Cultivation of a wild species of *Ganoderma* and comparative analysis of its bioactive compounds in Microwave Assisted and Hot Water based Extraction procedures.

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AIMS AND OBJECTIVES

- Successful cultivation of a wild species of *Ganoderma* using sawdust as the substrate.
- Comparative analysis of yields of bioactive compounds from Microwave Assisted Extraction and Hot Water Extraction as an effective method.
- Evaluation of the antioxidant activity of the extracts obtained through Microwave Assisted Extraction and Hot Water Extraction.

INTRODUCTION

- *Ganoderma* spp. is a member of the group Basidiomycetes, belonging to the Ganodermataceae family, and is the most significant medicinal mushrooms in the world.
- Currently the commercial valuation of *Ganoderma* spp. fruiting bodies is approximately Rs. 4,000 to 5,000 per Kilogram [1].
- Pharmacological tests have revealed the actions and properties of *Ganoderma* spp., such as immunomodulation, induction of cytokine production, anti-tumor, anti-radiation, anti-allergic anti-inflammatory, anti-oxidant, anti-parasitic, benefiting the cardiovascular, endocrine, respiratory and metabolic systems, etc [2].
- A relatively novel and promising technique called Microwave-Assisted Extraction (MAE) was used to extract bioactive components from *Ganoderma* sp. and its yield was compared with Hot Water Extraction process [3,4].
- MAE is more efficient due to shorter extraction time, less solvent consumption, improved extraction yield [5].

MATERIALS AND METHODS

- A piece of tissue from wild type mushroom *Ganoderma casuarinicola* of was isolated and transferred to sterilized Potato Dextrose Agar (PDA) media; after sufficient mycelial growth, it was transferred to Potato Dextrose Broth (PDB).
- Substrate was prepared and packed in polypropylene (PPE) bags; liquid media from PDB was inoculated to each PPE bag.
- After complete mycelia run in dark (packets turned white), PPE bags opened and then kept in light with frequent watering.
- After few days various stages of fruiting body development were noted.
- Cultivated fruiting bodies were grinded to fine powder.
- Hot water extract: 2gm of powdered sample was heated with 20 ml of boiled distilled water for 5,10,20mins; MAE: 2gm of powdered sample taken in 20ml of distilled water and kept in microwave for 2,4,6 mins. Both filtered by Whattman paper.
- Phenol, flavonoid, β-carotene and lycopene tests were done with high, medium and low microwave voltages; since best results were observed in high voltage, further tests were performed with high voltage settings.
- Phenol, carbohydrate, and protein test were done with both MAE and HWE extracts with different time variations. Spectrophotometric analysis was performed, and the OD values at specific nanometre were noted down.
- Total antioxidant capacity, DPPH, and ABTS tests were done to examine the antioxidant potentials of the extracts.
- Comparative analysis were done for the results of the tests performed with MAE and HWE extracts.

RESULTS

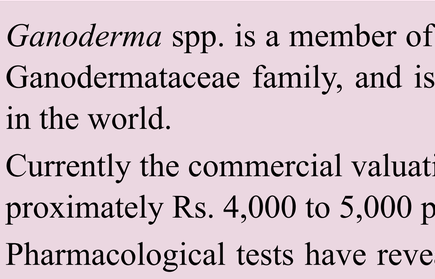

Figure 2: Comparison between concentration of BSA [Blue denotes MAE 2,4,6 minutes; Yellow denotes HWE 5,10,20 minutes]

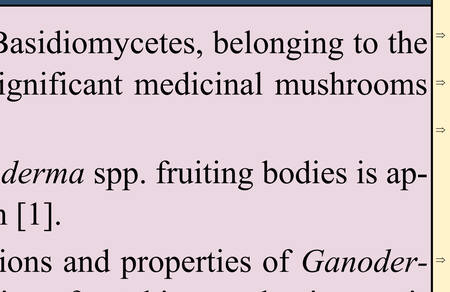

Figure 3: Comparison between concentration of Gallic Acid [Blue denotes MAE 2,4,6 minutes; Yellow denotes HWE 5,10,20 minutes]

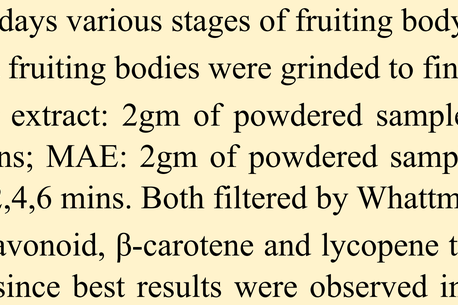

Figure 4: Comparison between concentration of Glucose [Blue denotes MAE 2,4,6 minutes; Yellow denotes HWE 5,10,20 minutes]

- **Successful cultivation of *Ganoderma casuarinicola* was done.**
- **Higher concentration of Carbohydrate and Phenol was observed in case of MAE extracts compared to Hot Water extracts; except in Protein, where higher concentration of BSA was observed in HWE than in MAE.**
- **Highest concentration was observed in 2mins MAE extracts and in 10 mins HWE in case of phenol, carbohydrate and proteins.**
- **Antioxidant results: In Total Antioxidant Capacity (TAC) highest concentration of ascorbic acid was observed in 2mins MAE again and 10 mins HWE again.**
- **DPPH and ABTS results: Comparing EC50 values of all 6 samples, showed lowest concentration of obtaining EC50 in 2mins MAE and 10 mins HWE.**

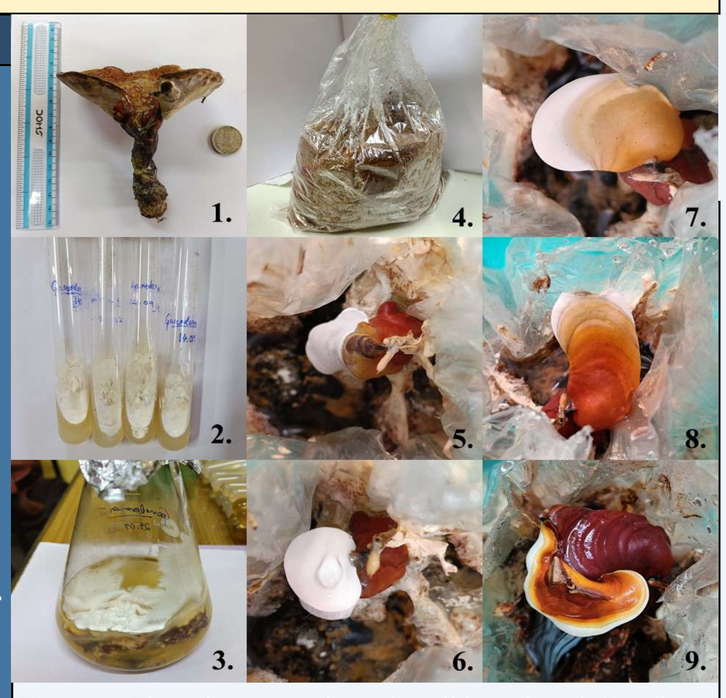

Figure 1: The culture procedure of a wild species of *Ganoderma* mushroom along with its different growth stages [1– Collected specimen, 2– Pure Culture on PDA, 3– Mycelial Culture on PDB, 4– Sterilised substrate, 5-9– Growth stages of Ganoderma].

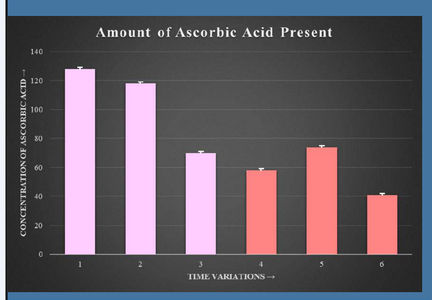

Figure 5: Comparison between concentration of Ascorbic Acid [Pink denotes MAE 2,4,6 minutes; Red denotes HWE 5,10,20 minutes]

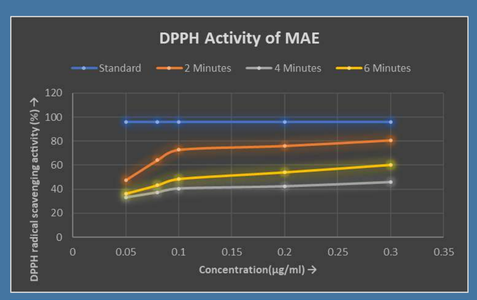

Figure 6: DPPH radical scavenging activity of MAE 2,4,6 minutes against concentration gradient.

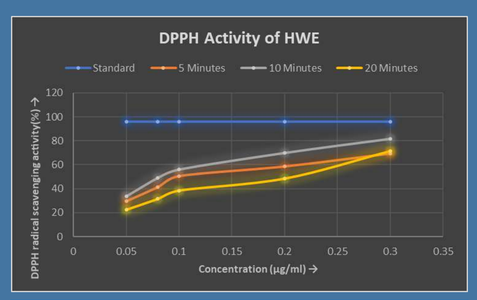

Figure 7: DPPH radical scavenging activity of HWE 5,10,20 minutes against concentration gradient.

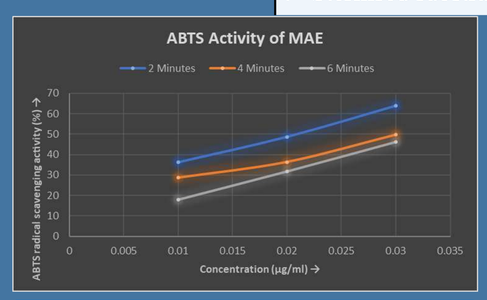

Figure 8: ABTS radical scavenging activity of MAE 2,4,6 minutes against concentration gradient.

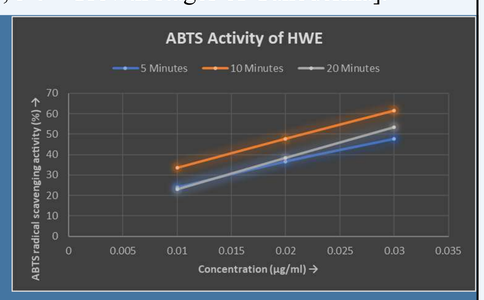

Figure 9: ABTS radical scavenging activity of HWE 5,10,20 minutes against concentration gradient.

CONCLUSION

- Artificial cultivation of *Ganoderma* sp. was successful but further studies should be done to generate more fruiting bodies in less amount of time.
- Among the 2 mins, 4 mins, 6 mins MAE extracts in high voltage settings, better results were obtained in 2 mins thus concluding that increasing the heat decreases the concentration of bioactive components.
- Since better results were obtained in case of MAE compared to HWE, so MAE can act as a potential extraction method for future extraction processes.
- The cultivation of Ganoderma sp. has been very successful in the recent times, and efforts are being put in to make it even more successful.
- The average cost of 1kg of Ganoderma sp. is sold for an astounding price of approximately 5000 INR, and thus the ones growing it can make a large amount of profit.
- These cultivated mushrooms are further used by large pharmaceutical companies to make various kinds of infusions, medicines, poultices, etc., and the selling point of these products earn them a lot of money.
- The consumers on the other hand, who consume these products are also benefited as these are very healthy, and medicinally important.
- Further studies must be conducted on anticancer, antitumor and other medicinally crucial activities so that this mushroom can live up to its name ‘Mushroom of Immortality’.

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