khatun, M.J. Mahal, M.S.A. Bhuiyan, S.M. Mou and R. Jahan, 2012c. Survey and scientific evaluation of medicinal plants used by the Pahan and Teli tribal communities of Natore district, Bangladesh. African Journal of Traditional, Complementary and Alternative Medicines, 9: 366-373.
44. Rahmatullah, M., M.N.K. Azam, Z. Khatun, S. Seraj, F. Islam, M.A. Rahman, S. Jahan, M.S. Aziz and R. Jahan, 2012d. Medicinal plants used for treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. African Journal of Traditional, Complementary and Alternative Medicines, 9: 380-385.
45. Rang, H.P., M.M. Dale, J.M. Ritter and P.K. Moore, 2003. Pharmacology, Churchill Livingstone, 5th Edition, pp: 562-563.
46. Rao, M.K., M.S. Rao and G.S. Rao, 2007. Treatment with *Centella asiatica* (Linn) fresh leaf extract enhances learning ability and memory retention power in rats. Neurosciences (Riyadh), 12: 236-241.
47. Reynolds, J.E.F., 1982. "Martindale: The Extra Pharmacopoeia", The Pharmaceutical Press, 28th edition; p 245.
48. Sainath, S.B., R. Meena, Supriya, Ch., K.P. Reddy and P.S. Reddy, 2011. Protective role of *Centella asiatica* on lead-induced oxidative stress and suppressed reproductive health in male rats. Environmental Toxicology and Pharmacology, 32: 146-154.
49. San, A.M., S. Thongpraditchote, P. Sithisarn and W. Gritsanapan, 2013. Total phenolics and total flavonoids contents and hypnotic effect in mice of *Ziziphus mauritiana* Lam. seed extract. Evidence Based Complementary and Alternative Medicine, 2013: 2013: 835854, doi: 10.1155/2013/835854.
50. Sathi, S.I., S. Rahman, M.A. Shoyeb, K. Debnath, M.A. Haque, Z. Khatun, M.S. Hossain, M.M.R. Shelley and M. Rahmatullah, 2012. A preliminary study of the antihyperglycemic and antinociceptive potential of *Tagetes patula* L. (Asteraceae) stems. Advances in Natural and Applied Sciences, 6: 1515-1520.
51. Shanmugasundaram, P. and S. Venkataraman, 2005. Anti-nociceptive activity of *Hygrophilous auriculata* (Schum) Heine. African Journal of Traditional, Complementary and Alternative Medicines, 2: 62- 69.
52. Shinomol, G.K. and Muralidhara, 2008. Prophylactic neuroprotective property of *Centella asiatica* against 3-nitropropionic acid induced oxidative stress and mitochondrial dysfunctions in brain regions of prepubertal mice. Neurotoxicology, 29: 948-957.
53. Shoha, J., H. Jahan, A.A. Mamun, M.T. Hossain, S. Ahmed, M.M. Hossain, S. Rahman, R. Jahan and M. Rahmatullah, 2010. Antihyperglycemic and antinociceptive effects of *Curcuma zedoaria* (Christm.) Roscoe leaf extract in Swiss albino mice. Advances in Natural and Applied Sciences, 5: 6-8.
54. Siddique, Y.H., G. Ara, T. Beg, M. Faisal, M. Ahmad and M. Afzal, 2008. Antigenotoxic role of *Centella asiatica* L. extract against cyproterone acetate induced genotoxic damage in cultured human lymphocytes. Toxicology In Vitro, 22: 10-17.
55. Sulaiman, M.R., M.K. Hussain, Z.A. Zakaria, M.N. Somchit, S. Moin, A.S. Mohamad and D.A. Israf, 2008. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia, 79: 557-561.
56. Sutradhar, B.K., M.J. Islam, M.A. Shoyeb, H.N. Khaleque, M. Sintaha, F.A. Noor, W. Newaz and M. Rahmatullah, 2011. An evaluation of antihyperglycemic and antinociceptive effects of crude methanol extract of *Coccinia grandis* (L.) J. Voigt. (Cucurbitaceae) leaves in Swiss albino mice. Advances in Natural and Applied Sciences, 5: 1-5.
57. Visweswari, G., K.S. Prasad, P.S. Chetan, V. Lokanatha and W. Rajendra, 2010. Evaluation of the anticonvulsant effect of *Centella asiatica* (gotu kola) in pentylenetetrazol-induced seizures with respect to cholinergic neurotransmission. Epilepsy & Behavior, 17: 332-335.

58. Wan, J., X. Gong, R. Jiang, Z. Zhang and L. Zhang, 2013. Antipyretic and anti-inflammatory effects of asiaticoside in lipopolysaccharide-treated rat through up-regulation of heme oxygenase-1. Phytotherapy Research, 27: 1136-1142.