Bangladesh Pharmaceutical Journal ***17****(1): 51-54, 2014*

Antimicrobial, Cytotoxic, Thrombolytic and Antioxidant Activities of *Syzygium fruticosum* (Roxb.) DC.

Somaia Haque Chadni[1], Abdullah Al Hasan[2] and A.T.M. Zafrul Azam[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Pharmacy, Southeast University, Dhaka-1213, Bangladesh

Abstract

The crude methanol extracts of the bark and leaves of *Syzygium fruticosum* (Roxb.) DC. were partitioned with petroleum ether, carbon tetrachloride, chloroform and water for biological investigation. In the brine shrimp lethality bioassay, carbon tetrachloride soluble fraction of leaf extract (LCTF) showed significant lethality having the LC_{50} value 0.65 µg/ml. In free radical scavenging activity screening (DPPH assay), chloroform fraction of methanolic extract of bark of *S. fruticosum* showed the highest free radical scavenging activity with IC_{50} value 20.01 µg/ml. In the microbiological investigation, only chloroform soluble fraction of bark (BCF) and aqueous fraction of bark (BAF) showed mild antimicrobial activity with zone of inhibition ranging from 7 to 14 mm as compared to standard ciprofloxacin (zone of inhibition of 50 mm). In the study for thrombolytic property, different extracts of *S. fruticosum* revealed varying degrees of thrombolytic activity ranging from 33.46% to 62.51%.

Key words: *Syzygium*, antimicrobial, brine shrimp lethality, free radical scavenging, thrombolytic activity.

Introduction

Syzygium fruticosum is a flowering plant of the Myrtaceae family. This family encompasses plants whose characteristics are of high industrial, pharmaceutical, scientific, and cultural importance. It is found in India, Myanmar, Thailand, China, Bangladesh and some other Asian countries. Traditionally, it is used as folk remedy for the treatment of stomachic, diabetes and bronchitis (Ruan *et al.*, 2008; Jain *et al.*, 2010).

Literature review showed that different species of *Syzygium* possess a variety of biological activities. The leaves of *S. cumini* revealed anti-allergic, antioxidant, anti-inflammatory and analgesic properties (Ruan *et al.*, 2008; Jain *et al.*, 2010). The fruit and bark extracts of *S. aromaticum* and the seed extracts of *S. aqueum, S. aromaticum* and *S. jambos* showed antioxidant, anti-allergic, antidiabetic, antihyperlipidemic, gastroprotective and analgesic activities (Muruganandan *et al.*, 2001; Ruan *et al.*, 2008; Jain *et al.*, 2010; Singh and Gupta, 2007; Ravi *et al.*, 2004; Kasiappan *et al.*, 2005). Many *Syzygium* species showed α-glucosidase inhibitory activity (Omar *et al.*, 2012), antihyperlipidemic effect (Sharma *et al.*, 2011), antiatherosclerotic potential (Tanwar *et al.*, 2011), antibacterial activity (Machado *et al.*, 2005), anti-HIV activity (Kusumoto *et al.*, 1995) and so on. The seed extracts of *S. fruticosum* displayed antioxidant and anticancer properties (Islam *et al.*, 2013).

On the other hand, chemical investigations of *Syzygium* species have revealed the presence of flavonoids, gallic acid, ellagic acid, glycosides triteripenoids and saponins (Bhatia and Bajaj, 1975). β-sitosterol, betulinic acid, mycaminose, crategolic (maslinic) acid, *n*-hepatcosane, *n*-nonacosane, *n*-hentriacontane, noctacosanol, *n*-triacontanol, *n*-dotricontanol, quercetin, myricetin, myricitrin and the flavonol glycosides myricetin 3-O-(4″-acetyl)-α-L-rhamnopyranosides, acylated flavonol glycosides have been isolated from the leaves of *S. cumini* (Mahmoud *et al.*, 2001; Sagrawat *et al.*, 2006). However, until now no phytochemical studies of *S. fruticosum* could be found in literature.

The present work was an endeavor to screen the methanolic extract (ME) of *S. fruticosum* and its different fractions for probable antibacterial, cytotoxic, thrombolytic and antioxidant activities, and we, here in, report the results of our preliminary investigations.

Correspondence to: A.T.M. Zafrul Azam, Tel. +880-1710-880064; Fax: +880-2-8615583; E-mail: zafrulazam@du.ac.bd

Materials and Methods

Plant material: Whole plant of *S. fruticosum* (Roxb.) DC. was collected from Debidwar, Comilla, Bangladesh in the month of August, 2009, and was identified at the Department of Botany, University of Dhaka, Bangladesh.

Extraction: The powdered leaf and bark (1000 g) of *S. fruticosum* were separately soaked in methanol (4 L) for 15 days. Solvent-solvent partitioning of the residues were done by using the protocol designed by Kupchan and modified by VanWagenen *et al.* (1993). For bioassay the crude extract of bark and leaf (5gm each) was separately dissolved in 10% aqueous methanol and partitioned by using petroleum ether, carbon tetrachloride and chloroform leaving an aqueous soluble material. The fractions were designated as BPF, BCTF, BCF and BAF from bark and LPF, LCTF, LCF and LAF from leaf, respectively.

Antimicrobial activity: The samples were tested for antimicrobial activity by the standardized disc diffusion method (Bauer *et al.*, 1966). The screening was done against some strains of bacteria. The results thus obtained were compared with standard antibiotic, ciprofloxacin.

Cytotoxicity screening: In brine shrimp lethality bioassay (Meyer *et al.*, 1982) dimethyl sulfoxide (DMSO) was used as solvent as well as negative control while vincristine sulfate (VS) served as the positive control. For cytotoxicity screening, DMSO solutions of the test samples were applied against *Artemia salina* in a 1-day *in vivo* assay. For the experiment, 4 mg of each of the test samples was dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml) were obtained by serial dilution technique.

Antioxidant activity: The free radical scavenging activity (antioxidant capacity) of the test samples were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH), the detailed procedure could be found elsewhere (Brand-Williams *et al.*, 1995).

Thrombolytic activity: Following the method developed by Prasad *et al.* (2007) *w*hole blood was drawn from healthy human volunteers (n=10) without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed sterile Eppendorf tubes and was allowed to form clots. After clot formation, the serum was completely removed without disturbing the clot. To each Eppendorf tube containing pre-weighed clot, 100 µl aqueous solutions of different extracts along with the crude extracts were added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control Eppendorf tubes. All tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. The released fluid was removed and Eppendorf tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

$$\% \text{ clot lysis} = (\text{Weight of the lysis clot} / \text{Weight of clot before lysis}) \times 100$$

Results and Discussion

Antimicrobial activity: Antibacterial activity of all test samples were investigated against five gram positive bacteria namely, *Bacillus cereus, B. megaterium, B. subtilis, Sarcina lutea & Staphylococcus aureus* and eight gram negative bacteria namely, *Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, S. typhi, Shigella boydii, S. dysenteriae, Vibrio mimicus & V. parahaemolyticus.* In this study, the chloroform soluble fraction of bark (BCF) and aqueous soluble fraction of bark (BAF) showed mild to moderate antimicrobial activity with zone of inhibition from 7 to 14 mm as compared to standard ciprofloxacin, which displayed zone of inhibition of 50 mm. So, these fractions (BCF, BAF) can be further studied to explore potent antimicrobial agents.

Cytotoxicity screening: The median lethal concentration (LC_{50}) of the test samples after 24 hr exposure was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration. Vincristine sulfate (VS) was used as positive control and the LC_{50} was found 0.37 µg/ml for VS. Among the extractives of *S. fruticosum* the carbon tetrachloride soluble fraction of leaf extract showed most significant lethality having the LC_{50} value 0.65µg/ml.

Antioxidant activity: In this investigation, chloroform soluble fraction of methanolic extract of bark showed the highest free radical scavenging activity with IC_{50} value 20.01 µg/ml (Table 1).

Table 1. IC_{50} values of the test samples and standard.

Test samples	IC_{50} (µg/ml)
Standard	20.36
BME	27.35
BPF	24.35
BCTF	25.52
BCF	20.01
BAF	66.39
LME	26.84
LPF	29.74
LCTF	72.43
LCF	41.45
LAF	20.92

The average values of three replicates are presented as mean.

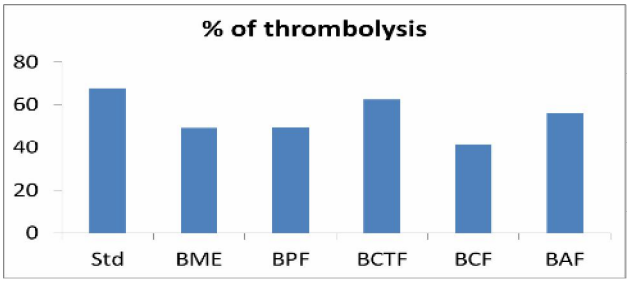

Figure 1. Thrombolytic activity of bark extractives of *Syzygium fruticosum.*

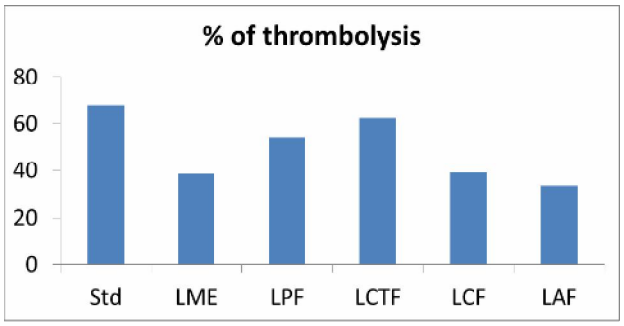

Figure 2. Thrombolytic activity of leaf extractives of *Syzygium fruticosum.*

Thrombolytic activity: Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots and subsequent incubation for 90 minutes at 37°C, showed 67.67% lysis of clot. On the other hand, distilled water treated as negative control exhibited a negligible percentage of lysis of clot (9.14%). In this study, different test samples of *S. fruticosum* exhibited varying degrees of thrombolytic activity ranging from 33.46% to 62.51% (Figure 1 and Figure 2).

Therefore, it can be concluded from the preliminary studies that some of the test samples obtained from *S. fruticosum* revealed mild to moderate antibacterial activity while significant antioxidant, cytotoxic as well as thrombolytic activities.

References

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**, 493-496.

Bhatia, I.S., and Bajaj, K.L. 1975. Chemical constituents of the seeds and bark of *Syzygium cumini*. *Planta Med.* **28**, 346–352.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* **28**, 25-30.

Islam, S., Nasrin, S., Khan, M.A., Hossain, A.S.M.S., Islam, F., Khandokhar, P., Mollah, M.N.H., Rashid, M., Sadik, G., Rahman, M.A.A. and Alam, A.H.M.K. 2013. Evaluation of antioxidant and anticancer properties of the seed extracts of *Syzygium fruticosum* Roxb. growing in Rajshahi, Bangladesh. *BMC Complementary and Alternative Med.* **13**, 142-151.

Jain, A., Sharma, S., Goyal, M., Dubey, S., Jain, S., Sahu, J., Sharma, A. and Kaushik, A. 2010. Anti-inflammatory activity of *Syzygium cumini* leaves. *Int. J. Phytomed.* **2**, 124-126.

Kasiappan, R., Subbaih, R. and Subramanian, S. 2005. Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin induced diabetes rats. *Food Chem. Toxicol.* **43**, 1433-1439.

Kusumoto, I.T., Nakabayashi, T., Kida, H., Miyashiro, H., Hattori, M., Namba, T. and Shimotohno, K. 1995. Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. *Phytotherapy Res.* **9**, 180-184.

Machado, K.E., Filho, V.C., Tessarolo, M.L., Mallmann, R., Meyre-Silva, C. and Bella Cruz, A. 2005. Potent antibacterial activity of *Eugenia umbelliflora*. *Pharmaceut. Biol.* **43**, 636-639.

Mahmoud, I.I., Marzouk, M.S., Moharram, F.A., El-Gindi, M.R., and Hassan, A.M. 2001. Acylated flavonol glycosides from *Eugenia jambolana* leaves. *Phytochemistry* **58**, 1239-1244.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E. and McLaughlin, J.L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* **45**, 31-34.

Muruganandan, S., Srinivasan, K., Chandra, S., Tandan, S.K., Lal, J. and Raviprakash, V. 2001. Anti-inflammatory activity of *Syzygium cumini* bark. *Fitoterapia* **72**, 369-375.

Omar, R., Li, L., Yuan, T. and Seeram, N.P. 2012. α-Glucosidase inhibitory hydrolyzable tannins from *Eugenia jambolana* seeds. *J. Nat. Prod.* **75**, 1505–1509.

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. *Complement. Alternat. Med.* **7**, 7- 36.

Ravi, K., Sivagnanam, K. and Subramanjan, S. 2004. Antidiabetic activity of *Eugenia jambolana* seed kernels on streptozotocin induced diabetic rats. *J. Med. Food* **7**, 187-191.

Ruan, P.Z., Zhang, L.L., Lin and M.Y. 2008. Evaluation of the antioxidant activity of *Syzygium cumini* leaves. *Molecules* **13**, 2545-2556.

Sagrawat, H., Mann, A.S. and Kharya, M.D. 2006. Pharmacological potential of *Eugenia jambolana*: A review. *Pharmacog. Mag.* **2**, 96-105.

Sharma, S.B., Tanwar, R.S., Nasir, A. and Prabhu, K.M. 2011. Antihyperlipidemic effect of active principle isolated from seed of *Eugenia jambolana* on alloxan-induced diabetic rabbits. *J. Med. Food* **14**, 353–359.

Singh, N. and Gupta, M. 2007. Effects of ethanolic extract of *Syzygium cumini* (L.) seed powder on pancreatic islet of alloxan diabetic rats. *Ind. J. Exp. Biol.* **45**, 861-867.

Tanwar, R.S., Sharma, S.B., Singh, U.R. and Prabhu, K.M. 2011. Antiatherosclerotic potential of active principle isolated from *Eugenia jambolana* in streptozotocin-induced diabetic rats. *Evidence-Based Complementary Alternative Med.* 2011.

VanWagenen, B.C., Larsen, R., Cardellina, J.H., Randazzo, D., Lidert, Z.C. and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J. Org. Chem.* **58**, 335-337.