



## Antioxidant, free radical scavenging, antibacterial and cytotoxic compound from the leaves of *Syzygium fruticosum*

Mst. Samima Nasrin<sup>1</sup>, Md. Golam Mostofa<sup>2</sup>, Md. Harun-Or-Rashid<sup>3</sup>, Md. Shofiqul Islam<sup>4</sup> and A. H. M. Khurshid Alam<sup>5\*</sup>

<sup>1</sup>Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, Chittagong-4318, Bangladesh

<sup>2</sup>Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

<sup>3</sup>Department of Pharmacy, World University of Bangladesh, 151/8, Green Road, Panthapath, Dhaka-1205

<sup>4</sup>Department of Pharmacy, Bangobondhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh

### Abstract

The leaves of *Syzygium fruticosum* (LSF) were extracted with 80% methanol to get the crude methanolic extract of LSF (LSF-M80). The LSF-M80 was successively fractionated with petroleum ether, chloroform, ethyl acetate, and finally with water to get four fractions. Based on the presence of phytochemical constituents, ethyl acetate fraction of LSF-M80 was subjected to column chromatography that led to identify and characterize a compound named gallic acid methyl ester (compound 1, C-1), and a mixture of two compounds. The LSF-M80 and C-1 showed strong antibacterial activity against a number of Gram (+) and Gram (-) bacteria. The LSF-M80 and C-1 also showed cytotoxic activity. Strikingly, the C-1 exhibited higher ferrous reducing antioxidant and DPPH free radical scavenging activities when compared with the reference standards like ascorbic acid (AA), gallic acid (GA), and butylated hydroxytoluene (BHT). In this study, we for the first time report the presence of gallic acid methyl ester and its higher antioxidant and free radical scavenging activity than the standards AA, GA, and BHT in *Syzygium fruticosum*.

**Keywords:** *Syzygium fruticosum*, Gallic acid methyl ester, Antioxidant, Antibacterial activity, Cytotoxicity

### Corresponding author: A. H. M. Khurshid Alam

Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh Tel: +8801719634499

Email: [khurshid.jaist@gmail.com](mailto:khurshid.jaist@gmail.com)

**Citation:** A. H. M. Khurshid Alam, et al (2018). Antioxidant, free radical scavenging, antibacterial and cytotoxic compound from the leaves of *Syzygium fruticosum*. Int J Pharm Sci & Scient Res. 4:6, 69-73.

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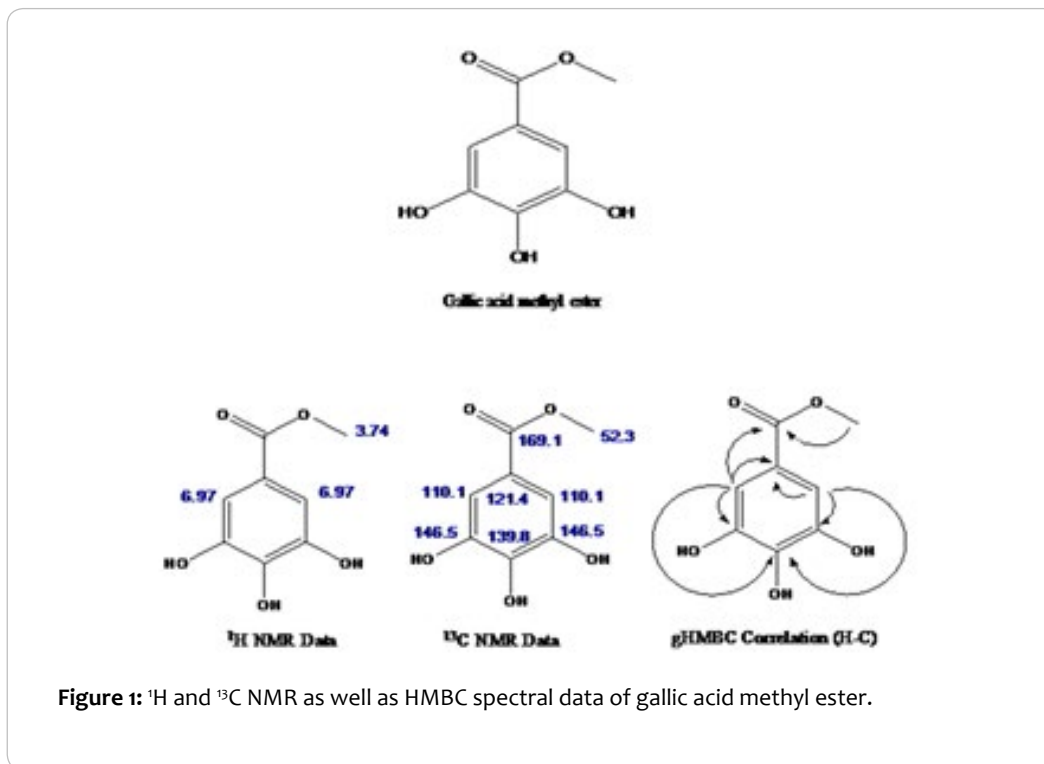
**Received:** July 25, 2018

**Accepted:** August 06, 2018

**Published:** October 31, 2018

### Introduction

*Syzygium fruticosum* Roxb. (Abbreviated as SF) <sup>[1]</sup>, a member of the Myrtaceae family, is a tree and is widely distributed all over the Bangladesh. The plant has a folkloric reputation for promoting diabetes mellitus, bloody dysentery, diarrhea, inflammation, ulcers etc <sup>[2-3]</sup>. A comprehensive literature review revealed that there is no chemical and biological reported data on this plant. However, fatty acids <sup>[4]</sup>, steroids <sup>[5]</sup>, terpenoids <sup>[6]</sup>, flavonols <sup>[7]</sup>, flavonoids <sup>[8]</sup>, phenolics <sup>[9]</sup> and tannins <sup>[10]</sup> were found in the genus of SF. In this study, we report the isolation and characterization of a compound named gallic acid methyl ester (compound 1, C-1), and its antioxidant, free radical scavenging, antibacterial, and cytotoxic activities.



## Materials and Methods

### Collection of plant

The leaves of SF (LSF) were collected from the Rajshahi University Campus and were identified by an expert taxonomist at National Herbarium, Dhaka, Bangladesh where a voucher specimen was deposited (Accession number: 1326).

### Preparation of Plant extract

The LSF were washed with fresh water to remove dirty material and were sun-dried occasionally for several days. Then the dried LSF were crushed into coarse powder (1000 gm) by grinding machine. The powdered materials were then placed in an amber colored extraction bottle and soaked with 3 liters of 80% methanol. The bottle and its contents were sealed and kept for 7 days with occasional shaking and stirring. The whole mixture was filtered through cotton followed by Whatman No. 1 filter papers, and was then concentrated with a rotary evaporator under reduced pressure at 40°C to afford a crude methanolic extract of 30 gm LSF (LSF-M80). The 30 gm of LSF-M80 was successively fractionated with petroleum ether, chloroform, ethyl acetate and finally with water to get four fractions: petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and aqueous fraction (AQF).

### Chromatographic separation

The EAF of LSF-M80 showed three prominent spots on TLC plates

using UV, IR and vanillin-sulfuric acid spray reagent. The extract (4.82 gm) was diluted with small amount of ethyl acetate and subjected to a column of silica gel and eluted with toluene with increasing portions of ethyl acetate, then with ethyl acetate, methanol and finally with water. Depending on the similar TLC behavior, different elutes were combined together and the fractions were designated as F-1, F-2, F-3, F-4, F-5, F-6, F-7, F-8 and F-9. The fractions F-1, F-2 and F-3 were present in very negligible amount. Therefore, fractions F-4, F-5, F-6, F-7, F-8 and F-9 were selected for further investigation. Among these fractions, F-4 and F-6 were subjected to PTLC to get pure compound and showed one distinct spot of each on the TLC plate using different solvent systems of toluene : ethyl acetate and when exposed in I<sub>2</sub> vapor. The fractions were then subjected to PTLC and eluted with the above solvent systems. After evaporation of solvent under reduced pressure, F-4 and F-6 turn into deep brown colored mass and needle shaped white crystal, respectively. Further TLC analysis of these compounds in different solvent systems also showed single spot, which might be the indication of single compounds.

### Antibacterial and cytotoxicity assay

Antibacterial and cytotoxic activities were tested by disk diffusion assay method<sup>[11-13]</sup> and by brine shrimp lethality assay<sup>[16-17]</sup>, respectively. All bacteria (listed in Table 1) were obtained from the stock cultures of the Microbiology Lab, Department of Pharmacy, University of Rajshahi, Bangladesh.

Phytochemical Constituents	PEF	CHF	EAF	AQF
Flavonoids	++	+	++++	++
Phenolics	++	+	++++	++
Saponins	-	-	+++	++
Tanins	+++	-	+++	+++
Glycosides	++	-	++	++
Steroids	+++	+	+	+

**Table 1:** Phytochemical test results of different extractives of LSF. (-) indicates not present, (+) indicates present

### Antioxidant and free radical scavenging assay

The ferrous reducing antioxidant capacity [18] and DPPH radical scavenging activity [19] were examined by standard spectrophotometric methods.

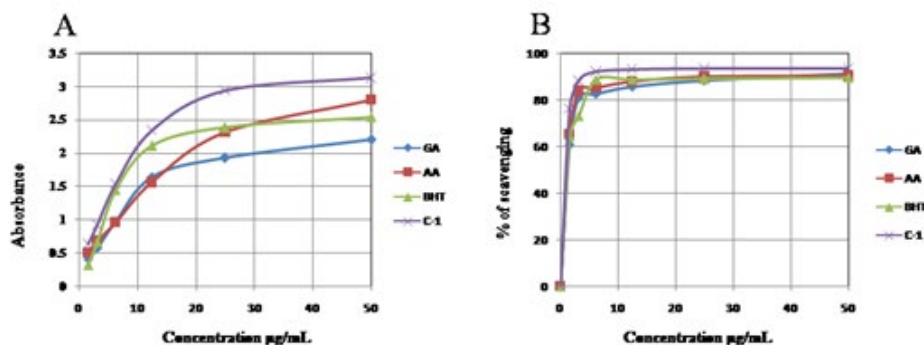
### Results and Discussion

The leaves of *Syzygium fruticosum* (LSF) were extracted with 80% methanol to get the crude methanolic extract of LSF ((LSF-M80). The LSF-M80 was successively fractionated with petroleum ether, chloroform, ethyl acetate, and finally with water to get four fractions: petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and aqueous fraction (AQF). Qualitative phytochemical screening revealed the presence of flavonoids, phenolics, saponins, tannins, steroids, and glycosides (Table 1). Based on this result, EAF was subjected to column chromatography to give gallic acid methyl ester (compound1, C-1).

C-1 was obtained as white crystal. It appeared as brown colored spot on the TLC plate with I<sub>2</sub>. The C-1 is soluble in methanol and sparingly soluble in ethyl acetate and chloroform, but insoluble in petroleum

ether and n-hexane. The R<sub>f</sub> value of the C-1 was 0.6. From the <sup>1</sup>H NMR spectrum of C-1 (in CD<sub>3</sub>OD), it was found that the C-1 possesses an aromatic signal at  $\delta$  6.97 (2H, s) and one methoxyl signal at  $\delta$  3.74 (3H) ppm, indicating the presence of a substituted aromatic moiety. The <sup>13</sup>C NMR spectrum showed four aromatic signals at  $\delta$ 110.09, 121.47, 139.84 (C-4) and 146.56 ppm, one carboxylic ester at  $\delta$  169.11 ppm and one methoxyl carbon at  $\delta$ 52.37. These <sup>1</sup>H and <sup>13</sup>C NMR spectral data as well as HMBC correlation and previous literature report [15] suggested the structure of C-1 was gallic acid methyl ester.

The results of antibacterial activity [11-12] of LSF-M80 and C-1 are given in the Table 2. The LSF-M80 and C-1 showed significant antibacterial activity against all tested pathogenic bacteria at a concentration of 200  $\mu$ g/disc. The MIC value was determined by serial dilution technique [13]. The MIC values of LSF-M80 were found to be 32  $\mu$ g/ml against all tested bacteria; on the other hand, the MIC values of C-1 were 8  $\mu$ g/ml against *Staphylococcus aureus*, *Escherichia coli*, *Shigella sonnei* and *Pseudomonas aeruginosa* and 16  $\mu$ g/ml against *Bacillus cereus* and *Shigella boydii*. The antibacterial activity of C-1 was similar to previously published data [14].



**Figure 2:** Ferrous reducing antioxidant capacity (A) and free radical scavenging activity (B) of C-1 and standards

Bacteria	Diameter of the zone of inhibition (mm)				
	LSF-M80		C-1		K
	100 µg/disc	200 µg/disc	100 µg/disc	200 µg/disc	30 µg/disc
<b>Gram (+)</b>					
1	5	9	18	26	23
2	8	11	19	23	25
<b>Gram (-)</b>					
3	10	13	14	24	22
4	7	10	18	27	26
5	7	9	15	28	22
6	5	11	8	19	25

**Table 2:** In vitro antibacterial activity of LSF-M80 and C-1

LSF-M80= 80 percent methanolic extract of leaves of *Syzygium fruticosum*; C-1= Compound-1; K= standard kanamycin. The control disc containing the solvent had no zone of inhibition.

The LSF-M80 and C-1 also showed a strong cytotoxic effect with ED<sub>50</sub> of 14.0 and 4.0 µg/ml, respectively. Strikingly, the C-1 showed higher ferrous reducing antioxidant and free radical scavenging activities when compared with the standards like ascorbic acid (AA), gallic acid (GA), and butylated hydroxytoluene (BHT) suggest that the C-1 could be used as reference standard as well as could be considered as a drug to treat several diseases caused by free radical.

### Conflict of Interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

### Acknowledgments

The authors want to give thank Dr. Mukhlesur Rahman, Senior Lecturer, School of Health Sport and Bioscience, University of East London for taking NMR data and characterize the compound. The authors also wish to thank the National Herbarium, Dhaka, Bangladesh for identification of the plant.

**Funding:** The authors declare that they have no fund for this research.

### References

1. Elliot RW, Jones DL, Blake T. (2010) Encyclopaedia of Australian plants suitable for cultivation. Vol 9, Australia: Port Melbourne: Lothian Press, 160-161.
2. Ruan PZ, Zhang LL, Lin MY. (2008) Evaluation of the antioxidant activity of *Syzygium cumini* leaves. *Molecules*, 13, 2545-2556.
3. Jain A, Sharma S, Goyal M, Dubey S, Jain S, Sahu J, Sharma A, Kaushik, A. (2010) Anti-inflammatory activity of *Syzygium cumini* leaves. *International Journal of Phytomedicine*, 2, 124-126.
4. Daulatabad CMJ, Mirajkar AM, Hosamani KM, Mulla GMM. (2006) Epoxy and cyclopropanoid fatty acids in *Syzygium cumini* seed oil. *Journal of the Science of Food and Agriculture*, 43, 91-94.

5. Sikder MAA, Kaiser MA, Rahman MS, Hasan CM, Al-Rehaily AJ, Rashid MA. (2012) Secondary metabolites from seed extracts of *Syzygium cumini* (L.). *Journal of Physical Science*, 23, 83-87.
6. Chang CW, Wu TS, Hsieh YS, Chao PL. (1999) Terpenoids of *Syzygium formosanum*. *Journal of Natural Product*, 62, 327-328.
7. Timbola AK, Szpoganicz B, Branco A, Monache FD, Pizzolatti MG. (2002) A new flavonol from leaves of *Eugenia jambolana*. *Fitoterapia* 73, 174-176.
8. Tian LW, Xu M, Wang D, Zhu H, Yang C, Zhang Y. (2011) Phenolic constituents from the leaves of *Syzygium forrestii* Merr. and Perry. *Biochemical Systematic and Ecology*, 39, 156-158.
9. Afify AEMR, Fayed SA, Shalaby EA, El-Shemy HA. (2011) *Syzygium cumini* (pomposia) active principles exhibit potent anticancer and antioxidant activities. *African Journal of Pharmacy and Pharmacology*, 5: 948- 956.
10. Zhang LL, Lin YM. (2009) Antioxidant tannins from *Syzygium cumini* fruit. *African Journal Biotechnology*, 8, 2301-2309.
11. Bauer AW, Kibry WMM, Sherris JC, Turck M. (1996) Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45, 493.
12. Barry AL. (1976) Principle and practice of microbiology. Philadelphia 7 Lea and Fabager.
13. Reiner R. (1982) Antibiotics. An introduction F. Switzerland. Basle 7 Hoffman La Roche and Co.
14. Al-Zahrani SHM. (2012) Antibacterial activities of gallic acid and gallic acid methyl ester on methicillin-resistant *Staphylococcus aureus*. *Journal of American Science*, 8, 7-12.
15. Kamatham S, Kumar N, Gudipalli P. (2015) Isolation and characterization of gallic acid and methyl gallate from the seed coats of *Givotia rottleriformis* Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells

16. Meyer BN, Ferrigni NR, Putnam JE, et al. (1982) [Brine shrimp: a convenient general bioassay for active plant constituents](#). *Planta Med*, 45, 31.
17. McLaughlin JL. (1992) [Bench-top bioassays for the discovery of bioactive compounds in higher plants](#). *Brenesia* p. 220.
18. Jayanthi P, Lalitha P. (2011) [Reducing power of the solvent extracts of \*Eichhornia crassipes\* \(Mart.\) Solms](#). *International Journal of Pharmaceutical Science*, 3,126-128.
19. Blois MS. (1958) [Antioxidant determinations by the use of a stable free radical](#). *Nature*; 181, 1199-1200.