

Mushroom as new repository for small bioactive molecules for anticancer efficacy

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ABSTRACT

Approximately 270 species of mushrooms have been reported as potentially useful for human health. However, few mushrooms have been studied so far in treating various diseases. Like other natural regimens, the mushroom treatment appears safe, as could be expected from their long culinary and medicinal use. At present, cancer is a leading cause of death worldwide. Nearly 10 million deaths were recorded globally from different cancers in 2020. We have screened the anti-proliferative and apoptotic potentials of more than thirty species of mushroom for their anticancer activity. The result reflects that a wild and edible mushroom, *Astraeus hygrometricus*, selectively induces robust apoptosis in MOLT-4 cells by targeting the G0/G1 stage of the cell cycle. A unique triterpene, astrakurkurone, isolated from this mushroom induces robust apoptosis by disrupting the mitochondrial membrane potential, augmenting reactive oxygen species (ROS) and RNS production. Induction of apoptosis is through activation of the intrinsic mitochondrial pathway by upregulating BIM, a pro-apoptotic protein, and down-regulating XIAP, a survival protein. It also downregulates the phosphorylated AKT, which down-regulates phosphorylated FOXO and phosphorylated GSK3 β in the treated cells leading to apoptosis. Therefore, it can be envisaged that mushroom-derived bioactive molecules will hold great promise in cancer treatment in the future.

Keywords: Mushroom, Cancer, Apoptosis, Cell cycle, Reactive oxygen species.

Indian Journal of Physiology and Allied Sciences (2023);

DOI: 10.55184/ijpas.v75i02.164

ISSN: 0367-8350 (Print)

INTRODUCTION

In the 21st century, cancer is identified as a primary cause of death globally and imposes a significant barrier to increasing the life expectancy of the human population worldwide.¹ According to the American Cancer Society projection, the United States may witness 1.9 million new cancer cases in 2022 and a fresh cancer death of 0.6.² Nearly 10 million deaths were recorded globally from different cancers in 2020. In the sphere of major death-causing factors, cancer is placed in the first or second position in 91 out of 172 countries.¹

Currently, cancer treatment is mainly confined to the use of various chemotherapeutic drugs along with a combination of various radiotherapy methods.³ An ideal chemotherapeutic drug should be target specific and act towards the cancer cells irrespective of a normal healthy cell. However, most of the available chemotherapeutic drugs are not accurately cancer-cell-oriented and manifest various side effects, ultimately hindering post-treatment patient recovery.⁴ So, there is an urgency to develop a new safe anticancer drug, preferably from natural sources with selective specificity, to counter the negative consequence of the existing drug.

Aspiring to find out useful natural anticancer drugs it was found that the mushrooms harbor a wide variety of secondary metabolites, various phenol and flavonoid molecule, glycosides, polysaccharides, tocopherols, alkaloids, carotenoids, volatile oils, organic acids and have the potentiality to serve as an alternative reservoir for naturally occurring antioxidants, anti-inflammatory, cardiovascular, anti-microbial, immunomodulation, anticancer, and anti-diabetic drug.⁵⁻⁸

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How to cite this article: Paul S. Mushroom as new repository for small bioactive molecules for anticancer efficacy. *Indian J Physiol Allied Sci.* 2023;75(2):76-79.

Conflict of interest: None

Submitted: 23/02/2023 **Accepted:** 20/03/2023 **Published:** 25/06/2023

Mushrooms have a promising ethnobotanical history of natural medicine and a successful record of combating cancer. Many mushrooms, such as *Agaricus*, *Antrodia*, *Cordyceps*, *Inocybe*, *Pleurotus*, *Russula*, and *Schizophyllum*, have a history of successful anticancer agents.⁹ But, till today, only about ten percent of mushroom species are introduced to scientific communities, and barely one percent of them are screened for therapeutic purposes. So, comparing its colossal diversity, this reliable alternative anticancer harbor was found to have been neglected for a long time.

Various mushrooms collected from different regions of West Bengal were screened against multiple cancer cell lines.

West Bengal is situated in the Eastern region of India, lies at 21°38' and 27°10' of North latitude and between 85°30' and 89°53' East longitude. The great Himalayas mountain stretches to the northern part of the state, whereas the Bay of Bengal borders it from the southern region. It is the only state in India with a cost line and the Himalayas. Due to the presence of the hill, the northern portion of the state experience a humid subtropical climate; in contrast,

the southern part encounters a tropical Savannah type of climate. The dual influence of the mountains and the sea and the impact of riverine topography and geography made the climate of West Bengal suitable for creating vast biodiversity.

Aspiring to find out the potential anticancer drug more than 30 mushroom species collected from different locations of West Bengal were screened against various cancer cell lines, viz. leukemic cell lines (MOLT-4, Reh, and NALM-6), breast cancer cell line (MCF-7), lung carcinomic cell line (A549), hepatocellular carcinomic cell line (Hep G2), and to determine its selectivity one normal cell line (BEAS-2B) and PBMC (Peripheral Blood Mononuclear Cells) isolated from a healthy donor was taken in consideration.

Mushroom Collection

Wild edible mushrooms *Amanita bannigiana*, *A. vaginata*, *Astraeus hygrometricus*, *A. asiaticus*, *Rusulla albonigra*, *R. cyanoxantha*, *R. delica*, *R. nigricans*, *R. lepida*, *R. sanguinea*, *R. emetica*, *Termitomyces heimii*, *T. microsperma*, *T. eurhizus* and non-edible

mushroom *Pisolithus arhizus*, *Scleroderma cepa* were collected from different locations of the laterite region of West Bengal. Edible mushrooms *Calocybe india*, *Macrocybe crassa*, *M. gigantea*, *Lentinus squarrosulus*, *Volvaria volvacea*, and non-edible mushrooms, such as *Daldenia concentrica*, *Phallus indusiatus*, *Schizophyllum commune* were collected from Gangatic and Vindhyan floodplain region. From the Northern Hilly region and Terai-Teesta floodplain region, six edible mushrooms viz., *Armillaria mellea*, *Auricularia auricula-judae*, *Coprinus comatus*, *Fistulina hepatica*, *Grifola frondosam*, and *Cordyceps sinensis* were collected for study (Figure 1).

Astraeus hygrometricus emerged as the most effective mushroom

Our screening result revealed that mushrooms collected from the laterite region showed better efficacy than the other four regions. Out of 30 mushrooms, seven mushrooms showed differential selective efficacy against various cancer cell lines. *T. heimii* and *P. arhizus* showed promising efficacy against Hep G2 and MCF-7 cells, respectively.¹⁰ In the case of A549 cells, *P. indusiatus* and *A. hygrometricus* showed promising efficacy. However, three mushrooms, *A. hygrometricus*, *L. squarrosulus*, and *M. crassa* processed a prominent selective effectivity against various leukemic cell lines.^{3,6} Accumulating all data our study revealed that *A. hygrometricus* emerged as the most potent mushroom and showed selective effectiveness against different cancerous cell lines irrespective of normal cells. MOLT-4 was found to be the most sensitive leukemic cell line. Mushroom-induced selective apoptosis in MOLT-4 cells, whereas no significant change was observed in normal BEAS-2B cells. So, from this extensive and comprehensive screening data, it can be concluded that the edible mushroom *A. hygrometricus* has the immense potential to become a new resource of the future novel anti-leukemic

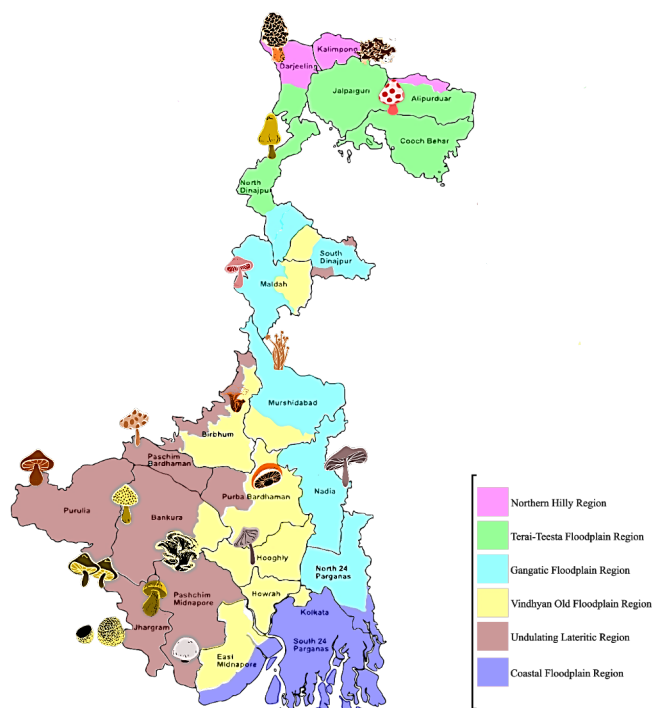


Figure 1: Different soil types and possible occurrence of mushrooms in West Bengal

drug for combating the incurable disease Leukaemia. The apoptogenic potentiality and selective anti-leukemic efficacy of the crude extract of *A. hygrometricus* intrigued us to isolate the novel lead molecule behind this astounding ability.

Acute lymphoblastic Leukemia (ALL)

Acute lymphoblastic leukemia (ALL) is a disorder that arises from malignant lymphoid progenitor cells of either T or B cell origin. T-ALL is a type of leukemia categorized as a biologically heterogeneous disease concerning phenotype, gene expression profile, and activation of aberrant cell signal transduction pathways. T-ALL is identified as the most common childhood malignancy, although its prevalence is also found in adults at a considerable rate.^{11,12} In the sphere of childhood leukemia, steady progress has been achieved toward effective treatment with 65–75% cure rates. But, on the other side, in the case of adult lymphoblastic leukemia, poor prognosis, the expense of the therapy, complexity, and toxic side still impose baffling challenges. The pathogenetic events leading to ALL include both sporadic (e.g., genetic lesions or mutations) to congenital (e.g., exposure to agents like pesticides, power frequency magnetic fields, harmful additions) factors that altered various key 'pro-oncogenes,' which under normal developmental conditions functions as an indispensable participant in most signatory pathways of development, activation, and differentiation that lead blood cells to maturity.^{11,13,14}

Primary Chemotherapeutic agents in leukemia

The significant players of induction therapy against leukemia are vincristine, corticosteroids, anthracyclines, etc. For early intensification, the most frequently used drug include L-asparaginase drugs, such as cytarabine, methotrexate, and cyclophosphamide; those have limitations due to adverse outcomes. So, developing a safe alternative anti-leukemic drug from natural sources is urgent.

Astrakurkurone, a miraculous triterpene, isolated from this mushroom.

Aiming to separate the lead molecule behind this anti-leukemic efficacy, we first profile the total metabolites present in the crude extract of *A. hygrometricus*. The gas-chromatography profile and subsequent mass spectrometric analysis initially detected more than a hundred primary metabolites cumulatively present in methanolic and ethyl acetate extract. Further, subsequent fractionization and rapid chromatography finally isolate astrakurkurone, a triterpene, from this wild edible mushroom.

Astrakurkurone, trigger mitochondria-dependent apoptosis in MOLT-4 cells

A unique triterpene, astrakurkurone, processed a significant selective efficacy against various cancer cell lines, but it showed a noticeable impact in the leukemic cells, particularly the MOLT-4 cell. It induced robust apoptosis in MOLT-4 cells by targeting the G0/G1 stage of the cell cycle, but no noticeable changes were observed in PBMC isolated from normal donors and BEAS-2B cells. Further, disruption of the mitochondrial membrane potential, ROS, and RNS augmentation were noticed in both time and concentration-dependently, responding to astrakurkurone treatment. Astrakurkurone manifested a mitochondria-dependent intrinsic apoptotic pathway by downregulating various cell survival mediators such as XIAP and upregulation the pro-apoptotic protein. Further, our investigation noticed that phosphorylated AKT was significantly downregulated in response to astrakurkurone, which ultimately de-phosphorylated downstream mediators of AKT-pathway, such as FOXO and GSK3 β , which are essential for cellular survival in MOLT-4 cells and eventually leading to robust apoptosis. Our investigation confirmed that astrakurkurone further cleaved the Bcl-2 proteins, the controller of apoptosis, in a time-dependent manner to induce cellular death. So, it can be inferred that the novel triterpene, astrakurkurone isolated from wild edible mushroom *A. hygrometricus* produces anti-leukemic efficacy in MOLT-4 cells through the mitochondria-dependent apoptotic pathway by building an elevated amount of ROS.

CONCLUSION

The entire study comprehensively established that among thirty different mushrooms collected from different locations of West Bengal tested against seven human cancer cell lines, *A. hygrometricus* exhibits a significant growth-inhibitory

effect on MOLT-4 cells with tolerable collateral damage to BEAS-2B and PBMC isolated from a healthy donor. MOLT4 represents an aggressive, robust, and resistant form of T cell Acute Lymphoblastic Leukemia, where PI3K-AKT was frequently observed to be deregulated, thus leading to the pathogenesis of this form of leukemia.

Our findings establish a primary mechanism for astrakurkurone, novel terpenoids isolated from *A. hygrometricus*. Astrakurkurone induces robust apoptosis in MOLT-4 cells through the involvement of intrinsic apoptosis by enhancing cellular ROS and RNS and through the mitochondria-dependent pathway. Astrakurkurone induces apoptosis by downregulating some crucial PI3K/AKT pathway proteins. This brings us to conclude that the mushroom-derived natural compound astrakurkurone is highly useful for combating incurable leukemia and holds great promise for drug development. Therefore, it can be envisaged that mushroom-derived bioactive molecules will have great promise in cancer treatment.

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