An insight in to isolation of natural products derived from macrofungi as antineoplastic agents: A Review

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Abstract

Macrofungi have been valued as medicinal provisions for humankind and are a rich source of natural anticancer compounds. However, the isolation of such compounds from macrofungi is challenging. This review highlights the importance and challenges that meet during an isolation of anticancer compounds from macrofungi. Moreover, it exhibits the impact of potential anticancer compounds and antioxidants derived from different kinds of mushrooms in decreasing the risk of cancer. It also displays the capacity to develop more effective anticancer drugs using natural antineoplastic agents of macrofungal origin.

Keywords: Macrofungi, Anticancer activity, Anticancer compounds, Antioxidants, Cancer, Antineoplastic drugs

Introduction

Epigeous or hypogeous fungi containing multicellular sporocarps that are visible to the naked eye are commonly referred to as macrofungi.[1,2] Majority of the species of macrofungi belong to phylum Basidiomycetes and some species to phylum Ascomycetes. This includes the Agaricales in the broad sense (mushrooms and relatives), Aphyllophorales (polypores, tooth fungi, coral fungi, etc.,), gasteromycetes (puffballs, etc.), and some groups of Ascomycetes, primarily discomycetes (cup fungi) Xylariaceae, and the genus Cordyceps.[3,4] However, the word mushroom conveys different senses to different human fraternities living in different regions of the world. In some western countries, mushrooms are referred only to the edible or button mushrooms, whereas all other cultivated forms are referred to as specialty, exotic or alternative mushrooms. On the contrary, microbiologists who work on mushroom biology of United States of America, indicate that the macrofungi with distinctive fruiting structures are directly known as mushrooms.[5] Chang and Miles described the mushrooms as macrofungi which can produce easily distinguishable and idiosyncratic fruiting bodies in its own distinctive way, growing above or underground.[6] According to the definition of Das, mushrooms can be used as a general term for the fruiting body of macrofungi

(Ascomycota and Basidiomycota) and embody a reproductive juncture of mushroom's life cycle.[7]

Mushrooms can be classified in to four categories; edible mushrooms, medicinal mushrooms, poisonous mushrooms or toadstools and other mushrooms. The fleshy and edible mushrooms fall in to the edible mushroom category (eg: Agaricus bisporus) and mushrooms which possess medicinal properties belong to the medicinal mushrooms such as Ganoderma lucidum. Thirdly, mushrooms that are being poisonous fall in to poisonous mushrooms (eg: Amanita phalloides) and a miscellaneous category that tentatively grouped as other mushrooms.[3,8] In the current study, almost all macrofungi species investigated have distinctive fruiting structures and current study focuses broadly on medicinal mushrooms. Mushrooms are commonly encountered in phylum basidiomycetes. They generate a large variety of reproductive structures identified as sporocarps or fruiting bodies. Interestingly, they have vast range of colors, sizes and shapes. Generally, they use fascinating methods for dispersion of their millions of spores. [9]

Among the estimated number of macrofungi species on earth (~140,000), only 14,000 (10 %) have been identified yet. About 3000 species from over 30 genera of these known species are known as prime edible mushrooms. So far, around 100 species were grown experimentally. Among them, about 30 mushrooms have been cultivated on a commercial basis and 60 species were cultivated on economic base concepts. However, only 15 species were shaped on an industrial scale.[10,11] The proportion of beneficial mushrooms among the undiscovered mushrooms have been estimated to be only 5%, implying 7000 yet unexplored beneficial mushrooms species will be hidden on earth.[10,12] As less proportion of macro fungi have been well investigated among the known species, it is very important to carry out further investigation of these undiscovered species due to its enormous benefits to mankind. The traditional use of macro fungi in generating bioactive metabolites has long been established and the experience in ethno medicinal use of macro fungi suggests the greater potential of mushrooms for successful bioprospecting.[11,12]

Medicinal Properties of Macrofungi

Macrofungi are an unlimited and largely untapped source of biologically active agents which has a high potential to be used therapeutically as medicinal provisions. Scientific explanation on the functions of mushroom derived metabolites in human body is increasingly being established. [13] Generally, macrofungi are growing in dark and highly competitive environments. They defend themselves from hordes of microbes attacks by generating natural protective substances. Hence, it is not astonishing that mushrooms are a rich source of important biologically active compounds. [14]

Medicinal mushrooms are used as a chief resource containing therapeutic substances in remedy of various kinds of human diseases. From ancient times, mushrooms have been used in traditional medicine. Mushrooms comprise wealthy nutritional value with elevated protein content, vitamins, minerals, fibers, trace elements with less calories and low cholesterol.[15,16] Mushrooms are known to contain bioactive substances such as antibacterial, antifungal, antiinflammatory, anticancer, antiviral, antiparasitic, antioxidant, antiproliferative, cytotoxic, antidiabetic, anti-HIV, hypocholesterolemic, anticoagulant and hepatoprotective compounds.[15-17] Some ordinary bioactive compounds isolated from these macrofungi encompass glycolipids, flavonoids, aromatic phenols, compounds derived from shikimic acid, polyketides, fatty acid derivatives, polyacetylamine, sesterterpenes and nucleosides.[18] Among the known species of mushrooms, approximately 2,000 species have been considered as safe for human

consumption.[19] The potent beneficial effects of mushrooms on human health are implemented either directly as antioxidants or via hindrance of alterations underlying key pathological states such as cancer, cardiovascular diseases, neurodegenerative diseases, diabetes, hypercholesterolemia and other degenerative diseases.[20] Currently, some of them are used as nutraceuticals which are natural food supplements having a potential value in maintaining good health and boosting immune system. Generally, they are consumed as medicines in the form of capsules or tablets.[21,22]

Isolation and identification of secondary metabolites from macrofungi

A variety of secondary metabolites are produced by macro fungi in response to external stimuli including nutritional or climatic alterations. Generally, they accumulate in some parts of the fungal body and exhibit highly varied structural differences.[23] Hence, the isolation and separation methods of bioactive compounds can be lengthened and tiresome. As a way out for this troublesome, the isolation procedures of natural products are combined with various separation techniques which is based on the solubility, volatility and stability of the preferred compound. The initial step in the procedure of isolating secondary metabolites from macrofungi is to extract those from the cellular environment using organic solvents. Majority of the biomasses produced by macrofungi are naturally available in the forms of inert, insoluble and as polymeric material.[24] Therefore, the biologically active secondary metabolites should be released and solubilized in the matrix, resulting initial crude organic extract. The choice of solvent or solvents for the extraction provides the primary basis for the sample preparation. Highly lipophilic components are extracted by using low-polarity solvents (eg. hexane, chloroform), whereas high-polarity solvents such as alcohols yield a spectrum of non-polar and polar compounds from the matrix.[24]

Secondly, desired components will separate from the crude extract. This is performed using liquid-liquid partition or by a number of low-resolution chromatography methods such as size-exclusion and normal phase column chromatography. The final purification steps will be facilitated by concentrating the components of interest. Generally, isolation of active compounds from extracts is carried out via bioassay guided fractionations where fractions obtained after each chromatographic or solvent–solvent fractionation is subjected to the relevant bio assays to locate the active fractions which were used for the next fractionation steps.[25] The third stage of the procedure generally engages a high-resolution method to separate the preferred compounds among the other components in the extract as some undesired compounds of the mixture may enclose some closeness to the isolated compounds. The optimization of the separation method becomes vital to accomplish adequate resolution in the final preparative isolation. Commonly, the final step is performed using high-pressure liquid chromatography (HPLC), droplet counter-current chromatography (DCCC), counter-current chromatography (CCC), centrifugal partition chromatography (CPC).[25]

One and two-dimensional NMR experiments, proton NMR (1H-NMR), 13C-NMR, Distortionless Enhancement Polarization Transfer 13C NMR (DEPT13C-NMR), H-H Correlation Spectroscopy (COSY), Heteronuclear Multiple Quantum Correlation (HMQC), Heteronuclear Multiple Bond Correlation (HMBC) and Nuclear Overhauser Effect Spectroscopy (NOESY) are used to determine chemical structures of the targeted compounds.[26] Mass spectrometric methods such as High Resolution Electrospray Ionization Mass Spectrometry (HREIMS) are used in obtaining the high resolution mass spectrum of the compound. Nuclear magnetic resonance spectroscopy (NMR spectroscopy) is being used as the outstanding research technique for elucidation of the structures of isolated organic compounds by using magnetic properties of certain atomic nuclei. Distinctively, NMR spectroscopy provides detailed information regarding the

chemical structure, molecular dynamics, reaction state of the molecules and chemical environment of targeted molecules.[27]

Prevalence of cancer and causes

A large category of neoplastic diseases are collectively known as cancer and it has become one of the most debilitating diseases in the world. Currently its prevalence is only second to the myocardial infarction.[28] Cancer is essentially considered as a genetic disease of cells since both sporadic (non-hereditary) and hereditary forms of cancer are characterized by the accumulation of genetic mutations resulting in unscheduled and unregulated proliferation of cells.[29] Proteins encoded by tumorous genes are often involved in apoptosis, regulation of cell proliferation and differentiation. Apoptosis plays a key role in activation of numerous biological events including maintaining tissue homeostasis, deleting excess or damaged cells to prevent tumor induction.[30] Generally, there are two types of tumor suppressor genes which safeguard the cell from tumor induction, namely gate keepers and care takers. Gate keepers are tumor suppressor genes involved in carrying out cell cycle arrest or induce apoptosis and caretakers are involved in DNA repair machinery. When tumor suppressor genes fail to maintain genomic stability, numerous mutations will be accumulated. As a consequence, the chances of obtaining mutated forms of proto-oncogenes will increase. Thereby, unnecessary and incorrect signals will be transferred to the cells that command to carryout uncontrolled growth and division while evading apoptosis (programmed cell death). This is marked as the onset of tumerogenesis.[31]

Although the root of cancer is many and diverse, the genotypic alterations of cancer cells are commonly demonstrated as six hallmark features in cell composition. They can be described as self-sufficiency in growth stimulatory signals, unresponsiveness to growth inhibitory signals, stimulation of angiogenesis, evading apoptosis, high replicative potential and activation of invasion and metastasis. These events collectively contribute to the development and progression of malignant tumors. In the quest for understanding cancer biology and cancer genetics, unraveling of the involvement of apoptosis in tumourigenesis has become one of the milestones. [32] Genes and proteins governing apoptosis (specifically Bcl-2 family members and caspases) are being targeted in the development of novel anti-cancer agents. Accordingly, the Bcl-2 family of proteins, consisting of pro and anti apoptotic members, were found to reciprocally regulate the release of apoptogenic factors such as cytochrome c which is found in mitochondrial intermembrane space. Consequently, downstream caspases are activated causing morphological changes in apoptotic cells.[32] The framework of apoptotic signal transduction involves three main pathways; (1) intrinsic (2) extrinsic and (3) granzyme B signaling pathways. These pathways are activated separately and ultimately converge into a common, conserved mechanism mediated by a family of cystein proteases known as caspases.[33]

The cell membrane death receptors such as fibroblast antigen signaling receptors (Fas), death receptor (DR4) and tumor necrosis factor receptor (TNFR) facilitate the extrinsic pathway of apoptosis.[34] The intrinsic apoptotic pathway is implemented in an intrinsic fashion in response to high levels of cellular stress conditions including cellular damage, cytokine deprivation, and exposure to cytotoxic drugs. These intracellular stimuli can stimulate the activation of different members of Bcl-2 family leading to release of apoptogenic factors through mitochondria. The Granzyme B pathway is executed via interconnection between both death receptor-mediated and mitochondrial pathway.[35]

Given the importance of Bcl-2 family in apoptosis, the role of different members in making the decision between life and death of the cells, was studied extensively.

Intriguingly, it has been found that the subtle interplay between pro and anti-apoptotic members of this family dictates the fate of cells, as heterodimerization leads to hindrance of their respective functions.[36] Specifically, the ratio between pro-apoptotic and anti-apoptotic proteins are found in equilibrium in normal cells, and deregulation of the balance between these proteins implicated in cancer. Despite the remarkable advances in understanding molecular biology and molecular genetics of cancer, number of deaths caused by cancer becomes gradually increased worldwide. Cancer is considered as one of the world's leading causes of death, with an estimation of 15 million people being diagnosed by 2020.[37]

Antioxidant potential of macrofungi

As human body continously exposes to a variety of stress conditions, it generates free radicals and other reactive oxygen species (ROS) via diverse physiological and biochemical processes. Free radicals such as hydroxyl (OH●), superoxide (O_2●-), hydroperoxyl (OOH●), alkoxy (RO●) and peroxy (ROO●) radicals can act as reactive oxygen species. The presence of free radicals in minute quantities is essential in the regulation of signal transduction and gene expression. However, excess amount of free radicals is directly harmful to living cells causing oxidative damage to biological molecules including DNA, proteins and lipids. [38,39] Mainly, ˙OH and ˙O_2- radicals involve in the oxidative damage, induced in biological systems. Non-free radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and other reactive nitrogen species (RNS) including nitric oxide (NO●), nitrogen dioxide (NO_2), and peroxynitrite (ONOO●) also produce in the human body during cell metabolism causing toxicity to living cells.[40,41]

The human body is mainly defensed from radical mediated toxicity by the action of natural antioxidants in the body.[42] Conversely, the endogenous mechanisms engaged in the free radical scavenging in cells occasionally become unstable and insufficient to counteract the free radicals produced excessively. Therefore, it results in overproduction of free radicals leading to be a vital cause for lethal conditions such as cancer and other degenerative diseases linked to ageing.[43,44] Antioxidants derived from mushrooms can act as a key defender for radical intervened toxicity produced in human cells. Mushrooms and plants comprise of a spectrum of radical scavenging metabolites including polyphenolic substances (phenolic acids and flavonoids), terpenoids (carotenoids) and vitamins.[45,46] Among the antioxidant compounds generated by macrofungi, phenols and flavonoid substances are predominant due to larger capacity for scavenging free radicals. The chemical structure of polyphenols consisting of an aromatic ring with hydroxyl substituents is mainly correlated with free radicals scavenging.[47,48] Epidemiological analyses have proven that many of these antioxidant substances have strong anticancer potential.[49] Therefore, the intake of antioxidants is in some way connected with reduced threat of cancer and other dreadful conditions related to ageing.[50]

Human cancer cell lines

HeLa cells were the first human cells which were successfully cloned. HeLa cells were obtained from an African-American woman Henrietta Lacks (1920-1951) who was the unwitting source of first human cancerous cell line.[51] HeLa cells could be used for conducting many experiments and signified an enormous boon to medical research.[51] Even though, HeLa is the most commonly used cell line, currently, different cell lines are being used in cancer research other than HeLa cell line. Cell line MCF-7 derived from breast cancer cells of a 69 year old woman, VERO originated from kidney epithelial cells of a African green monkey, Hep-G2 derived from human liver cancer cells,

JURKAT originated from T lymphocyte cells of 14 year old boy with T cell leukemia, HEK-293 derived from human embryonic kidney cells, HEp-2 originated from human laryngeal carcinoma cells, RD cells derived from 7 year old child with rhabdomyosarcoma and CC-1 cells originated from rat liver epithelial cells are some important cancer cell lines that are frequently used in cytotoxicity studies. [52]

Anticancer activity exerted by anticancer compounds isolated from macrofungi

The medicinal use of macrofungi has been renowned in Japan, China, Russia and Korea as well as in the western world countries including United States and Canada. [53] Currently, fungal preparations and compounds derived from macrofungi are used in Chinese folk medicine in healing cancer and they act as adjuvants to chemotherapy, radiotherapy and surgery [53,54]. So far, around 650 species of macrofungi have been recognized to possess antitumor activity 55. The anticancer activity of the mushroom was first exhibited by Lucas et al., who analyzed the extracts of sporocarps of Boletus edulis for sarcoma 180 cell line in mice. [56,57] Currently, antitumor application of macrofungi preparations and substances unquestionably make it a fast-track research area worth mass awareness.

Despite the presence of novel antineoplastic agents, cancer yet remains as the second primary root of death distressing millions of people annually.[58] The current cancer treatments such as radiotherapy, surgery, chemotherapy and hormonal therapy have ended up with a modest progress in falling the mortality caused by cancer with distasteful side effects.[59] Thus, there is a fresh surge in the interest of natural products including mushrooms due to their strong anticancer activity. Substances derived from natural sources have recently been paid much attention, in the hope of discovering more effective anti cancer agents with less toxicity.[60] Nowadays, many cancer patients rely on complementary and alternative medicine (CAM) with the hope of finding a remedy for cancer or simply to boost their quality of lives. CAM is an integration of complementary treatments that can use alongside with the conventional treatments (radiotherapy, chemotherapy and surgery) or as an alternative healing method for standard medical therapies. Intriguingly, Ayurvedic medicine derived from natural products has been massively contributed to the development of modern treatment for cancer.[61] Thus, integration of ancient understanding on cancer therapy with evolving knowledge of molecular and genetic basis of cancer leads to development of more effective treatment methods.

Natural metabolites isolated from macrofungi have been successfully used in the discovery and advancement of valuable drug leads for cancer.[62] Calvacin was the most frequently used natural product derived from a mushroom, which display strong antitumor activity. It has been isolated from the Calvatia gigantean which belongs to giant puffballs. Its activity was found against various experimental tumors, including Sarcoma 180, mammary adenocarcinoma 755, leukemia L-1210, and HeLa cell lines.[63] Currently, the medicinal value of mushrooms is being studied worldwide for their competency to attack cancer. Therefore, discovering novel antitumor substances from mushrooms has become a matter of great significance.[64]

Lentinan, originated from Lentinula edodes, krestin from Trametes versicolor, schizophyllan derived from Schizophyllum commune, the Maitake D-fraction (β-glucan) from Grifola frondosa, are permitted in Japan as prescription drugs in remedy of cancer. [65] Ganoderma lucidum is also an essential medicinal macrofungi used today, commended as “mushroom of immortality”. Polysaccharide (GLPS) fractions that are derived from G. lucidum have been recognized to own potent cytotoxic effect.[66] Furthermore, it has been reported that this mushroom consumption slows down the improvement of cancer

later in life implying an inverse relationship between mushroom intake and the threat of developing breast or gastric cancer.[67]

This antitumor activity of mushrooms has attributed partially to the high molecular weight polysaccharides which are carbohydrate polymers (500–2000 kDa) occur as components of the fungal cell wall.[68,69] Apart from this, glycoproteins (polysaccharide-protein complexes) have also exhibited promising anticancer properties by directly influencing the immune system via stimulation of macrophages, natural killer cells and T lymphocytes.[70,71] In recent times, a polysaccharide isolated from Cordyceps jiangxiensis has displayed a direct anticancer effect in vitro against SGC-7901 cell line (human gastric carcinoma cell line).[68]

So importantly, polyphenolic substances isolated from mushrooms have been recognized to possess strong anticancer properties. For instance, hispolon and hispolon derivatives isolated from the fungus Phellinus igniarius have been reported to have apoptosis effect on human epidermoid KB cells.[74] Hispolon is a yellow pigment belongs to flavonoid group which was first isolated from Inonotus hispidus in 1996.[75] Hispolon can obstruct chemiluminescence response of human mononuclear cells and inhibit mitogen-induced proliferation of spleen lymphocytes of mice.[75]

Other fungal high molecular weight compounds, such as flammulin, lectins and velutin have been reported to have direct activity against tumor cells in vitro.[76] However, a number of low molecular weight fungal metabolites also exhibit anticancer activity. For example, a tricyclic sesquiterpene isolated from Omphalotus olearius namely illudin, has been used as the primary structure for the cancer drug irofulven. Currently, it is being used as a semisynthetic cancer drug in preclinical and clinical trials owing to its effects on cancer linked kinases enzymes and activity of apoptotic cells.[80,81,82] Additionally, cordycepin, derived from some Cordyceps species, has demonstrated an potent anticancer effect on diverse cancer cell lines.[83,84] The experimental tumors that are apoptosed by anticancer compounds isolated from different kind of mushrooms are mentioned in (Table 1).

Discussion and Conclusion

This review provides a fruitful analysis on anticancer properties of secondary metabolites isolated from medicinal macrofungi and mycological research centered on cancer therapy. Intriguingly, it reveals scientific evidences for using natural compounds derived from medicinal mushrooms as antineoplastic agents. The preference of various separation steps to isolate secondary metabolites from mushroom is of greater importance as it directly affects the duration of isolation process. The analytical-scale optimization of the separation parameters makes the procedure shorter and convenient. Moreover, this review highlights the capacity to develop more effective anticancer drugs using natural antineoplastic agents derived from macrofungi.

Conflict of Interest

The authors declare that they have no conflict of interest that competes with any of the contents of the manuscript.

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Table 1. The experimental tumors that are apoptosed by anticancer compounds isolated from different kind of mushrooms.

Name of the natural product	Scientific name of the medicinal mushroom/	Name of experimental tumors	References

Calvacin	*Calvatia gigantean*	Mammary adenocarcinoma 755, Sarcoma 180, leukemia L-1210, HeLa cell lines	[63]
Lentinan	*Lentinula edodes*	Leukemia cells U-937, breast cancer cells MDA-MB-231	[68]
Schizophyllan	*Schizophyllum commune*	Leukemia cells U-937, breast cancer cells MDA-MB-231	[68]
Krestin	*Trametes versicolor*	T24 human urinary bladder transitional carcinoma cell	[69]
Polysaccharide (GLPS) fractions	*Ganoderma lucidum*	Leukemia L1210, Lewis lung carcinoma	[66], [70]
Fungal polysaccharide	*Cordyceps jiangxiensis*	Human gastric carcinoma cell line SGC-7901	[68]
Inoscavin A	*Fulviformes fastuosus*	RD sarcoma cells	[72]
Ergone	*Phellinus repandus*	RD sarcoma cells, HepG-2 cells	[73]
Hispolon and derivatives	*Inonotus hispidus, Phellinus igniarius*	Human mononuclear cells, human epidermoid KB cells	[74], [75]
Lectins	*Pleurotus Ostreatus*	Antihepatoma and antisarcoma cells	[77]
Cordycepin	*Cordyceps* sp	Human breast cancer MDA-MB-231 cells, human OEC-M1 oral cancer cells	[78], [79]