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Inhibitory activity of *Hericium erinaceus* extracts against some bacterial triggers of multiple sclerosis and selected autoimmune diseases

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INTRODUCTION & AIM

Hericium erinaceus (Bull.) Persoon (HE) is a mushroom that is used to treat a variety of medical conditions (Sokol et al., 2016; Stamets, 1993; Wang et al., 2014) including Alzheimer's disease (AD) (Tsai-Teng et al., 2016), Parkinson's disease (PD) (Pp et al., 2020) and peripheral nerve injury (Wong et al., 2012). However, there is a lack of research into its effects against autoimmune diseases, including multiple sclerosis (MS). This study aims to explore the inhibitory activity of HE extracts against some bacterial triggers of MS and selected autoimmune diseases (Cock & Cheesman, 2019). Thus, this study aims to be the first to our knowledge to explore the anti-microbial properties of HE extracts on bacteria that have been shown to be the triggers for MS development (Cock & Cheesman, 2019).

(a) (b) (c) (d)

Figure 1: *Hericium erinaceus in various mediums; a.* HE mycelium on agar. b. HE mycelium colonising grain. c. Grow bags at various fruiting stages in greenhouse. d. Harvested fruiting body

METHOD

HE was cultivated by sourcing a sample from a reputable source (Little Acre, Brisbane, QLD, Australia), growing onto agar as shown in figure 1 (a), expanding the mycelium in broth, using the broth to inoculate grain (b), which once colonised was used to inoculate grow bags filled with enriched substrate (Master's mix) (c). When the grow bags were fully colonised, they were placed into a humidity-controlled greenhouse for fruiting. Fruiting bodies were collected (d), dehydrated and ground to a powder. The HE fruiting body powder was extracted by maceration with various solvents: methanol, deionised water, ethyl acetate, hexane and chloroform, and these extracts were tested for biological activities. Antimicrobial activity was tested against *Acinetobacter baylyi* and *Pseudomonas aeruginosa*, triggers for the development of MS (Cock & Cheesman, 2019), and *Proteus mirabilis, Klebsiella pneumoniae* and *Streptococcus pyogenes*, triggers for the development of rheumatoid arthritis, ankylosing spondylitis, and rheumatic fever (Cock & Cheesman, 2019) using disc diffusion assays (figure 2), at concentrations of 24.8, 18.5, 4.2, 5.5 and 5.5 mg/mL respectively, using negative and positive controls, water and Chloramphenicol 10mg respectively, and their minimum inhibitory concentrations (MIC) were quantified using liquid micro-dilution assays (figure 3), at concentrations ranging from one quarter to 512th of the stock concentration. The toxicity of the HE extracts was evaluated using the *Artemia nauplii* lethality assay (figure 4), with negative and positive controls, sea water and Potassium dichromate 1mg/ml respectively.

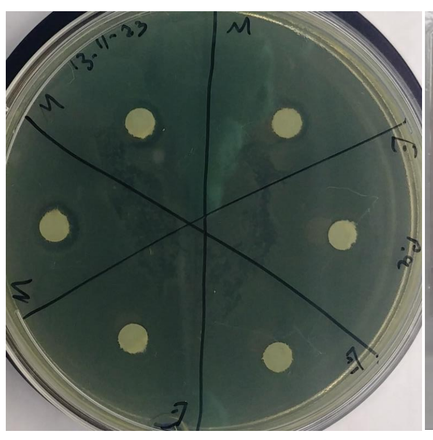

Figure 2: *P. aeruginosa disc diffusion plate showing ZOI for Methanol and Ethyl acetate*

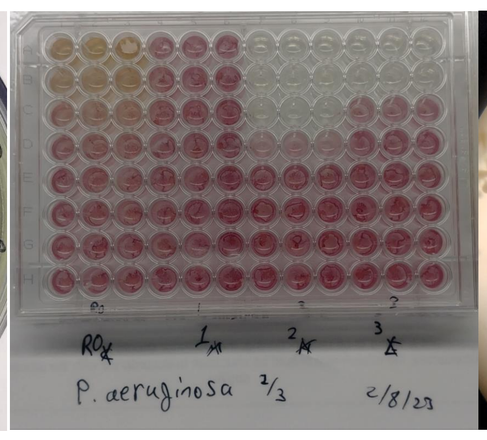

Figure 3: *P. aeruginosa* liquid micro-dilution plate

Figure 4: Newly hatched *Artemia nauplii larvae*

RESULTS & DISCUSSION

In the disc diffusion assay, all of the extracts have shown general antibacterial activity against a few pathogenic bacteria, showing zone of inhibition ranging from 6.5 to 10 mm at stock concentrations, the greatest specificity was shown by methanol extract of HE on *P. mirabilis*, mean zone of inhibition 10 mm, at 24.8 mg/ml (Table 1). In the liquid micro-dilution assay, water and methanol extracts have shown some antibacterial activity on *P. aeruginosa*, at concentrations of 2.3 and 3.1 mg/ml respectively, MIC for negative and positive controls (sea water and Potassium dichromate 1mg/ml respectively) were 0 and 0.01 to 0.63 mg/ml respectively (Table 2). Percent death is 0% for most extracts at stock concentration (Table 3), and at 50% concentration percent death is 0% for all extracts, which is above 1000µg/ml, thus toxicity results support existing data, that HE can be classed as non-toxic. All the experiments were done in triplicates.

Table 1: Disc diffusion assay results

Extract / Bacteria	Water		Methanol		Chloroform		Ethyl acetate		Hexane		positive control	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
A. baylyi	7.83	0.17	7.67	0.33	6.83	0.17	6.50	0	6.67	0.17	19.00	0
P. aeruginosa	7.83	0.33	9.00	0	7.00	0	7.83	0.17	7.67	0.33	10.67	0.33
P. mirabilis	7.00	0	7.83	0.60	8.33	0.33	7.83	0.60	7.67	0.33	16.00	0.58
K. pneumoniae	7.17	0.44	0	0	0	0	0	0	0	0	22.67	0.33
S. pyogenes	7.00	0	0	0	0	0	0	0	0	0	8.67	0.33

Table 2: Liquid micro-dilution assay results

Extract / Bacteria	Water	Methanol	Tetracyclin	Ciprofloxacin	Gentamicin
	MIC mg/ml	MIC mg/ml	MIC mg/ml	MIC mg/ml	MIC mg/ml
A. baylyi	0	0	0	0	2.5
P. aeruginosa	2.30	3.10	0.039	0.156	0.0097
P. mirabilis	4.63	0	0	0.00061	0.039
K. pneumoniae	4.63	0	0.15625	0.15625	0.009766
S. pyogenes	0	0	0	0	0.625

Table 3: Artemia lethality assay results for stock concentration extracts

Extract 100%	% death 1	% death 2	% death 3	Mean	Std error of mean
Water 18.5mg/mL	9.52	16.67	5.56	10.58	3.25
Methanol 24.8mg/mL	100	100	94.44	98.15	1.85
Ethyl acetate 4.2mg/mL	0.00	6.25	0.00	2.08	2.08
Hexane 5.5mg/mL	0	0	0	0	0
Chloroform 5.5mg/mL	0	0	0	0	0
Positive control	100	100	100	100	0
Negative control	0	0	0	0	0

We can infer from the liquid micro-dilution results that there are multiple compounds in HE with anti-bacterial properties against *P. aeruginosa,* as there was an anti-bacterial effect in both the water and methanol extracts.
The inhibitory activities of the HE extracts against some bacterial triggers of MS, and their lack of toxicity, highlights their potential in the prevention and treatment of these diseases. Further studies such as anti-inflammatory assays will confirm the effects of these extracts against other aspects of MS progression and other neurological conditions.

CONCLUSION

The inhibitory activities of the HE extracts against some bacterial triggers of MS, and their lack of toxicity, highlights their potential in the prevention and treatment of these diseases. Further studies such as anti-inflammatory assays will confirm the effects of these extracts against other aspects of MS progression and other neurological conditions.

FUTURE WORK / REFERENCES

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