Jagannath University Journal of Science
Volume 07, Number I, December 2020, pp. 9-13
https://jnu.ac.bd/journal/portal/archives/science.jsp
ISSN 2224-1698

JnUJSci

Chemical and Biological Screening of Endophytes from Bark of *Gynura procumbens*

Research Article

Mohammad Mostafizur Rahman[1], Muhammad Abdullah Al-Mansur[2], Shanta Easmin[1], Tahmina Afroz[1], Md. Shahinul Haque[3], Md. Mizanur Rahman[4], Md. Aminul Haque[1*]

[1]*Department of Chemistry, Jagannath University, Dhaka, Bangladesh*
[2]*INARS, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh*
[3]*Department of Chemistry, Bangladesh University of Health Science (BUHS), Bangladesh*
[4]*Department of Chemistry, Jatir Janak Bangabandhu Sheikh Mujibur Rahman College, Bangladesh*

Received: 01 October 2020
Accepted: 24 November 2020

Abstract : Three endophytic fungal strains were isolated from the bark a of medicinal plant *Gynura procumbens*. For chemical and biological studies, **Sh-1** fungal strain was sub-cultured in a large scale on a potato-carrot semi-solid agar media. Silica gel column chromatography was performed to isolate bioactive compound. One steroid compound Ergosterol (**S-1)** was isolated from **Sh-1** fungal strain. The chemical structure of the isolated compound was established by analyses of different spectroscopic methods such as IR, ^{1}H and ^{13}C NMR. Biological screening (antibacterial and antifungal assays) were performed for **Sh-1** fungal extract which displayed mild growth inhibitory against three bacteria i.e. *E. coli* (12 mm), *S. aureus* (8 mm), *B. subtilis* (7 mm) and one fungi *P. aeruginosa* (8 mm).

Keywords : *Endophytes ● Gynura procumbens ● Ergosterol ● Antibacterial activity ● Antifungal activity*

1. Introduction

In the last few decades, scientists have started to understandthat plants may act as a reservoir of uncountable numbers of organisms recognized as endophytes (Bacon and White 2000). Endophytes are kinds of bacteria or fungi which live in between the intercellular spaces of living host plant tissues without causing detectable disease (Strobel 2002). Endophytes are found almost in all terrestrial plants and play a significant role for the development of hosts (Strobel 2003; Saikkonen *et al.*, 2004). Several endophytes might produce biologically active substances which would be involved in a host-endophyte association. Now a days, it is clearly understandable that the host specificity is a fact in endophyte-higher plant relationships (Bacon and White 2000). As a result, these secondary metabolites produced in nature may ultimately have medicinal applications. Recent evidence showed that secondary metabolite production from an endophyte is not accidental but appear to be composed with ecological niche (Gloer 1997). So, efforts are continuing all over the world to isolate endophytes and evaluate their natural products. Endophytic fungi have been studied due to the pharmaceutical potential of their secondary metabolites. Various endophytic fungi species were known as sources of anticancer, antibiotic, insecticidal, antioxidant, antiviral and immunomodulation compounds (Shukla *et al.*, 2014). *Gynura procumbens* is a familiar medicinal plant of Bangladesh. The plant is usually used in the

* Corresponding author: Md. Aminul Haque
Email address: amin2k12@chem.jnu.ac.bd

regular treatment of numerous health aliments like fever, kidney diseases, constipation, viral skin diseases, rheumatism, migraine, rashes, and cancer (Rosidah *et al.*, 2009). The leaves of *Gynura procumbens* have anti-hyperglycemic (Akowuah *et al.*, 2002), anti-herpes simplex virus (Nawawi *et al.*, 1999), anti-hyperlipidemic (Zhang *et al.*, 2000), anti-inflammatory (Iskander *et al.*, 2002), anti-hypertensive, and anti-allergy properties. In the present study, as part of our progressing research *Gynura procumbens* was selected for isolation of endophytic fungi. The chemical and biological properties of *Gynura procumbens* were studied due to its bioactive potential with respect to antimicrobial activity.

2. Materials and Methods

Instrument

For column chromatographic technique, column grade G-60 (60-120 mesh with 0.04-0.063 mm particle size, Ar. 7734, ASTM, Merck-Germany) silica gel was used as stationary phase. For ^{1}H and ^{13}C NMR spectra recording, a Bruker 400 MHz spectrometer were used taking deuterated chloroform ($CDCl_3$) as the internal standard. Preparation of media was done under a Laminar flow (Biological Safety Cabinet; Thermo Forma. Class 11 A1). HIRAYAMA autoclave (Hirayama Mfg. Corp.) was used for media sterilization.

Collection of plant and sterilization process

Young medicinal plant *Gynura procumbens* was collected from local nursery of Dhaka, Bangladesh. Different plant parts were cut into small fragments. The surface of each plant barks was sterilized using 70% EtOH, 3% NaOCl (sodium hypochlorite), and sterile H_2O **(**Schulz *et al.*, 1993**)**. The clean plant parts were successively kept into respective solution for 3 min. The surface sterilization efficiency for every segment of tissue was confirmed by imprint method (**Figure 1**) **(**Schulz *et al.*, 1993**)**. Potato-carrot agar medium was used for culture and growth of endophytic fungi. The medium was prepared using grated potato and carrot (200 g/L each) boiled for 30 min. The boiling mixture was cooled and smashed. The extract was adjusted to a volume of 1L by the addition of water. 17.5 g/L agar was used for the solidification of media.

Isolation of endophytic fungi

The plant parts obtained from surface sterilization process were inoculated in potato-carrot agar media autoclaved on sterilized petri dish. After 20 days of inoculation period, three fungal strains from the barks were isolated. In the replication process of common fungi, one strain (**Sh-1**, **Figure 2**) was found to show optimum growth for further cultivation. This fungal strain isolated from bark showed maximum growth in minimum time and solvent extract showed spots which were well separated in thin layer chromatography (TLC). **Sh-1** was chosen for chemical and biological screening.

Culture extraction

Sh-1 was fermented in large scale (3 inch ×3 inch, 5×100 petri dishes, 20 days, room temperature) at potato-carrot agar semi-solid medium. The fungal broth from 500 Petri dishes was homogenized using an Ultra-Turrax. The resultant mixture was extracted with EtOAc (ethyl acetate). The EtOAc was collected by filtration and the filtrate was evaporated to dryness.

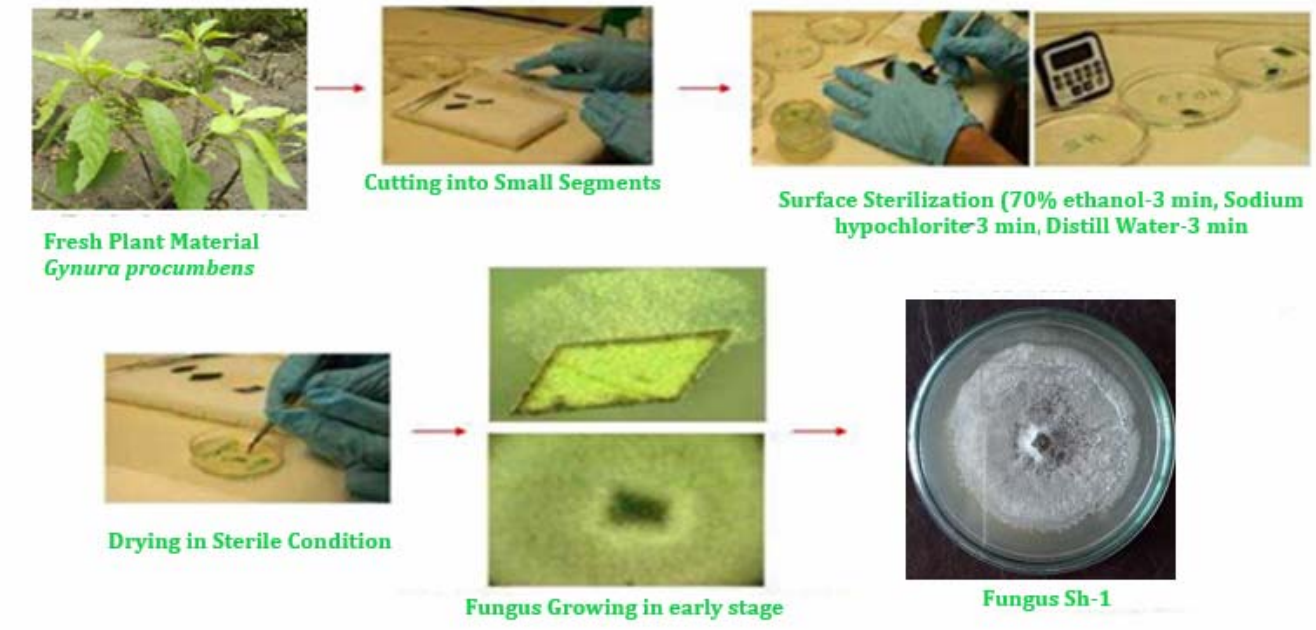


Figure 1: Surface sterilization and inoculation.

Figure 2: Fungi (**Sh-1**) isolated from *Gynura procumbens*.

Biological activity

Antibacterial Activity assay

Sh-1 fungal strain extract was studied for antibacterial activity against six bacteria- *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Bacillus coccus, Bacillus subtilis* and *Clostridium tetani* in agar disc diffusion assay **(**Balouiri *et al.*, 2016**)**. Standard tetracycline was used as positive control. The zones of inhibition produced by compounds and tetracycline were recorded in mm and compared. 7-9 mm, 10-12 mm, 13-15 mm, and above 15 mm zone of inhibition were considered as insignificant, mild, moderate and significant activity, respectively **(**Rob *et al.*, 2005**).**

Antifungal activity assay

Crude fungal extracts of the plant *G. procumbens* were tested for antifungal activity using disc diffusion method. The crude fungal extract of ethyl acetate was tested for antifungal activity against fungi *Pseudomonas aeruginosa*. All the extract was tested at 400 µg/disc concentration.

Chemical studies of the extract

The crude extract (600 mg) was dissolved in *n*-hexane and silica gel was added to it. A column (60 cm) was packed with normal phase G-60 silica gel using *n*-hexane as the equilibrating solvent. The dried extract mixed with silica gel was carefully applied to the top of the column and initial elution was bring out with *n*-hexane. After the application of sample, solvent having increasing polarities from 100% *n*-hexane to 100% ethyl acetate (EtOAc) was added. After running pure EtOAc, mixture of EtOAc and methanol was run. The eluted fractions were collected in test tubes, monitored by TLC and on the basis of R_f values, eleven different fractions (F-1 – F-11) were obtained. Among the fractions, the F-3 portion which was eluted with 20% EtOAc (*n*-hexane/ethyl acetate 8:2) gave single spot in TLC with a R_f value ~0.3 (*n*-hexane/ethyl acetate 8:2) and obtained as pure compound **1** (**S-1**) (10 mg).

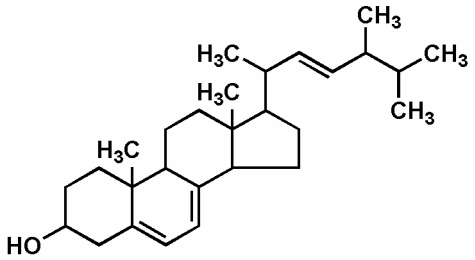
Properties of isolated compounds

Compound 1 (S-1): white needles and crystalline (10 mg), IR (KBr): ν 3428, 2955, 1652, 1458, 1370, 1055, 960, 840 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 0.62 (3H, s, H-18), 0.80 (3H, d, H-26), 0.82 (3H, d, H-27), 0.91 (3H, d, H-28), 0.93 (3H, s, H-19), 1.03 (3H, d, H-21), 3.63 (1H, bm, H-3α), 5.16 (1H, dd, H-22), 5.19 (1H, dd, H-23), 5.37 (1H, m, H-7) and 5.56 (1H, dd, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 38.39 (C-1), 31.98 (C-2), 70.48 (C-3), 40.79 (C-4), 139.79 (C-5), 119.61 (C-6), 116.30 (C-7), 141.37 (C-8), 46.26 (C-9), 37.04 (C-10), 21.12 (C-11), 39.10 (C-12), 42.84 (C-13), 54.57 (C-14), 23.01 (C-15), 28.31 (C-16), 55.74 (C-17), 12.07 (C-18), 16.29 (C-19), 40.45 (C-20), 21.12 (C-21), 135.58 (C-22), 131.99 (C-23), 42.84 (C-24), 33.10 (C-25), 19.97 (C-26), 19.67 (C-27) and 17.63 (C-28).

3. Results and Discussion

Three endophytic fungal were isolated from the bark of the plant *G. procumbens.* One steroid compound (**S-1**) was isolated from one of the endophytes **Sh-1**. In the IR spectra, O-H stretching, C=C stretching, >C=O stretching appeared at 3428 cm^{-1}, 1652 cm^{-1} and 1055cm^{-1} respectively. In the ^1H NMR spectra, compound **S-1** showed signals at 0.62, 0.80, 0.82, 0.91, 0.93, and 1.03 ppm for the presence of six methyl groups. Signal at 3.63 (m) ppm assigned to 3α-H of the steroidal nucleus **(**Greca *et al.*, 1990; Shoeb *et al.*, 2013**)**. Signals at 5.16 (dd) ppm and 5.19 (dd) ppm showed that 22-H and 23-H were connected by a double bond. Spectrum showed two broad signals at 5.37 ppm and 5.56 ppm which is typical for 6-H and 7-H connected to double bond of a steroidal nucleus **(**Greca *et al.*, 1990**)**. The ^{13}C NMR spectrum of the compound **S-1** gave 27 signals due to the presence of 28 carbons. Signal at 42.84 ppm was very intense due to the presence of two overlapping carbons. The signal at 70.48 ppm was characteristic signal of C-3 bearing the –OH group. The signals were found due to presence of quaternary carbons, methine group, methylene carbons, and methyl carbons, respectively. Combining spectroscopic data (^1H and ^{13}C NMR) and comparing with those of known steroids it was found that the **S-1**

matches the data of Ergosterol. Therefore, structure of **S-1** was identified as Ergosterol **(**Kuo *et al.*, 2002**)**



Ergosterol

Table 1: Inhibition zones of the sample against bacteria.

Bacterial strain	Diameter of zone of inhibition (mm)	
	Crude extract (400 µg/disc)	Tetracycline
Bacillus coccus	5	42
Escherichia coli	12	38
Salmonella typhimurium	6	33
Staphylococcus aureus	8	35
Bacillus subtilis	7	40
Clostridium tetani	6	40

Table 2: Inhibition zones of the sample against fungi.

Fungal strain	Diameter of zone of inhibition (mm)	
	Crude extract (400 µg/disc)	Ketoconazole
Pseudomonas aeruginosa	8	33

Ethyl acetate extract of the strain was found to be most active against three of the test bacteria in antibacterial activity assays, exhibiting good activities against *E. coli* (12 mm), *S. aureus* (8 mm), and *B. subtilis* (7 mm). This positive result suggests that all the fungal extracts may contain antibacterial metabolites. Results are shown in **Table 1**. For antifungal activity assay, all the extract was tested at 400 µg/disc concentration and they exhibited mostly good activity against one of the test fungi *P. aeruginosa* (**Table 2)**. Determination of the relationship between the endophyte and host plant is subject to further study.

4. Conclusions

In conclusion, a notable steroid compound Ergosterol was isolated from the cultures of the endophytes bark of *Gynura procumbens.* The fungal extract was found to show moderate growth inhibitory activity against microbes *E. coli* (12 mm). To the best of our knowledge, the study of endophytic fungi from the *Gynura procumbens* is reported here for the first time in Bangladesh.

Acknowledgement

Authors are grateful to Jagannath University for the financial support for the research work and INARS, BCSIR for their research collaboration with lab facility.

References

Akowuah GA, Sadikun A, Mariam A. 2002. Flavonoid Identification and Hypoglycaemic Studies of the Butanol Fraction from *Gynura procumbens*. *Pharm Biol* 40: 405-410.

Bacon CW, White JF. 2000. Microbial Endophytes, *Marcel Dekker*. New York: 3-29.

Balouiri M, Sadiki M, Ibnsouda SK. 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis* 6 (2): 71-79.

Gloer JB. 1997. Application of fungal ecology in the search for new bioactive natural products. *Environmental and Microbial Relationships* (MYCOTA, 2nd Edition): 257-283.

Greca MD, Monaco P, Previtera L. 1990. Stigmasterol from *Typha latipolia*. *J. Nat Prod* 53: 1430-1435.

Iskander MN, Song Y, Coupar IM, Jiratchariyakul W. 2002. Antiinflammatory screening of the medicinal plant *Gynura procumbens*. *Plant Foods Hum Nutr* 57: 233-244.

Kuo YC, Lin LC, Don MJ, Liao HF, Tsai YP, Lee GH, Chou CJ. 2002. Cyclodesipeptide and dioxomorpholine derivatives isolated from the insect-body portion of the fungus Cordyceps cicadae. *J Chin Med* 13: 209-219.

Nawawi A, Nakamura N, Hattori M, Kurokawa M, Shiraki K. 1999. Inhibitory effects of Indonesian medicinal plants on the infection of herpes simplex virus type 1. *Phytother Res* 13: 37-41.

Rob T, Rahman MR, Mosihuzzaman M, Rahman KM, Sultana N. 2005. *J Bangladesh Chem Soc* 18, 64-69.

Rosidah, Mun FY, Amirin S, Mariam A, Gabriel AA, Mohd ZA. 2009. Toxicology evaluation of standardized methanol extract of *Gynura procumbens*. *Ethnopharmacol* 123: 244-249.

Saikkonen K, Wali P, Helander M, Faeth SH. 2004. Evolution of endophyte-plant symbioses. *Trends Plant Sci* 9: 275-280.

Schulz B, Wanke U, Drager S, Aust HJ. 1993. Endophytes from herbaceous plants and shrubs: Effectiveness of surface sterilization methods. *Mycological Res* 97: 1447-1450.

Shoeb M, Nusrat SE, Khondker M. 2013. Chemical investigation of *Coryphataliera* Roxb. *Bangladesh J Bot* 42: 51-53.

Shukla ST, Habbu PV, Kulkarni VH, Jagadish KS, Pandey AR, Sutariya VN. 2014. Endophytic microbes: A novel source for biologically/ pharmacologically active secondary metabolites. *Asian J. Pharmacol. Toxicol* 02 (03): 01-16

Strobel GA. 2002. Rainforest endophytes and bioactive products. *Crit Rev Biotechnol* 22: 315-333.

Strobel GA. 2003. Endophytes as sources of bioactive products. *Microbes Infect* 5: 535-544.

Zhang XF, Tan BK. 2000. Effects of an ethanolic extract of *Gynura procumbens* on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. *Singapore Med J* 41: 9-13.