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Isolation of fungal endophytes from *Monsonia angustifolia* and screening for their antimicrobial and extracellular activities

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INTRODUCTION & AIM

Fungal endophytes are microorganisms which inhabit the internal tissue of any living plants without causing any noticeable harm towards the plant itself (Aamir *et al al*., 2020). These fungal endophytes have been reported to produce secondary metabolites to possess various biological activities including antimicrobial, antioxidant, anti-inflammatory, cytotoxicity and anticancer activities with potential application in pharmaceutical industries (Spina *et al.,* 2023). In addition to the bioactive secondary metabolites, fungal endophytes have also been reported to produce various extracellular enzymes **(**Yadav *et al.*, 2022**)**. The extracellular enzymes produced by the fungal endophytes have been widely applied in various industries including food processing, pharmaceutical and agricultural industries (Shankar *et al.,* 2019).

The medicinal plant *Monsonia angustifolia* commonly known as Tee ya thaba, is dispersed over some provinces in South Africa. The stems and the leaves of *M. angustifolia* are commonly used by different communities to prepare natural remedies for the treatment of eye infections, haemorrhoids, anthrax, and diarrhoea by the local people in South Africa (Fouche *et al.,* 2015). Despite the many benefits possessed by this plant, the diversity of the fungal endophytes associated with this plant as well as the ability of the fungal biological to produce the secondary metabolites endophytes and extracellular enzymes have not been explored.

Consequently, the aim of the present study was to isolate and screen the antimicrobial and extracellular enzymatic activities of fungal endophytes from *M. angustifolia*.

METHOD

Plant collection and fungal endophytes isolation

- The healthy and symptom-free leaves, stems and spikes of *M. angustifolia* were surface sterilised and incubated on potato dextrose agar (PDA) and yeast nitrogen base (YNB) for 7 days at 25 °C.

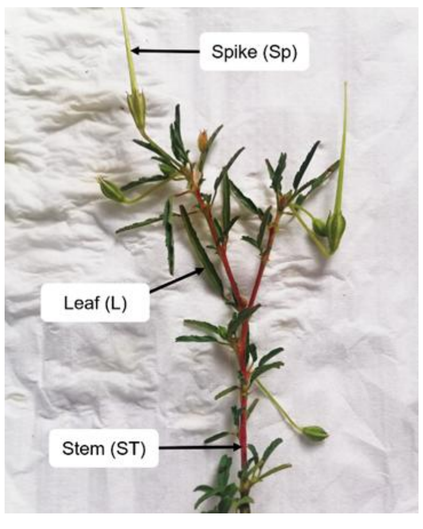

FIGURE 1 The different parts of *M. angustifolia* used for isolation of filamentous fungi and yeasts endophytes.

Fermentation and extraction of secondary metabolites

- The pure cultures of filamentous fungi and yeast endophytes were cultured in Potato Dextrose Broth for 14 days and Malt Extract Broth for 3 days, respectively at 25 °C. Ethyl acetate was then used for the secondary metabolite extractions.

Antimicrobial of the crude extract from endophytes

- The minimum inhibitory concentration (MIC) of the fungal endophytes crude extracts was determined using broth micro-dilution method against *Enterococcus faecalis* ATCC 29212, E*scherichia coli* ATTCC 25922, *Klebsiella pneumoniae* ATTCC 13883, *Pseudomonas aeruginosa* ATTCC 15422*, Staphylococcus aureus* ATTCC 25923 and the yeast *Candida albicans* ATTCC 10231

The extracellular enzymatic activity of the endophytes

- Agar plate method was used to qualitatively assess the ability of the fungal endophytes to produce extracellular enzymes namely, the amylases, cellulases, laccases, lipases, pectinases, proteases and xylanases.

RESULTS & DISCUSSION

Isolation & identification

TABLE 1 The number of fungal and yeast endophytes isolates from various parts of *M. angustifolia.*

Plant part	Isolates	Number of isolates
Leaves	Filamentous fungi	10
	Yeast	0
Stems	Filamentous fungi	10
	Yeast	7
Spikes	Filamentous fungi	1
	Yeast	1
Total		29

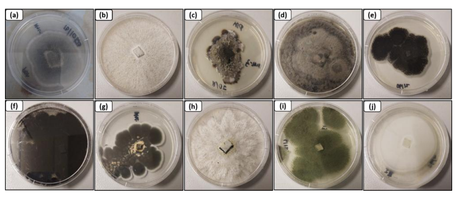

FIGURE 2 Morphological features of the filamentous fungal endophytes isolated from the leaves of *M. angustifolia* on PDA. (a) MaL-1, (b) MaL-2, (c) MaL-3, (d) MaL-4, (e) MaL-8, (f) MaL-9, (g) MaL-11, (h) MaL-12, (i) MaL-13 and (j) MaL-14.

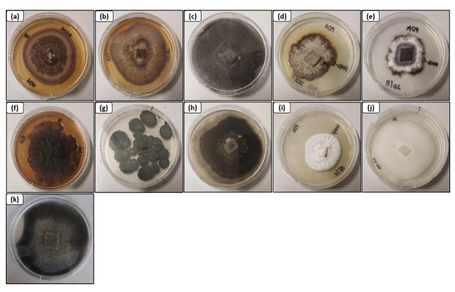

FIGURE 3 Morphological features of the filamentous fungal endophytes from the stems and the spikes of *M. angustifolia* on PDA. (a) MaST-1, (b) MaST-2a, (c) MaST-3, (d) MaST-5, (e) MaST-6, (f) MaST-7, (g) MaST-8, (h) MaST-10, (i) MaST-11, (j) MaST-13 and (k) MaSP-1.

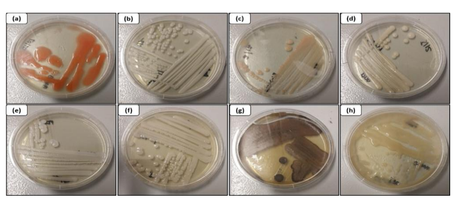

FIGURE 4 Morphological features of yeast endophytes from the stems and spikes of *M. angustifolia* on YM agar. (a) MaST-16, (b) MaST-17, (c) MaST-18, MaST-19, (d) MaST-20, (e) MaST-21, (f) MaST-22 and (g) MaSP-3.

Antimicrobial and extracellular activity of yeast endophytes

TABLE 3 Minimum inhibitory concentrations of ethyl acetate crude extracts from filamentous fungal endophytes

Filamentous fungi	*S. aureus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
A. alstroemeriae MaL-1	-	1.25	-	-	-	1.25
D. ueckerae MaL-2	-	-	-	1.25	-	1.25
D. miriciae MaL-3	-	-	-	1.25	-	1.25
A. alternata MaL-4	-	-	-	-	-	2.50
A. costaricensis MaL-8	2.50	2.50	-	-	-	2.50
A. aculeatus MaL-9	-	1.25	-	-	-	1.25
T. purpureogenus MaL-11	-	2.50	-	2.50	2.50	1.25
D. novem MaL-12	-	1.25	-	1.25	-	1.25
A. aflatoxiformans MaL-13	1.25	2.50	-	1.25	2.50	1.25
G. candidum MaL-14	-	-	-	1.25	-	1.25
E. sorghinum MaST-1	-	1.25	-	1.25	2.50	1.25
E. thailandicum MaST-2a	-	1.25	-	1.25	2.50	2.50
A. alternata MaST-3	-	-	-	-	-	2.50
C. pavettae MaST-5	-	2.50	-	1.25	-	2.50
D. miriciae MaST-6	2.50	1.25	-	2.50	2.50	1.25
C. raphigera MaST-7	2.50	1.25	0.31	0.63	1.25	1.25
P. crustosum MaST-8	-	1.25	-	-	-	2.50
A. alstroemeriae MaST-10	-	2.50	-	2.50	-	2.50
S. terricola MaST-11	-	2.50	-	2.50	-	1.25
G. candidum MaST-13	-	1.25	-	2.50	-	1.25
A. alternata MaSP-1	-	1.25	1.25	2.50	-	1.25
DSMO	2.50	-	2.50	1.25	1.25	2.50
Chloramphenicol	0.04	0.02	0.02	0.31	0.02	
Amphotericin B						0.04

TABLE 2 Enzymatic indices (EIs) of the endophytic filamentous fungi isolated from *M. angustifolia.*

Filamentous fungi	Amylase	Cellulase	Laccase	Lipase	Pectinase	Protease	Xylanase
A. alstroemeriae MaL-1	1.42 ± 0.62	0.27 ± 0.07	0.49 ± 0.45	0.20 ± 0.05	0	1.25 ± 0.27	1.08 ± 0.27
D. ueckerae MaL-2	0	0.22 ± 0.30	1.56 ± 0.26	0.25 ± 0.03	0	0.23 ± 0.13	0.18 ± 0.03
D. miriciae MaL-3	0.68 ± 0.37	0.43 ± 0.06	2.09 ± 0.22	0.71 ± 0.25	0.65 ± 0.80	0.75 ± 0.25	0
A. alternata MaL-4	1.40 ± 0.09	0.27 ± 0.07	0.10 ± 0.08	0.16 ± 0.01	0.36 ± 0.04	0.63 ± 0.05	0.44 ± 0.22
A. costaricensis MaL-8	0	0.07 ± 0.03	0	0	0.26 ± 0.06	0	1.17 ± 0.15
A. aculeatus MaL-9	0	1.23 ± 0.04	0	1.22 ± 0.45	0.84 ± 0.27	0.03 ± 0.05	0.94 ± 0.10
T. purpureogenus MaL-11	2.06 ± 0.49	0	0	0	0.26 ± 0.09	0.12 ± 0.10	2.94 ± 0.72
D. novem MaL-12	0.01 ± 0.03	0.02 ± 0.00	0.65 ± 0.21	0.22 ± 0.04	0.02 ± 0.00	0	0.22 ± 0.04
A. aflatoxiformans MaL-13	0.39 ± 0.09	0.10 ± 0.00	0	1.79 ± 0.26	0.09 ± 0.01	0.06 ± 0.05	0.10 ± 0.01
G. candidum MaL-14	0	0	0	0	0.01 ± 0.02	0	0.22 ± 0.03
E. sorghinum MaST-1	0.36 ± 0.10	0	0	0.21 ± 0.04	0	0.07 ± 0.00	0.09 ± 0.05
E. thailandicum MaST-2a	0.84 ± 0.06	0	0	2.00 ± 0.00	0.37 ± 0.06	0	0.25 ± 0.02
A. alternata MaST-3	0.67 ± 0.17	0	0.60 ± 0.43	0.18 ± 0.03	0	0.54 ± 0.06	0.91 ± 0.23
C. pavettae MaST-5	0.38 ± 0.18	1.60 ± 0.12	0	0	0	0.10 ± 0.09	0.30 ± 0.13
D. miriciae MaST-6	0.04 ± 0.04	0.25 ± 0.04	0.71 ± 0.59	0.31 ± 0.04	0.07 ± 0.02	0.76 ± 0.21	0.07 ± 0.05
C. raphigera MaST-7	1.94 ± 0.60	1.73 ± 0.52	0.47 ± 0.12	1.94 ± 0.38	0.88 ± 0.04	0.11 ± 0.03	0.21 ± 0.05
P. crustosum MaST-8	0	1.18 ± 0.08	0	0.51 ± 0.02	0	0	0.93 ± 0.42
A. alstroemeriae MaST-10	1.34 ± 0.58	0.60 ± 0.47	0.06 ± 0.10	0.51 ± 0.09	0.28 ± 0.10	0.05 ± 0.04	0.21 ± 0.10
S. terricola MaST-11	1.42 ± 0.07	2.81 ± 0.36	0	0.89 ± 0.19	0.40 ± 0.06	0.98 ± 0.17	1.36 ± 0.14
G. candidum MaST-13	0	0.05 ± 0.00	0	0	0	0	0.14 ± 0.07
A. alternata MaSP-1	0.35 ± 0.19	0.53 ± 0.01	0	0.50 ± 0.00	0.22 ± 0.23	0	0.86 ± 0.14
Percentage	71%	76%	43%	76%	67%	67%	95%

Antimicrobial and extracellular activity of filamentous fungi

TABLE 5 Minimum inhibitory concentrations of ethyl acetate crude extracts from yeast endophytes .

Yeast endophytes	*S. aureus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
R. mucilaginosa MaST-16	0.31	0.02	0.31	1.25	0.02	0.63
L. elongisporus MaST-17	0.31	0.04	0.63	1.25	0.02	0.63
R. nothofagi MaST-18	0.31	0.04	0.63	1.25	0.02	0.63
P. terrestris MaST-19	0.63	0.08	0.63	1.25	0.02	0.63
M. guilliermondii MaST-20	0.63	0.08	0.63	1.25	0.02	1.25
P. guilliermondii MaST-21	0.31	0.08	0.63	1.25	0.02	0.63
A. pullulans MaST-22	0.31	0.04	0.63	1.25	0.02	0.63
T. fusiformis MaSP-3	0.63	0.04	0.63	1.25	0.02	0.63
DSMO	2.50	-	2.50	1.25	1.25	2.50
Chloramphenicol	0.04	0.02	0.02	0.16	0.02	
Amphotericin B						0.04

TABLE 3 Enzymatic indices (EIs) of the endophytic yeasts isolated from *M. angustifolia*.

Yeast endophyte	Amylase	Cellulase	Laccase	Lipase	Pectinase	Protease	Xylanase
R. mucilaginosa MaST-16	0.31 ± 0.10	0.57 ± 0.06	0	0	0	0	1.13 ± 0.25
L. elongisporus MaST-17	0.25 ± 0.00	0.50 ± 0.08	0	0.55 ± 0.08	0	0	1.08 ± 0.29
R. nothofagi MaST-18	0.13 ± 0.06	0.57 ± 0.09	0	0.57 ± 0.12	0.20 ± 0.08	0.21 ± 0.12	0
P. terrestris MaST-19	0.13 ± 0.06	0.25 ± 0.00	0	0.37 ± 0.19	0	0.23 ± 0.06	1.01 ± 0.18
M. guilliermondii MaST-20	0.33 ± 0.00	0.33 ± 0.16	0	0.45 ± 0.13	0.25	0	0.64 ± 0.12
P. guilliermondii MaST-21	0.35 ± 0.17	0.50 ± 0.06	0	0.37 ± 0.06	0.27 ± 0.10	0	0.71 ± 0.00
A. pullulans MaST-22	0	0.73 ± 0.00	0.57 ± 0.06	0.74 ± 0.12	1.04 ± 0.16	0	1.38 ± 0.00
T. fusiformis MaSP-3	0	0.33 ± 0.12	0	0.03 ± 0.06	0.23 ± 0.09	0	0.69 ± 0.13
Percentage	75%	100%	13%	88%	63%	25%	88%

CONCLUSION

The plant *M. angustifolia* contains high number of different fungal endophytes with varying degrees of inhibitory activities against Gram-negative bacteria, Gram-positive bacteria and pathogenic yeast. Further these fungal endophytes also have the ability to produce different extracellular enzymes. Therefore, these findings encourages further study the obtained fungal endophytes for the use in food, agriculture and pharmaceutical industries.

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