Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (7) Issue (4) (2022), (253-256) **online: ISSN 2356–976x**
http://bjas.journals.ekb.eg

Bioactive Natural Products from Endophytic Fungi with Anticholinesterase Potential

W.M.Alsallami, M.G.Hassan[2], A.A.Hamed[1], M.O.Abdel-Monem[2] and M.E.El Awady[3]
[1]Microbial Chemistry Department, National Research Centre, El-Buhouth St. 33, Dokki-Cairo 12622, Egypt.
[2]Botany and Microbiology,Dept., Faculty of Science, Benha Univ., Benha, Egypt
[3]Microbial Biotechnology Dept, National Research Centre, El-Buhouth St. 33, Dokki-Cairo 12622, Egypt.
E.mail: Sunflash2014@yahoo.com

Abstract:
According to growing evidence, fungi, are a rich source of natural chemicals with a diverse variety of biological roles. As part of our effort to separate and identify physiologically active chemicals from naturally occurring sources, 45 endophytic fungi were isolated from various sources. Surface sterilization of the obtained samples and culturing of the sterilized samples on two selective media: were used to isolate endophytic fungi. First media was malt agar media (malt extract 15 g, sea salt 24.4 g, agar 15 g, distilled water up to 1 L, pH 5.2). and potato dextrose agar (prepared as per container with the addition of sea salt 24.4 g/L). Filter-sterilized nalidixic acid (50 mg/L) and chloramphenicol (200 mg/L) were added to all media. To obtain the fungal crude extracts, the isolated fungal strains were grown on potato dextrose broth media for 15 days at 28oC. The anticholinesterase activity of the obtained ethyl acetate crude extracts was tested. Seven of the identified fungus strains showed significant anticholinesterase activity. The most potent fungal strain H1 was cultivated at 25°C on potato dextrose agar (PDA), with the key morphological traits assessed using a light microscope.

Key words: Bioactive Natural Products; Endophytic Fungi; Anticholinesterase

1.Introduction.

Endophytic fungus can be found in almost every plant species on the planet. They live in the tissues of the host plant, which they have acquired through symbiotic or pathogenic connections. Many macromolecules involved in encouraging plant development or providing plant protection are produced by these microorganisms, according to reports **[1]. Schulz and Boyle [2]** characterized endophytes as "fungi that invade plants inside without causing visible harm, exist mutualistically, ubiquitously in plants, and produce a variety of chemicals that can inhibit diseases."
Endophytic fungi are a type of symbiotic relationship between fungi and their hosts **[6].** Symbiosis is defined as the coexistence of multiple species in which both hosts and symbionts benefit **[7].** Many endophytic fungi protect their hosts by activating plant defence mechanisms against a variety of diseases. Endophytes are known to produce an antibiotic chemical that suppresses pathogen growth, or they may compete for space and nourishment with pathogens. Barley plants associated with the endophyte *Piriformospora indica*, for example, have shown to be resistant to the vascular disease *Fusarium culmorum* and the leaf pathogen Blumeria graminis **[8].** Endophytes are a diverse group of organisms that constitute a rich supply of bioactive organic products with potential for investment in the pharmaceutical and agricultural industries **(9).**
Acetylcholinesterase inhibitor (AChEI) is a therapeutically relevant enzyme inhibitor that has been utilised in Alzheimer's disease treatment (AD). Alzheimer's disease (AD) is a progressive neurological illness that causes memory loss, language deterioration, reduced visuo-spatial skills, poor judgement, and other symptoms in senior people **[3].**
Continous search for novel cholinesterase (AChE and BuChE) inhibitors from natural sources, such as plants and microorganisms living in atypical biological settings, is ongoing. Endophytes are chemical synthesizers that create a variety of biologically active metabolites with distinct structures, including as alkaloids, benzopyranones, flavonoids, glycosides, phenolic acids, quinones, steroids, terpenoids, tetralones, and xanthones. Endophytes are becoming more popular as natural product sources with unique chemical skeletons and bioactivities **[4].**

2.Materials and Methods.

2.1. Sample collection.

Plant samples were obtained from several locations around the Wadi-Elnatrun Valley. The samples were tagged and brought to the lab in an ice box. Plant parts (stem, root, and leaves) were collected and removed with a sterile knife, then stored in sterile polyethylene bags at 4°C until used for further research. Within 24 hours after collection, all plant samples were processed.

2.2. Isolation of Endophytic Fungi

Isolation of endophytic fungi from plant parts was done according to the method of **Kyeremeh *et al*. [10].**

2.3. Small scale fermentation.

Rice medium was employed to obtain crude extracts of all isolated fungus; 50 g of rice was steeped in 100 mL distilled water, then prepared and autoclaved. Within each flask, 1ml of endophytic fungal cultures

were injected in the media individually. Flasks were then incubated at 30°C in a static environment for 15 days.

2.4. Extraction of bioactive natural product

Extraction was carried out with Ethyl acetate, where the organic phase was collected and kept for drying at 37°C [**11].**

2.5. Determination of Anticholinesterase activity of fungal endophytic crude ethyl acetate extract

The inhibition of acetylcholinesterase and butyrylcholinesterase was measured using a modified Ellman's technique. **[14]**

2.6Identification of most potent fungal strain using phenotypic study

Cultural and morphological parameters such as colony development pattern, conidial morphology, and pigmentation were used to identify the fungal isolates. **[13].**

3.Results

3.1. Plant samples collection

Plant samples were obtained from the Wadi El-Natrun depression's two large lakes (Al-Hamra and Al-Beida) (El-Beheira Governorate). The samples were brought into the lab, coded, and maintained in a cool area (5°C) until fungal strains were isolated. **Table 1 Figure** 1

Table (1) Wadi El-natroun sample data

Plant code	Plant name	location
1P	*Juncus rigidus*	Wadi El-Natrun depression
2P	*Hyoscyamus muticus* L.	(El-Beheira Governorate)
3P	*Fagonia Arabica*	

1P **2P** **3P**

Fig. (1) Wadi-El-natroun plant samples

3.2Isolation of endophytic fungi from plant samples

The plant samples were collected from Wadi-Elnatrun Valley, Egypt. The coplants was identified based on the morphological features as. 20 endophytic fungi have been isolated from the collected plants, coded and recorded. **Table (2)**

Table (2) Isolated fungi from collected plant.

No. of sample	Location	Isolates account	Isolated fungi code
1	*Juncus rigidus*	7	J1, J2, J3, J4, J5, J6, J7
2	*Hyoscyamus muticus* L.	9	H1, H2, H3, H4, H5 .H6. H7, H8, H9
3	*Fagonia arabica*	4	F1, F2,F3,F4
Total isolate			20

3.3Fermentation and extraction

To obtain the bioactive components, the isolated fungal strains were cultured on rice medium for small-scale fermentation. Inoculated fungal spore suspensions were inoculated into 250 mL Erlenmeyer flasks containing 25 g solid rice media and incubated for 15 days. Ethyl acetate was used to extract the cultures **(Table 3).** The ethyl acetate phase was evaporated until it was completely dry and then used for future research.

Table (3) Extraction of crude secondary metabolite from actinomycetes isolated from different samples.

Sample	Isolate code	Weight extract (mg)	Sample	Isolate code	Weight extract (mg)
	J1	5.0	***Hyoscyamus muticus* L**	H4	5.0
	J2	8.0		H5	0.7
Juncus rigidus	**J3**	3.5		H6	2.1
	J4	4.0		H7	0.8
	J5	10.1		H8	1.3
	J6	2.2		H9	0.7
	J7	3.9	***Fagonia arabica***	F1	2.3
***Hyoscyamus muticus* L**	**H1**	4.4		F2	5.2
	H2	2.9		F3	1.6
	H3	3.6		F4	2.8

3.4. Screening of anticholinesterase activity of fungal endophytic crude ethyl acetate extract

The isolated fungi's ethyl acetate extracts were tested for their ability to create AChEIs. Seven isolates (J1, J2, H1, H3, H4, and H6) of the 20 endophytic fungal strains inhibited AChE, with the H1 strain being the most effective, whilst little activity was seen in 13 cultures **(Table 4).**

Table (4) Anticholinesterase activity of fungal endophytic crude ethyl acetate

Extracts from isolate	***anticholinesterase activity***
J1	**+**
J2	**++**
J3	**-ve**
J4	**-ve**
J5	**-ve**
J6	**-ve**
J7	**-ve**
H1	**++++**
H2	**-ve**
H3	**++**
H4	**+**
H5	**+++**
H6	**+++**
H7	**-ve**
H8	**-ve**
H9	**-ve**
F1	**-ve**
F2	**-ve**
F3	**-ve**
F4	**-ve**

3.5. Fungal Phenotypic characteristics

The most potent strain fungus H1 colonies are fast growing, attaining a diameter of 5.0 cm on Malt Extract Agar in 7 days. Morphological characteristic indicating that the most potent fungi is *Cladosporium sp.*

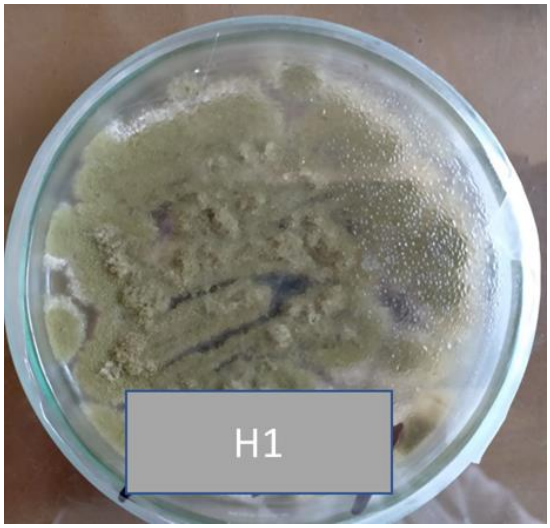

Fig.(2) Colony morphology of fungal isolate H1

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