IIUC Journal of Science and Engineering
Vol.-2, Issue-1, December 2024, pp. 81-88
ISSN: 3005-5873

Antimicrobial potentials of leaves of *Syzygium fruticosum* (Roxb.)

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Article info

Keywords
Syzygium fruticosum
Antimicrobial agent
Natural therapy
Antibiotic,
Gram positive
Gram negative bacteria

Abstract

The study was designed to investigate the antimicrobial principles of *Sygygium fruticosum* (Roxb.). The methanolic extract (CME) of leaves of *S. fruticosum* was successively fractionated with different organic solvents such as petroleum ether (PEF), chloroform (CHF) and ethyl acetate (EAF) to get four fractions: PEF, CHF, EAF and aqueous fraction (AQF). The antibacterial potentials of the extractives were determined using the agar well diffusion method against *Bacillus cereus*, *Stephylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella boydii*, and *Shigella sonnei*. The dilution method was used to calculate the minimum inhibitory concentration (MIC). The antibacterial activity of extractives was mild to moderate level against experimental bacterial strains. Among the extractives, EAF had the highest antibacterial efficacy against bacterial strains. The EAF's MIC value of 32 g/ml indicated that it might be employed as a powerful antibacterial agent.

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1. Introduction

In developing countries, a considerable proportion of people are suffering from food borne diseases which are caused by a range of gram negative bacteria (e.g *Salmonella typhi*, *E. coli* and *P. aeruginosa*) as well as gram positive bacteria (e.g *Staphylococcus aureus* and *Bacillus cereus*) (Braga et al., 2005; Doughari, Elmahmood, & Manzara, 2007; Pandey & Singh, 2011; Pirbalouti, Bahmani, & Avijgan, 2009; Rajendra, Rubin, & Rawat, 2012; Solomakos, Govaris, Koidis, & Botsoglou, 2008). Medicinal plants have been utilized to heal a variety of human ailments since ancient times. Despite massive availability of commercial antibiotic, its unavoidable side effects and concomitantly growing antimicrobial resistance paves the way

of searching for new antimicrobial agent from natural sources (Akinyemi, Oluwa, & Omomigbehin, 2006; Bhavnani & Ballow, 2000; Chopra, Hodgson, Metcalf, & Poste, 1997; Hart & Kariuki, 1998; Latha & Reddy, 2009). *S. fruticosum* is one of the 1200-1800 species of Myrtaceae family, widely distributed as an edible fruits tree all over Bangladesh (Ahmad et al., 2016; Moni et al., 2021b). Down through the ages, different parts of *S. fruticosum* have been traditionally used in ameliorating blood dysentery, diabetes mellitus, diarrhea, inflammation, stomachic and ulcer (Moni et al., 2021a). Besides these, literature review revealed that *S. fruticosum* along with some other species of the *Syzygium* genus possesses antifungal, antiviral, antiprotozoal, analgesic, antimalarial, cytotoxicity, thrombolytic (Uddin, Hossain, Reza, Nasrin, & Alam, 2022). *S. fruticosum* has a wide range of secondary plant metabolites, including polyphenolics, terpinoids, and glycosides, which play a great role in abovementioned activity. More specifically, it is evident from previous studies that gallic acid methyl ester was isolated from *S. fruticosum* leaves extract (Islam et al., 2013a; Nasrin, Mostofa, Harun-Or-Rashid, Islam, & Khurshid, 2018; Ruan, Zhang, & Lin, 2008; Uddin et al., 2022). Besides being used as anticancer, antiangiogenic, anti-inflammatory etc gallic acid and its derivatives have vital role in treating microbial infection (Choubey, Varughese, Kumar, & Beniwal, 2015). However, no comparative work is yet available on antimicrobial activity of different organic fractions of *S. fruticosum* leaves. Therefore, this study was designed to explore the antimicrobial activity of the methanol, chloform, petroleum ether, ethyl acetate and aqueous extract of *S. fruticosum* leaves against standard (Kanamycin) and different gram positive as well as gram negative bacteria.

2. Materials and methods

2.1 Plant collection and extract preparation

The *S. fruticosum* was collected from Kaptai hill tracts Rangamati district, Chittagong, Bangladesh. A taxonomist authenticated it, and it was deposited at the National Herbarium in Dhaka, Bangladesh, with the accession number: 1326. The grounded leaves were soaked in methanol for 7 days with shaking and stirring. Later, the crude methanolic extract (CME) of leaves of SF was successively fractionated with petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF) and aqueous fraction (AQF) following the Kupchan and Tsou protocol (Reza et al., 2021).

3. Antibacterial screening

3.1. Assay for antibacterial activity

Antibacterial activity of the CME and four organic fractions PEF, CHF, EAF and AQF were determined using the agar well diffusion method (Nasrin et al., 2018). Three different types of discs were prepared such as sample discs, standard discs and blank discs. Then test samples of CME, PEF, CHF and AQF were prepared through 7.5 mg of dried extracts/fraction was dissolved in 175 µl methanol. This gave a concentration of 600µg/10µl. Controls were run in simultaneously, with solvent being used to fill the well. After 24 hours, the plates were examined for zones of inhibition and the results were compared to kanamycin (30 mg/disc).

3.2. Determination of minimum inhibitory concentration (MIC)

The determination of MIC of the CME and PEF, CHF, EAF and AQF was carried out by the microdilution method (Valgas, Souza, Smânia, & Smânia Jr, 2007). The test bacteria, grown at 37^0C in nutrient agar medium, were diluted in sterile nutrient broth medium in such a manner that the suspension contained about 10^7cells/ml.

4. Results and discussion

Antibacterial screening is necessary to ensure the antibacterial spectrum of an agent against various types of pathogenic organisms including bacteria. Antibacterial screening is often conducted in two phases: a primary test to determine whether an activity is present or absent, and a secondary assay to quantify the relative potency reported as the minimum inhibitory concentration (MIC) value. The CME, PEF, CHF, EAF and AQF were tested for their antibacterial activity against two Gram-positive and four Gram-negative bacteria. Standard antibiotic, kanamycin (30 µg/disc), was used for comparison. The results of the extractives' antibacterial activity against the test bacteria are shown in Table I. Plant extracts have the potential to be antimicrobial due to the presence of specific bioactive substances such phenolics, tannins, flavonoids, and polyphenols (Nasrin et al., 2018). Results revealed that phenolics are the most powerful and efficient chemicals against bacteria and fungi out of all of these bioactive molecules (Naz et al., 2017). A similar correlation was found between the antibacterial activity data from the current study and the total phenolic contents of the samples (Islam et al., 2013b). Total phenolics of various fractions were correlated favorably with their antibacterial ability (Islam et al., 2013b).

Table I
***In vitro* antibacterial activity of CME and its four extractives**

No. and types of Bacteria		Diameter of the zone of inhibition (mm)					
		CME 600µg /disc	PEF 600µg/disc	CHF 600µg/disc	EAF 600µg/disc	AQF 600µg/disc	Kanamycin 30µg/disc
Staphylococcus aureus	Gram (+)	9	-	11	9	10	23
Bacillus cereus	Gram (+)	11	8	10	9	11	25
Pseudomonas aeruginosa	Gram (-)	10	9	22	14	10	22
Escherichia coli	Gram (-)	7	6	11	7	13	26
Shigella sonnei	Gram (-)	7	7	9	15	-	22
Shigella boydii	Gram (-)	-	-	8	8	8	25

Our study showed that all the extractives (PEF, CHF, EAF, AQF, CME) had antibacterial activity and among them CHF as well as EAF had the most significant antibacterial activity against all tested bacteria. The MIC of CHF was found to be 11, 10, 22, 11, 9, 8 against *S. aureus*, *B. cereus*, *P. aeruginosa, E. coli, S. sonnei* and *S. boydii,* respectively at 600 µg/disc. While EAF was showed a MIC of 9, 9, 14, 7, 15, 8 against *S. aureus*, *B. cereus*, *P. aeruginosa, E. coli, S. sonnei* and *S. boydii,* respectively at 600 µg/disc. The MIC of AQF was found to be 10, 11, 10, 13, 8 *S. aureus*, *B. cereus*, *P. aeruginosa, E.coli* and *S. boydi* respectively at 600 µg/disc but no antibacterial activity was found against *S. somnei*.. The MIC of CME was found to be 7, 10, 11, 9 against *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus*, respectively at 600 µg/disc but no antibacterial activity was observed against *S. boydii*. The MIC of PEF was found to be 8, 9, 6, 7 against *B. cereus*, *P. aeruginosa, E. coli,* respectively at 600 µg/disc, where it was not shown any activity against *S. boydii* and *Staphylococcus aureus* (Table I).

Table II
Determination of MIC values of CME and its two extractives against six bacteria

Name of the bacteria	**MIC in µg/ml**		
	CME (µg/ml)	**EAF (µg/ml)**	**CHF (µg/ml)**
1. *Staphylococcus aureus*	128	128	128
2. *Bacillus cereus*	128	64	128
3. *Pseudomonas aeruginosa*	128	128	32
4. *Escherichia coli*	128	256	128
5. *Shigella sonnei*	128	128	128
6. *Shigella boydii*	128	128	128

The EAF and CHF showed the most potent antibacterial activity. Therefore, these fractions were used to find out the minimum amount responsible for antibacterial activity. The MIC of EAF and CHF was

found to be 64 μg/ml against *B. cereus* and 32 μg/ml against *P. aeruginosa,* respectively. The CME of SF leaves exhibited significant antibacterial activity with MIC 128 μg/ml against all six bacterial strains used in this test (Table II).

5. Conclusions

In this investigation, we observed that this plant's ethyl acetate fraction (EAF) had strong antibacterial properties. The EAF contains secondary metabolites that have antibacterial properties. To establish *Syzygium fruticosum* Roxb. as an alternative treatment for the management of microbial infection, further mechanistic research will be necessary.

Disclosure statement

All the authors read and approved to submit the manuscript for this journal.

Funding

This research received no external funding.

Acknowledgments

Authors wish to thank the authority of Department of Pharmacy, University of Rajshahi, Rajshahi for their kind support in progress of the research. Author would like to thanks Prof. A H M Khurshid Alam for his guidelines during antimicrobial work.

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