Antioxidative, Thrombolytic and Cytotoxic Potentials of *Jatropha pandurifolia* Stem bark and *Syzygium reticulatum* Leaf

Nisrat Jahan[1], Md. Abdur Rahim[1], Sheikh Nazrul Islam[2], A.T.M. Zafrul Azam[1] and Monira Ahsan[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh
[2] Institute of Nutrition and Food Science, University of Dhaka, Dhaka 1000, Bangladesh

(Received: January 18, 2023; Accepted: June 13, 2023; Published (web): June 21, 2023)

ABSTRACT: The study attempted to illustrate the antioxidant, thrombolytic and cytotoxic properties of two different medicinal plants, *Jatropha pandurifolia* and *Syzygium reticulatum. In vitro* antioxidative activity was tested through qualitative and quantitative DPPH scavenging assay which revealed the radical scavenging activity. Methanolic stem bark extract of *Jatropha pandurifolia* (MSJP) and ethyl acetate leaf extract of *Syzygium reticulatum* (EALSR) showed moderate clot rupture activity, the value of which were 61.30 ± 2.35% (**P<0.01) and 63.81 ± 1.92% (**P<0.01) accordingly, whereas streptokinase showed 73.6 ± 0.76% clot lysis capability. IC_{50} value was 6.82 ± 0.99 µg/mL for butylated hydroxy toluene (BHT) wherein MSJP and EALSR showed 8.1± 1.44 (***P<0.001); and 10.34±2.12 (**P<0.01) µg/mL accordingly. In Brine shrimp cytotoxicity, MSJP, EALSR and vincristine sulphate exhibited mild activity with LC_{50}, the value of which were 5.73±2.85 (*P<0.05), 5.12±1.57 (*P<0.05) and 0.52 ± 0.18 µg/mL respectively. The results proved the presence of many bioactive compounds showing thrombolytic, antioxidative and cytotoxic activities.

Key words: *Jatropha pandurifolia*, *Syzygium reticulatum*, thrombolytic, antioxidative and cytotoxicity

INTRODUCTION

Medicinal plants utilized in conventional medication in different ailments through the course of advancement around the world are fascinating research subjects being a vast precursor of structurally multifaceted and versatile bioactive molecules. Thrombus or blood clot prevents blood perfusion by obstructing blood vessels causing a deficiency in blood flow and oxygen in target tissues that develop necrosis. The function of a thrombolytic drug is to dissolve thrombin in acutely occluded coronary arteries, pulmonary embolism (PE), atrial fibrillation, deep-vein thrombosis and mechanical prosthetic heart valves[1] thereby to ensure blood supply to myocardium to limit ischemia and to develop prognosis with minimum blood loss.[2] It is well noted that available thrombolytic agents in the market like streptokinase, urokinase, alteplase, anistreplase,[3] tissue-type plasminogen activator offer some limitations such as partial fibrin specificity, side effects, having large dose and in some cases coupled bleeding tendency.[4,5] Persistent research will offer novel insights and uphold evolution toward the devolution of the supreme thrombolytic therapy.[6,7] There has been some evidence to recommend that free radicals and some reactive nitrogen species prompt and amplify oxidation that may proceed to cell death mechanisms like apoptosis and necrosis[8] associated with other disorders including arthritis, Alzheimer's disease, atherosclerosis, diabetes and cancer.[9,10] Several reports suggest that antioxidant could decline oxidation as well as increase survival times.[11,12] The brine shrimp lethality bioassay is

Correspondence to: A.T.M. Zafrul Azam
Email: zafrulazam@du.ac.bd

Dhaka Univ. J. Pharm. Sci. **22**(1): 97-103, 2023 (June)
DOI: https://doi.org/10.3329/dujps.v22i1.67100

simple, rapid (24 h), robust, inexpensive and easy technique.[13] The bioassay is linked to have a good cytotoxic activity in carcinogenic tissue and represents some pesticidal activity.[14,15] Brine shrimp lethality bioassay is based on the mortality activity of test compounds on a brine shrimp organism and was first proposed by Michael *et al.*, and further modified later.[16,17,18]

Jatropha pandurifolia or J. integerrima Jacq. is an erect tiny, ornamental evergreen shrub having star-shaped red, pink, or vermilion blooms. In Asia, Latin America, and Africa it is also known as peregrine or spicy jatropha.[19] For era different parts of this plant are utilized by folks. Phytocompounds namely alkaloid like jatrophine, jatropham and curcin,[20] and diterpene like jatrophone, jatrophatrione, jatropholone A–B)[21,22] were identified from this plant. Furthermore, sitosterol, glycoside, lignin, tannin, saponin[23], fatty acids (palmitic, oleic, and linoleic acids), carotenoid, flavonoid etc.[24] were also reported. Pharmacological activities such as antiinflammatory, antituberculosis, antiplasmodial, cytotoxicity [25,26], anticancer[27,28], antimicrobial [29,30,31], antifungal antioxidant potentials[32] are revealed from different parts of the plant. Leaves, stem, bark, roots, and oil have long been used for a variety of diseases as styptic agent, emetic agent, antidote[33,34], purgative[35], moreover in warts, toothaches, ringworm, gastric emptying, rheumatic pain, gum bleeding, and skin diseases.[36,37]

Syzygium reticulatum (also known as *Eugenia reticulata* Wight) belongs to Myrtaceae family. This pant is widely distributed in Bangladesh (Sylhet), India (Assam, Meghalaya) and Myanmar. This is a medium-sized evergreen tree. Leaves are ovate-lanceolate with thick coriaceous nervation on both surfaces. It has drupe like fruit holding covered seeds.[38] Fruits and flowering period is from March to June. Wood is used for handles of tools and for making arches.

For *J. pandurifolia*, a few biological profiling studies were examined, but no report for *S. reticulatum* has yet been discovered. This study aims to examine the antioxidant, thrombolytic and cytotoxic properties of various parts of the two plants.

MATERIALS AND METHODS

Collection and identification of the plant. In August 2019, *J. pandurifolia* stem barks were collected from botanical garden, University of Dhaka, Bangladesh and *Syzygium reticulatum* was collected in 2022 from Lawachara, Sylhet. The plant components were recognized by a taxonomist (Ms. Nasrin Aktar), Bangladesh National Herbarium, where a voucher specimen was deposited for future reference (DACB Accession No. JP: 54445, SR: 65585)

Preparation of plant samples. The plant parts of both species were cleaned properly to remove dirt, shed dried and the air-dried parts were then crushed into coarse powder and the powder was preserved in a labeled airtight container with an identification label for further investigation. The powdered stem-barks of *J. pandurifolia* (3.0 kg) and leaves of *S. reticulatum* (2.0 kg) were soaked into ethyl acetate over the period of 15 days, filtered through a cotton plug followed by Whatman filter paper and finally concentrated using a Buchii rotary evaporator under minimal pressure.

Solvents and reagents. The solvents and reagents used in this investigation were analytical or laboratory grade. The organic solvents utilized in the tests were methanol, ethyl acetate and DMSO, which were distilled before use. Additionally, DPPH, streptokinase, butylated hydroxytoluene (BHT), vincristine sulphate and saline water were needed for the experiments.

a. Thrombolytic activity

5 mL of blood were taken in different pre weighed sterile vials each containing 1 mL of blood. Tubes were incubated at 37°C for 45 minutes [39], allowed to be clotted and weighed again. After clot formation, the clear portion was removed carefully. Clot weight was measured subtracting the previous two weights. 100 µL test samples (1 mg/100 µL water) were added separately to each clot containing vial. 100µL streptokinase (30,000 I.U) and 100 µL

distilled water were used as positive control and negative control, respectively. After incubation at 37°C for 90 minutes clot lysis was observed. The released clear plasma was removed and vials were again weighed to calculate the weight difference. The following calculation was used to measure clot lysis percentage.

% of thrombolytic activity = (wt. of clot after treatment / wt. of clot before treatment) × 100

b. Antioxidant activity

Qualitative antioxidant activity test. The plant extract was diluted in a suitable solvent and spotted on a TLC plate. A suitable mobile phase was prepared with different polarities. *n*-Hexane:ethyl acetate (2:1) as a non-polar solvent, $CHCl_3$:CH_3OH (5:1) as a medium polar solvent and $CHCl_3$:CH_3OH:H_2O (40:10:1) as a polar solvent were used. 0.02% w/v DPPH in methanol was used as a detector of antioxidative components.[40,41]

DPPH free radical scavenging assay. 2 mL of the different concentrations (500, 250, 125, 62.5, 31.25, 15.63, 7.83, 3.91, 1.96 and 0.98 µg/ mL) of the test samples were mixed with 2 mL of DPPH solution (20 µg/mL methanol), incubated (30 mins, 25^0C in dark place) for afterward UV absorbance measurement at 517 nm. IC_{50} values were calculated from the regression equation by plotting concentrations vs percentage of scavenging the free radicals. Butylated hydroxyl toluene (BHT) and methanolic DPPH solution were used as positive control and negative control, accordingly.[42] DPPH radical scavenging activity was calculated by the following equation:

$$I\% = 1- \{A_1 / A_0\} \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of test samples or standard. The experiment was repeated in triplicate at each concentration.

c. Brine shrimp lethality bioassay

The brine shrimp lethality bioassay was used to predict the cytotoxic activity[43] of pure compounds in 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.83, 3.91, 1.96 and 0.98µg/ml using serial dilution technique. The solutions of different concentrations were then added to 10 live nauplii in 5 ml simulated seawater into different vials. The vials were examined after 24 h using a magnifying glass and the number of existing live nauplii in each vial was counted. The percentage of mortality was calculated and plotted against LogC. Then lethal concentrations (LC_{50}) were calculated to measure cytotoxic activity. Saline water and vincristine sulphate (VS) were used as negative control and positive control, accordingly.

% mortality = (no. of dead nauplii/initial total no. of live nauplii) × 100

RESULTS AND DISCUSSION

a. Thrombolytic activity. In this study MSJP and EALSR showed moderate clot rupture activity valued as 61.30 ± 2.35% (**P<0.01) and 63.81± 1.92% (**P<0.01) accordingly wherein standard Streptokinase showed 73.6 ± 0.76% thrombus lysis capabilities. In this study, clot lysis activity of the crudes was evaluated as a part of revelation of cardioprotective drugs which was observed as the highest percentage of clot lysis capability. It is noted that numerous secondary metabolites such as flavonoids, alkaloids, tannins, saponins show thrombolytic properties as a natural reservoir.[44] The results are shown in Table 1.

Table 1. Thrombolytic activity of MSJP and EALSR.

Sample ID	% of clot lysis
MSJP	61.30** ± 2.35
EALSR	63.81**± 1.92
Streptokinase (STK)	73.6 ± 0.76
Negative control (water)	5.03± 0.70

(Values are expressed as mean ± SD, n = 3; Significance level among different groups at P ≤0.05 (*P<0.05; **P<0.01, ***P<0.001); Test groups were compared to standard group;

MSJP: Methanolic stem bark extract of *J. pandurifolia;* EALSR: Ethyl acetate leaf extract of *S. retilulatum*)

b. Antioxidant activity. In the qualitative antioxidant activity test, on TLC plates dense yellow spots were observed under UV spectroscopy which indicates the abundance of antioxidative compounds.

Reactive oxygen species (ROS) and other oxidants have been linked to a number of ailments and diseases, according to a large body of research.[45] Free radical reactions have a well-established function in disease pathophysiology and are known to contribute to a wide range of both acute and chronic human illnesses, including diabetes, atherosclerosis, aging, immunosuppression, and neurodegeneration.[46] The body's natural antioxidant capacity and ROS levels were out of balance, which led to the requirement of dietary and/or pharmacological supplementation, especially during a disease assault. Commonly superoxide dismutase, catalase enzymes or glutathione transferase [47] are role models having antioxidant activity in most medicinal plants in addition to phytochemicals like polyphenolic, proanthocyanidins, flavonoids, tannins, tocopherols etc. [48]

Table 2. Antioxidant activity of MSJP and EALSR.

Conc. (µg/ ml)	% of Scavenging		Free radical scavenging activity (IC_{50} in µg/ ml)		Butylated hydroxyl toluene (BHT)		
	MSJP	EALSR	MSJP	EALSR	Conc. (µg/ ml)	% of Scavenging	IC_{50} (µg/ ml)
1000	75.67	82.45	8.1***± 1.44	10.34**±2.12	200	95.79	6.82± 0.99
500	71.18	69.85			100	94.39	
250	68.6	82.39			50	92.28	
125	65.97	83.28			25	82.47	
62.5	61.37	79.1			12.5	74.06	
31.25	58.69	75.43			6.25	64.95	
15.63	55.82	90.3			3.125	37.61	
7.813	53.28	60.3			1.5625	19.38	
3.91	49.4	48.51			0.78125	1.16	
1.96	30.93	41.8					
0.98	14.01	38.88					

(Values are expressed as mean ± SD, n = 3; Significance level among different groups at P ≤0.05 (*P<0.05; **P<0.01, ***P<0.001); Test groups were compared to standard group;
MSJP: Methanolic stem bark extract of *J. pandurifolia;* EALSR: Ethyl acetate leaf extract of *S. retilulatum*)

The present study expressed half maximal inhibitory concentration i.e IC_{50} : 6.82± 0.99 µg/mL for butylated hydroxy toluene (BHT) wherein MSJP and EALSR exerted strong to moderate DPPH scavenging property with IC_{50}: 8.1± 1.44 (***P<0.001); and 10.34±2.12 (**P<0.01) µg/mL accordingly reporting noteworthy scavenging activity indicating the abundance of relevant phytochemicals. The results are presented in Table 2.

c. Cytotoxic activity. Brine shrimp lethality bioassay is a fast and quick method used to calculate the potential of any cytotoxic compound that could be lethal to living organism. The bioassay has a strong association with both pesticidal activity and cytotoxic activity in various human solid tumors. [49,50]

MSJP and EALSR exhibited mild lethality activity with LC_{50} value 5.73*±2.85(*P<0.05) and 5.12*±1.57 (*P<0.05) µg/ mL, respectively whereas vincristine sulphate showed LC_{50}: 0.52 ± 0.18 (µg/ mL). The findings demonstrate that both plants' crude extracts contain a large number of phytochemicals with cytotoxic activity. The results are projected in Table 3.

Table 3. Cytotoxic activity of MSJP & EALSR.

Conc. (µg/ ml)	Log C	% of mortality		LC_{50} (µg/ ml)		Vincristine Sulphate			
		MSJP	EALSR	MSJP	EALSR	Conc. (µg/ ml)	Log C	% mortality	LC_{50} (µg/ ml)
1000	3	100	100	5.73*±2.85	5.12*±1.57	40	1.602	100	0.52± 0.18
500	2.699	90	90			20	1.301	100	
250	2.398	80	70			10	1.000	100	
125	2.097	60	60			5	0.699	90	
62.5	1.796	50	30			2.50	0.398	80	
31.25	1.495	40	30			1.25	0.097	60	
15.63	1.194	30	20			0.63	−0.201	50	
7.813	0.893	30	20			0.31	−0.509	40	
3.91	0.591	20	20			0.16	−0.796	30	
1.96	0.292	20	20			0.078	−1.108	20	
0.98	-0.01	10	10						

(Values are expressed as mean ± SD, n = 3; Significance level among different groups at P ≤0.05 (*P<0.05; **P<0.01, ***P<0.001); Test groups were compared to standard group;

MSJP: Methanolic stem bark extract of *J. pandurifolia;* EALSR: Ethyl acetate leaf extract of *S. retilulatum*)

CONCLUSION

The outcomes recovered in this research confirmed the conventional curative uses of these plants in different ailments for treating different diseases. The thrombolytic, antioxidant and cytotoxic activities were unveiled that would be noteworthy analysis of concealed ethno-pharmacological effectiveness to ascertain numerous persuasive bioactive molecules.

ACKNOWLEDGEMENT

MA and ATMZA are grateful to the University of Dhaka, Bangladesh for funding the research project under Centennial Research Grant (CRG) 2021-22. The authors are thankful to Faculty of Pharmacy, University of Dhaka, Bangladesh for providing all the research amenities to bring out this work.

REFERENCES

1. Bristol-Myers Squibb Company. Princeton; 2010. Coumadin (warfarin sodium) tablets crystalline and coumadin (warfarin sodium) for injection prescribing information.
2. Laurence, D.R. and Bennett, P.N. 1992. *Clinical Pharmacology*. seventh edition. New York: Churchill Livingstone. 483.
3. Collen, D. 1990. Coronary thrombolysis: Streptokinase or recombinant tissue-type plasminogen activator? *Ann. Intern. Med*. **112**, 529-38.
4. Wu, D.H., Shi, G.Y., Chuang, W.J., Hsu, J.M., Young, K.C. and Chang, C.W. 2001. Coiled coil region of streptokinase gamma-domain is essential for plasminogen activation. *J. Biol. Chem.* **276**, 15025-15033.
5. Ali, R., Hossain. M., Runa. J. F., Hasanuzzaman, M. and Islam, M. 2014. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds.*Avicenna J. Phytomed.* **4**, 430-436.
6. Basta, G., Lupi, C., Lazzerini, G., Chiarelli, P., L'Abbate, A. and Rovai, D. 2004. Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. An *in vitro* study on blood of normal subjects and patients with coronary artery disease. *Thromb Haemost.* **91**, 1078-83.
7. Hussain, F., Islam, M. A., Bulbul, L., Moghal, M.M.R. and Hossain, M.S. 2014. *In vitro* thrombolytic potential of root extracts of four medicinal plants available in Bangladesh. *Anc. Sci. Life*. **33**, 162-164.
8. Chatterjee, S., Lardinois, O., Bhattacharjee, S., Tucker, J., Corbett, J. and Deterding, L. 2011. Oxidative stress induces protein and DNA radical formation in follicular dendritic cells of the germinal center and modulates its cell death patterns in late sepsis. *Free Radic Biol Med.* **50**, 988-999.
9. Clancy, D. and Birdsall, J. 2013. Flies, worms and the free radical theory of ageing. *Ageing Res. Rev.* **12**, 404-412.
10. Karthikeyan, R., Manivasagam, T., Anantharaman, P., Balasubramanian, T. and Somasundaram, S.T. 2011. Chemopreventive effect of *Padina* boergeseniiextractson ferric nitrilotriacetate (Fe-NTA)-induced oxidativedamage in Wistar rats. *J. Appl. Phycol.* **23**, 257-263.

11. Block, K.I., Koch, A.C., Mead, M.N., Tothy, P.K., Newman, R.A. and Gyllenhaal, C. 2008. Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of the evidence from randomized controlled trials. *Int. J. Cancer*. **123**, 1227-1239.
12. Ali, M.S., Amin, M.R., Kamal, C.M.I. and M.A. 2013. *In vitro* antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the *A. philippense* L. leaves. *Asian Pac. J. Trop. Biomed.* **3**, 464–469.
13. Ghisalberti, E.L. 1993. Detection and isolation of bioactive natural products. In: Colegate SM, Molyneux RJ, editors. Bioactive natural products: Detection, isolation and structure elucidation. New York: CRC Press. 15-8.
14. McLaughlin, J.L., Rogers, L.L. and Anderson, J.E. 1998. The use of biological assays to evaluate botanicals. *Drug Inform. J.* **32**, 513-24.
15. Apu, A.S., Muhit, M.A. Tareq, S.M. Pathan, A.H., Jamaluddin, A.T.M. and Ahmed, M. 2010. Antimicrobial Activity and Brine Shrimp Lethality Bioassay of the Leaves Extract of *Dillenia indica* Linn. *J. Young Pharm.* **2**, 50-53.
16. Michael. A.S., Thompson, C.G. and Abramovitz, M. 1956. Artemia salina as a test organism for bioassay. *Sci.* **123**, 464.
17. Vanhaecke, P., Persoone, G., Claus, C. and Sorgeloos, P. 1981. Proposal for a short-term toxicity test with Artemia nauplii. *Ecotoxicol. Environ. Saf.* **5**, 382–387
18. Wu, D.C. 2014. An important player in brine shrimp lethality bioassay: The solvent. *J. Adv. Pharm. Technol. Res.* **5**, 57-58.
19. Duke, J.A. 1985. Medicinal plants. Sci. **229**, 1036.
20. Luo, M.J., Yang, X.Y., Liu, W.X., Xu, Y., Huang, P., Yan, F. and Chen, F. 2006. Expression, Purification and Anti-tumor Activity of Curcin. *Acta. Biochim. et Biophy. Sinica.* **38**, 663-668.
21. Sutthivaiyakit, S., Mongkolvisut, W., Ponsitipiboon, P., Prabpai, S., Kongsaeree, P., Ruchirawat, S. and Mahidol. C. 2003. A novel 8, 9-secorhamnofolane and a new rhamnofolane endoperoxide from *Jatropha integerrima*roots. *Tetrahed. Lett*. **44**, 3637-3640.
22. Yang, J., Long, Y.O. and Paquette, L.A. 2003. Concise total syntheses of the bioactive mesotricyclic diterpenoids jatrophatrione and citlalitrione. J. *Am. Chem. Soc*. **125**, 1567-1574.
23. Kolawole, O.S. Rahaman, A.A.A. and Oladele, F.A. 2014. A numerical approach to the taxonomy of the genus *Jatropha* Linn. Using quantitative phytochemical constitutents, *Europ. J. Expt. Biol*. **4**, 71-76.
24. Kuspradini, H.,Rosiarto, A.M., Putri, A.S. andKusuma, I.W. 2016.Antioxidant and toxicity properties of anthocyanin extracted from red flower of four tropical shrubs. *Nusantara Bios*. 8, 135-140.
25. Sutthivaiyakit, S., Mongkolvisut, Prabpai, S. and Kongsaeree, P. 2009. Diterpenes, sesquiterpenes, and a sesquiterpene—coumarin conjugate from *Jatropha integerrima*. *J. Nat. Prod*. **72**, 2024-2027.
26. Akhter, A., Rahman, M.S. and Ahsan, M. 2008. Preliminary Antimicrobial and Cytotoxic Activities of n-Hexane Extract of *Jatropha pandurifolia*, Lat. *Am. J. Pharm.* **27**, 918-921.
27. Mbwambo, Z.H., Moshi, M.J., Masimba, P.J., Kapingu, M.C. and Nondo, R.S.O. 2007. Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. *BMC Compl. Altern. Med.* **7**, 9.
28. Moshi, M.J., Innocent, E., Magadula, J.J., Otieno, D.F., Weisheit, A., Mbabazi, P.K., Weisheit, A. and Nondo, R.S.O. 2010. Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania. *Tanzania J. Health Res*. **12**, 63-67
29. Gaikwad, R.S., Kakde, R.B., Kulkarni, A.U., Gaikwad, D.R. and Panchal, V.H. 2012. *In vitro* antimicrobial activity of crude extracts of Jatropha species, *Curr. Bot*. **3**, 09-15
30. Machaceep, S. and S. Machaceep. 1990. Nature Study Series. *Encyclopedia of Plants and Animals; Praepittaya: Bangkok*. **3**, 93.
31. Rampadarath, S., Puchooa, D. and Sanmukhiya, M.R. 2014. Antimicrobial, phytochemical and insecticidal properties of jatropha species and Wild *Ricinus communis* L. Found in Mauritius, *Int. J. Pharmacog. Phyto. Res*. **6**, 831-840.
32. Kuspradini, H.,Rosiarto, A.M., Putri, A.S. andKusuma, I.W. 2016. Antioxidant and toxicity properties of anthocyanin extracted from red flower of four tropical shrubs. *Nusantara Bios*. **8**, 135-140.
33. Shetty, S. Udupa, S.L. Udupa, A.L. and Vollala, V.R. 2006. Wound healing activities of bark extract of *Jatropha curcas* Linn in albino rats, *Saudi Med.* **27**, 1473-6.
34. Kirtikar, K.R. and Basu, B.D. 2002. Ind. Medi. Plants. Popular Prakashan, Dehradun.
35. Nayak, B.S. and Patel, K.N. 2010. Pharmacognostic studies of the *Jatropha curcas* leaves, *Int. J. Pharm. Tech. Res.* **2**, 140-143.
36. Pertino, M., Schmeda-Hirschmann, G. Rodriguez, J.A. and Theoduloz, C. 2007. Gastroprotective effect and cytotoxicity of semisynthetic jatropholone derivatives. *Planta Med*. **73**, 1095-1100.
37. Mujumdar, A.M. and Misar, A.V. 2004. Anti-inflammatory activity of *Jatropha curcas* roots in mice and rats, *Ethnopharmacol*. **90**, 11-15.
38. Borah, R.L. 2014. An Updated Account of the Name Changes of the Dicotyledonous Plant Species included in the Vol: I (1934-36) & Vol: II (1938) of “Flora of Assam”. *Plant Archives* **14**, 87-96.
39. Prasad, S., Kashyap, R.S. , Deopujari, J.Y. , Purohit, H.J. , Taori G.M. and Daginawala H.F. 2007. Effect of *fagonia arabica* (dhamasa) on *in vitro* thrombolysis. *BMC Complement. Altern. Med.* **7**, 36.
40. Sultana, M.S., Golder, M., Biswas, B., 2, Karmakar, U.K., Bokshi, B., Alam, M.J., Shahriar, M., and Sadhu, S.K. 2022. Antioxidative and antidiabetic potentials of the pneumatophores of *Heritiera fomes* Buch. Ham. *Dhaka Univ. J. Pharm. Sci.* **20**, 283-291.

41. Sadhu, S.K., Okuyama, E., Fujimoto, H. and Ishibashi, M. 2003. Seperation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. *Chem. Pharm. Bull.* **51**, 595-598.
42. Williams, W. B., Cuvelier, M.E. and Berset, C.L.W.T. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **28**, 25-30.
43. Meyer, B.N. Ferrigni, N.R. Putnam, J.E. Jacobsen *et al.* 1982. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*. **45**, 31-34.
44. Ali, M.R., Kuri, S., Das, A. and Islam, M. A. 2013. Preliminary phytochemical screening and *in vitro* thrombolytic potential of the methanolic extract of *Enhydra fluctuans*Lour (leaves). *Int. J. Pharmamed. Ind*. 1, 270-280.
45. Halliwell, B., Gutteridge, J.M.C. 1981. Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Lett*. **128**, 347-352.
46. Harman, D. 1998. Free radical theory of aging. Current status. Amster-dam: *Elsevier*, 3-7.
47. Meena, H., Pandey, H.K., Pandey, P., Arya, M.C. and Ahmed Z. 2012. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Baccopa monnieri* and *Centella asiatica. Ind. J. Pharmacol.***44**, 114-117.
48. Kasote, D. M., Katyare, S. S., Hegde, M. V. and Bae, H. 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *Int. J. Biol. Sci.* **11**, 982-991.
49. Ghisalberti, E.L. 1993. Detection and isolation of bioactive natural products. In: Colegate SM, Molyneux RJ, editors. *Bioactive natural products: Detection, isolation and structure elucidation. New York: CRC Press*, 15-18.
50. McLaughlin, J.L., Rogers, L.L., Anderson, J.E. 1998. The use of biological assays to evaluate botanicals. *Drug Inform J.* **32**, 513-524.