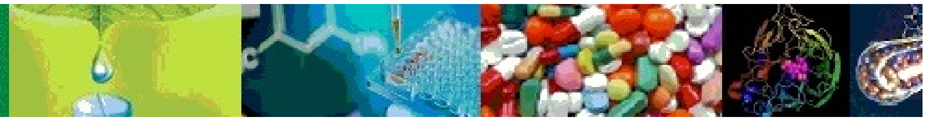
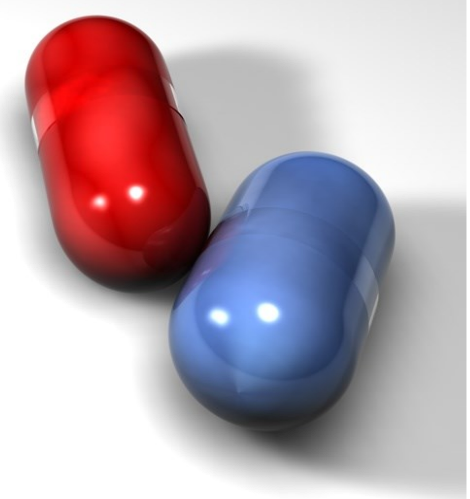
BDPS
Bangladesh Pharmacological Society



Bangladesh Journal of Pharmacology



Volume: 15; Number 4; Year 2020

Cite this article as: Jalil MTM, Ibrahim D. Antimicrobial activity of volatile compounds produced by endophytic fungi associated with *Ocimum sanctum.* Bangladesh J Pharmacol. 2020; 15: 100-12.

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088; DOI: 10.3329/bjp.v15i4.48496

Bangladesh J Pharmacol 2020; 15: 110-112

Letter to the Editor

Antimicrobial activity of volatile compounds produced by endophytic fungi associated with *Ocimum sanctum*

Sir,

Endophytic fungi are known as biosynthesizers inside their host plants (Owen and Hundley, 2004). Many endophytes capable of secreting bioactive compounds that may be used against pathogenic microorganisms (Daoud et al., 2013). The endophytic fungi *Chaetomium cupreum* (isolated from *Mussaenda luteola*; Shylaja et al., 2018), *Cryptosporiopsis ericae* (isolated from *Coptis chinensis;* Zhang et al., 2018), *Phoma* sp. (isolated from *Fucus serratus;* Hussain et al., 2014), *Nigrospora sphaerica* URM-6060 (isolated from *Indigofera suffruticosa;* Santos et al., 2015), *Trichoderma virens* and *Aspergillus terreus* (isolated from Cyperaceae sp.; Ratnaweera et al., 2018).

The essential oil of *Ocimum sanctum* showed significant antimicrobial agent (Sharma et al., (2014). The current study focused on the evaluation of antimicrobial activity of bioactive compounds produced by endophytic fungi isolated from the medicinal plant, *Ocimum sanctum.*

To test the antimicrobial production, plate to plate method was carried out (Stinson et al., 2003). The inoculum suspension of the test microorganism was spread on the agar media respectively. Then, the 14-days-old endophytic isolate cultures were physically attached to the agar plates seeded with test microorganisms. The two plates were sealed using two layers of Parafilm® and kept at 4ºC for 7 days to allow the complete fumigation process of the volatile compounds. Then, the plates were transferred and incubated at 30ºC for 48 to 96 days for test fungi and at 37ºC for 24 hours for bacteria and yeasts. For fungi, the diameter of the test fungal culture was measured as an indication of the growth rate, whereas the colony-forming units were counted for yeast and bacterial cultures. Untreated test microorganisms were used as a control. Besides, the mycelia of the test fungi that showed the positive result were transferred to fresh potato dextrose agar media whereas the single colony of the test bacteria and yeast

Table I

Antimicrobial activity of volatile compounds produced by endophytic fungi against pathogenic microorganisms

Test microbes	%Inhibition			
	IBRL OS-27	IBRL OS-64	IBRL OS-94	IBRL OS-98
Bacteria (Gram positive)				
MRSA ATCC 33591	94.3 ± 1.3	82.5 ± 2.0	87.3 ± 1.2	-
S. aureus	94.2 ± 1.2	84.3 ± 3.1	89.4 ± 0.9	-
S. mutans	88.3 ± 2.7	-	95.2 ± 1.1	-
Bacteria (Gram negative)				
K. pneumoniae ATCC 13883	-	-	-	58.5 ± 2.0
S. typhimurium	-	-	-	80.4 ± 0.8
Y. enterocolitica	-	70.9 ± 3.1	66.3 ± 2.2	-
Yeast				
C. albicans IBRL	26.2 ± 2.1	-	-	-
C. utilis IBRL	-	-	64.4 ± 4.1	44.2 ± 3.5
Fungi				
T. rubrum IBRL SA1	-	-	37.6 ±2.6	-
M. fulvum IBRL SD3	-	-	45.7 ± 3.6	-

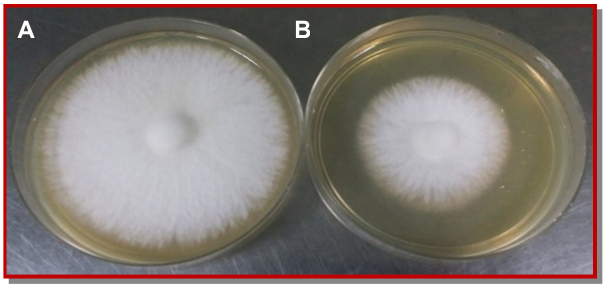


Figure 1: The growth of *Microsporum fulvum* IBRL SD3 for 20 days without (A) or with (B) fumigation to the culture of endophytic fungus, IBRL OS-94

were streaked onto fresh nutrient agar and Sabouraud dextrose agar, respectively to maintain their viability.

Table I shows only four (IBRL OS-27, IBRL OS-64, IBRL OS-94, and IBRL OS-98) out of 148 endophytic fungal isolates (volatile in nature) from *O. sanctum* exhibited significant antimicrobial activity against several test microorganisms. The highest inhibition percentage was observed on Gram positive bacteria for all the endophytic fungal isolate with the inhibition value ranged from 82 to 95%, except for IBRL OS-98. As for Gram negative bacteria, only isolate IBRL OS-98 was able to inhibit both *Klebsiella pneumoniae* ATCC 13883 and *Salmonella typhimurium* with the inhibition of 58.5 and 80.4%, respectively. However, isolate IBRL OS-64 and IBRL OS-94 were only able to inhibit *Yersinia enterocolitica* with the inhibition value of 70.9 and 66.3%, respectively.

For yeast, isolates IBRL OS-94 and IBRL OS-98 were able to inhibit *Candida utilis* IBRL compared to other isolates with a moderate inhibition of 64.4 and 44.2%, respectively. Besides that, the isolate IBRL OS-94 was observed to have the capability to inhibit *Trichophyton rubrum* IBRL SA1 and *Microsporum fulvum* IBRL SD3 with an inhibition value of 37.6 and 45.7%, respectively. The results indicated that most of the fungal isolates exhibited better inhibitory activity towards Gram positive bacteria. It is noteworthy to observe that the Gram negative bacteria, *Salmonella typhimurium* was not susceptible to both of the fungal isolates, IBRL OS-27 and IBRL OS-98. The finding was in contrast to the previous data of disc diffusion assay (data not shown) whereby both isolates showed a good inhibitory effect on Gram negative bacteria. This may be explained by the different constituent of volatile compounds with a different mode of action and most probably the compounds were targeted regardless of the cell wall of bacteria.

Preliminary identification showed IBRL OS-27 might be categorized in the genus of *Trichoderma* spp. This type of species is a plant pathogen such as *T. harzianum* that could be characterized as a green mold that grows aggressively with white mycelium, which rapidly infected the host and might cause a soft decay (Sokovic and Van-Griensven, 2006). Fungal isolate IBRL OS-94 was morphologically characterized and identified as *Muscodor* sp. The isolate showed a vast volatile antimicrobial activity since it can inhibit bacteria, yeast, and fungi. It is noteworthy that the fungal isolate was observed to produce volatile bioactive compounds that able to kill *Microsporum fulvum* IBRL SD3 since it did not survive in the viability test. Figure 1 illustrates a significant growth reduction of *M. fulvum* IBRL SD3 (in diameter) fumigated with the fungal isolate IBRL OS-94 relative to growth control. Based on observation, the mycelial production of the test fungus (Figure 1A) was less dense compared to control (Figure 1B). *Muscodor* spp. is one of a well-known fungus that able to produce volatile antimicrobial compounds. The antimicrobial activity of volatile organic compounds produced by *Muscodor crispans* against *Pythium insidiosum was reported (*Krajaejun et al., 2012) They revealed that volatile organic compounds from *M. crispans* (B23) able to inhibit and thus killed *P. insidiosum in vitro*. Likewise, B23 also was observed to reduce the growth of all *P. insidiosum* isolates tested even in low concentrations and the inhibition rate was dose-dependent. According to Strobel (2006), ketones, lipids, esters, acids, and alcohols are major components of antimicrobial activity of volatile organic compound found in *Muscodor*.

The authors are grateful to Universiti Sains Malaysia for awarding the RUI research grant scheme (AC: 1001/PBIOLOGI/811326) to support this study.

Mohd Taufiq Mat Jalil and Darah Ibrahim

Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800, Minden, Penang, Malaysia.

Corresponding author:

email: taufiqjalil28@gmail.com

References

Daoud H, Sadrati N, Zerroug A, Dahamna S, Bouharati S. Screening of antimicrobial and antioxidant secondary metabolites from endophytic fungi isolated from wheat (*Tricum durum*). J Plant Protect Res. 2013; 53: 128-36.

Hussain H, Kock I, Al-Harrasi A, Al-Rawahi A, Abbas G, Green IR, Shah A, Badshah A, Saleem M, Draeger S, Schulz B, Khrohn K. Antimicrobial chemical constituents from endophytic fungus *Phoma* sp. Asian Pac J Trop Med. 2014; 699-702.

Krajaejun T, Lowhnoo T, Yingyong W, Rujirawat T, Fucharoen S, Strobel GA. In vitro antimicrobial activity of volatile organic compounds from *Muscodor crispans* against the pathogenic oomycete *Pythium insidiosum*. SE Asian J Trop Med Public Health. 2012; 43: 1474-83.

Owen NL, Hundley N. Endophytes: The chemical synthesizers inside plants. Sci Prog. 2004; 87: 79-99.

Ratnaweera PB, Walgama RC, Jayasundera KU, Herath SD, Abira S, Williams DE, Andersen RJ, de Silva ED. Antibacterial activities of endophytic fungi isolated from six Sri Lankan plants of the family Cyperaceae. Bangladesh J Pharmacol. 2018; 13: 264-272.

Santos IP, Silva LCN, Silva MV, Araujo JM, Cavalcanti MS, Lima VLM. Antibacterial activity of endophytic fungi from leaves of *Indigofera suffruticosa* Miller (Fabaceae). Front Microbiol. 2015; 6: 350.

Sharma V, Gupta RC, Singh B, Dhaliwal HS, Srivastava DK. Volatile oil composition and antimicrobial activity of essential oil of two varieties of *Ocimum sanctum* (L.) from Dhameta (Kangra): A North Indian region. Intl J Sci Res. 2014; 5: 142-47.

Shylaja G, Sasikumar K, Satiavelu A. Antimycobacterial potential of resorcinol type lipid isolated from *Chaetomium cupreum*, an endophytic fungus from *Mussaenda luteola*. Bangladesh J Pharmacol. 2018; 13: 114-19.

Sokovic M, Van-Griensven LJLD. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. Eur J Plant Pathol. 2006; 116: 211-24.

Stinson M, Ezra D, Hess WM, Sears J, Strobel G. An endophytic *Gliocladium* sp. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. Plant Sci. 2003; 165: 913-22.

Strobel G. *Muscodor albus* and its biological promise. J Ind Microbiol Biotechnol. 2006; 33: 514-22.

Zhang X, Zhang D, Liu J, Pan H, Qin J, Zhang Y. Antifungal effects of volatile organic compounds from the endophytic fungus *Cryptosporiopsis ericae* Cc-HG-7 isolated from *Coptis chinensis* Franch. Biocontrol Sci Technol. 2018; 28: 496-508.