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Dr. Rashmi A Joshi
Scientist, Patanjali Herbal Research Department, Patanjali Research Institute, Uttarakhand, India

Correspondence
Dr. Rashmi A Joshi
Scientist, Patanjali Herbal Research Department, Patanjali Research Institute, Uttarakhand, India

Antrodia camphorata with potential anti-cancerous activities: A review

Dr. Rashmi A Joshi

Abstract
Antrodia camphorata has long been used in traditional medicines of Taiwan for protection of diverse health related conditions as this fungal species possess over more than 78 active compounds which are responsible for its medicinal properties. These compounds are mainly terpenoids, polysaccharides, benzoquinone derivatives, succinic and maleic derivatives. The purpose of this review is to summarize available information about the anticancer activities of the crude extract and the different main bioactive compounds of *A. camphorata.*
The clinical trials of its crude extract or pure compounds on humans are either limited or not performed, but still based on the research activities *A. camphorata* can be considered as an alternative synergizer or phytotherapeutic agent in the treatment of cancer and reflects that the present situation promise to prepare some medicines from it so that the mankind can be benefited.

Keywords: Anticancer activities, cytotoxicity, polysaccharides, terpenoids

1. Introduction
Cancer is one of the most deadly disease in the 21st century and globally remains one of the leading cause of morbidity and mortality. After cardiovascular disease, it is the second noncommunicable diseases causing death [1-4] and is responsible for one in eight deaths worldwide, more than AIDS, tuberculosis, and malaria together [5] and accounting for about 8 million deaths worldwide [6]. Overall cancer incidence and mortality are higher in North America, Australia, New Zealand and Western Europe as compared to the rest of the world . In the United States, one in four deaths is attributed to cancer. It is estimated that by 2030 there will be 26 million new cancer cases and 17 million cancer deaths per year [7-9]. Cancer is a dreadful disease characterized by the irregular proliferation of the cells. It may be uncontrollable and incurable, and may occur at any time at any age in any part of the body. It is caused by a complex, poorly understood interplay of genetic and environmental factors. It begins with mutations in DNA, which instructs the cells how to grow and divide. Normal cells have the ability to repair most of the mutations in their DNA, but the mutation which is not repaired and causing the cells to grow becomes cancerous. From a scientist's standpoint, the environmental factors includes smoking, use of tobacco, unhealthy diet, doing not enough physical activity, infectious diseases as well as chemicals and radiation in our homes and workplace along with trace levels of pollutants in food, drinking water and in air, however the degree of risk from pollutants depends on the concentration, intensity and exposure [10]. As a cell progresses from normal to cancerous, the biological imperative to survive and perpetuate drives fundamental changes in cells behavior. So the actual cause of the disease in different sections is still to be explored clearly. Cancer is thus, a class of diseases classified by the type of cell that is initially affected. The most prevalent types of cancer include that of lung, liver, colon, cervical, prostate and breast cancer. Despite of all the considerable efforts it remains to be an aggressive killer. The treatment for cancer is highly costly and even chemotherapy and radiation therapy have not been so successful in controlling the cancer. The use of synthetic chemotherapeutic agents in use clinically have not succeeded in fulfilling the expectations. There is a constant demand to develop new, effective and affordable anticancer drugs. From the Vedic times till today plants have gained importance to fulfill this demand of providing a source of alternative medicine. Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products and the plant kingdom has been the most significant source. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives.

Currently, a few plant products are being used to treat cancer and globally, the incidence of plant derived products for cancer treatment is from 10% to 40% with this rate reaching 50% in Asiatic patients. In Europe alone expenditure for anticancer herbal products is estimated to be 5 billion dollars per year. Approximately five decades of systemic drug discovery and development have resulted in the establishment of a large collection of useful chemotherapeutic agents. To combat cancer United States National Cancer Institute has undergone 2069 anticancer clinical trials, in which over 150 drug combinations have been successfully recorded against cancer. Advances in the clinical researches for anticancer agents have been increased over the years and as a result numbers of drugs have been introduced [11-13].

Any member of the plant kingdom can be used as anticancer herb, but this word anticancer is with a broad brush and is a combined form of three actions which make a herb as anticancerous agent. The anticancerous activities includes the evaluation of cytotoxicity which refers to the action of herb or any agent against tumors in vitro (i.e., in laboratory cell cultures). Antitumor is for the action toxic to tumors in animals and anticancer is action against tumors in human trials. Besides this some important members of plant kingdom are responsible for increasing the immunity of the human system and relieves from the harmful effect of chemotherapy and radiations used during cancer treatment. These are called immunomodulators and thus play a significant role in treating the cancer patients. A myriad of many plant products exist that have shown very promising anticancer properties *in vitro*, but have yet to be evaluated in humans. Natural therapies, such as the use of plant derived products in cancer treatment, may reduce adverse side effects. Since cancer cells lose many of the regulatory functions present in normal cells, they continue to divide when normal cells do not. This feature makes cancer cells susceptible to chemotherapeutic drugs. Imperative organic compounds present in plants could exaggerate to diminish the toxicity caused due to chemotherapy. Task of modulating the adverse effect is feasible only through requisite perspective regarding the specificity of these molecules with combination therapy [11, 14].

According to Linnaeus the fungi belong the plant kingdom as they existed on minerals and no one knows how long the fungi have inhabited the earth. A mushroom is defined as a macro fungus with a distinctive fruiting body which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand [15, 16]. Mushrooms have been part of the normal human diet for thousands of years and, in recent times, the amounts consumed have risen greatly, involving a large number of species. Mushrooms are considered, all over the world, valuable health foods since they are poor in calories, fat, and essential fatty acids, and rich in proteins, vitamin and minerals. There are both edible and poisonous mushrooms commonly edible ones are *Antrodia camphorata*, *Agaricus bisporus*, *Lentinus edodus*, *Volvariella volvacea*, *Volvaria* sp, *Amanita vaginata*, *A. fulva*, *Bolus edulis* and many more [15]. The fruiting body and mycelium of different mushrooms contain different secondary metabolites such as terpenoids, steroids, polyphenol, polyketides, polyglucan, flavonoids, alkaloids, polysaccharides, and dietary fibers which exert several pharmacological activities [6, 16, 17].

Mushrooms have been used for centuries in Asian countries owing to their beneficial effects on health and longevity. Some species of mushrooms are effectively known to possess health promoting properties, e.g., antioxidant, antimicrobial, anticancer, cholesterol-lowering effects, and immunostimulatory effects and hence are called as medicinal mushrooms. Previous studies have reported that a single medicinal mushroom may produce both stimulatory and inhibitory effects on immune cells, depending on conditions, but the factors responsible for this apparent dichotomy remain obscure. Mushroom extracts have been extensively studied for their medicinal effects. Recently the mushrooms have been explored in cancer treatment as these extracts can produce direct cytotoxic effect on cancer cells. All over the world research is being conducted to evaluate the medicinal properties of the different isolates present in the different species of mushrooms. The preliminary research has shown that some isolates from the mushrooms posses properties against cardiovascular disease, cancer, viral, bacterial and parasitic infections. They also possess anti-inflammatory and anti-diabetic properties. Countries like Japan, Korea and China are widely using the extracts of mushrooms as potential adjuvants to radiation treatments and chemotherapy [18, 19].

This review focus on the *Antrodia camphorata*, a very common mushroom in Taiwan and China which is considered as medicinal mushroom as it possess various chemical compounds that have, in recent years, have shown promise as anticancer agents and their potential mechanism of action will be outlined soon.

2. Antrodia

The *Antrodia* clade was established by Hibbett and Donoghue in 2001 within the Polyporoid clade and included several brown-rot polypores (viz. *Antrodia, Daedalea*, *Piptoporus*, *Fomitopsis*, *Postia* including *Oligoporu*s, *Laetiporus*, *Auriporia, Neolentiporus* and *Phaeolus*. The sole exception among brown-rot taxa was *Grifola*, a white-rot polypore. There are about 50 species in this genus, roughly 29 species are known from Europe, 21 species in North America, and 18 species in east Asia, although more new species have been reported. Most species grow on the wood of coniferous trees, except for *A. albida*, which grows on the dead wood of deciduous trees [16]. This genus was classically defined for annual to perennial, resupinate to pileate polypores with a dimitic hyphal system, cylindrical to ellipsoid basidiospores and producing brown rot. The detailed phylogenetic study of *Antrodia* sp. showed that the genus was not monophyletic but separated in three groups. One was represented by *Antrodia vaillantii* and *A. gossypium* (group A), a second by *A. serialis*, *A. sinuosa, A. heteromorpha* and *A. malicola* (group B), and a third by *A. xantha* and *A. carbonica* (group C). Yu *et al.* in 2010 confirmed the polyphyly of *Antrodia* and the validity of *Taiwanofungus*, a polypore genus including endemic species that originally were described or included in *Antrodia* sp [20, 21]. *Antrodia* species are becoming increasingly important due to the potential pharmaceutical value of their biologically active ingredients.

Antrodia camphorata is also called as *Taiwanofungus camphoratus* or *Antrodia cinnamomea* as it grows slowly in the rotting inner trunk cavities of the rare, indigenous, endangered camphor tree *Cinnamomum kanehirai*, and causes brown heart rot. The common name of this species is ‘niu zhang zhi’ or’zhang-yi’,stout camphor fungus and also called as the fungus of fortune [16, 17]. *Cinnamomum kanehirai* is commonly called as Bull camphor tree and is a broad-leafed evergreen tree that grows at altitudes of 450-2000 meters in low-elevation mountainous terrain in the counties of Taoyuan, Mioli, Nantou, Kaohsiung and Taitung. In the wild the fruiting bodies grow slowly and are hardly noticeable until the

host tree falls down. In the order to harvest this species more easily, some people illegally fall the host trees and this illegal felling had severely threatened *Cinnamomum kanehirai* and the trees are now currently protected by the Taiwan government. This peculiar combination of slow growth and host-specific requirements makes this mushroom one of the highest priced food items in the world with prices up to US $5,000 per kilogram. The annual market is worth over $100 million (US) in Taiwan alone [6, 23, 28, 29].

Recently, many studies have indicated that its medicinal applications go far beyond the original usage. Therefore, demand for the fruiting bodies of *A. camphorata* has far exceeded the supply. The wild type material to be collected is very rare and costly so to replace the wild material, commercial cultivation of *A. camphorata* has been developed by using a variety of techniques and as a result artificial cultivation is developed as a substitute. At present *A. camphorata* is available in three ways, gathering in the wild fruiting bodies, wood or solid state cultivation, and submerged cultivations i.e., cultivating fruiting body, pure mycelium (grown by liquid fermentation), or mycelial biomass (mycelium and residual substrate). Levels of triterpenes are highest in the fruiting body products, which are also the most expensive, and lowest in the mycelial biomass products, with liquid fermentation mycelial products offering a cost effective intermediate option [25]. The preparation of media and culture conditions are important factors responsible for the yield and bioactivity of *A. camphorata*. Thus these artificial cultivation are able to solve the market demand.

In Taiwan *A. camphorata* is commercially available in the form of fermented wine or pure cultures in powdered, tablet and capsule form [26]. The metabolism of this fungus needs to be extensively examined so that more metabolites possessing bioactive compounds can be extracted out and more *in vivo* tests and randomized controlled clinical trials are carried out for medicinal use of this mushroom.

3. Taxonomic description

Antrodia camphorata was first identified and published by Zang and Su in 1990 as a new *Ganoderma* species, *Ganoderma camphorate*. According to morphology of its fruiting body and cultural characteristics, this fungus has been proposed the name of *A. camphorata*. In 2004, a phylogenetic analysis based on sequence data derived from large ribosomal subunit sequences of ribosomal RNA genes indicated that *A. camphorata* is distantly related to other species in *Antrodia* and, consequently, the fungus was transferred to the new genus *Taiwanofungus*. By using polymorphism analysis of internal transcribed spacer regions of the ribosomal RNA gene, *A. camphorata* was reconsidered as an *Antrodia* species and now the current taxonomic position of *A. camphorata* is as follows

Kingdom	:	Fungi
Phylum	:	Basidiomycota
Class	:	Homobasidiomycetes
Order	:	Aphyllophorales
Family	:	Polyporaceae
Genus	:	*Antrodia*
Species	:	*camphorata*

But still the nomenclature and exact taxonomy of its genus and species of *A. camphorata* is still the subject of debate and needs further research [27].

4. Medicinal Properties

Traditionally the medicinal properties of *Antrodia camphorata* have been well demonstrated particularly in Taiwan and the economic value of it mainly due to its medicinal use as it is one of the most valued medicinal fungus in Taiwan. The native Taiwanese used this species in the form of soup by cooking up it up and had been passed down for generation. The extracts have been used to ameliorate the effects of alcohol intoxication as well as well-known for its miraculous effect on a number of health conditions, especially those pertaining to the liver, abdominal pains, hepatitis B, cancerous growths hypertension, and hangover. It has been used in ancient indigenous traditions as an enhancer of body metabolism, strength, and longevity and as an ameliorant of fatigue, for the treatment of twisted tendons and muscle damage, terrified mental state, influenza, cold, headache, fever and many internally affiliated diseases [22]. *Antrodia camphorata* has also been used as a natural therapeutic ingredient in Traditional Chinese Medicine (TCM) for its antioxidative, anti-tumor, anti-cancer, anti-hepatitis, vasorelaxation, anti-inflammatory, cytotoxic, and neuroprotective activities. The mushroom is often used as an antidote to treat intoxication caused by food, alcohol, or drug poisoning by promoting the recovery of liver cells and enhancing the body's metabolism and effective in naturally stimulating the immune system. As a result from the realm of traditional medicine the scientific interest in *A. camphorata* and its curative properties originated and this source of ethnic medicine became an interesting mushroom for scientific research, which usually confirms the legitimacy of their usage. Studies have also shown that *Antrodia camphorata* has antioxidant properties and can reduce oxidative damage caused by free radicals that arise naturally due to the process of aging or the onset of diseases. This species has recently attracted pharmaceutical attention for its antitumor properties [24,28].

5. Mycological characteristics

The trophophase of *A. camphorata* occurs from June to October and the fruiting bodies assume different plate-like, bell-like, hoof-like or tower-like shapes. They are flat on the surface of wood at the beginning of growth and then the brim of the front edge rises to roll into plate-shaped or stalactites. The top surfaces are lustrous, brown to dark brown in color, with unobvious wrinkles, flat and blunt edges. The bottom sides are orange red or partially yellow with ostioles all over. The hyphae possess generative hyphae 2–3.5 μm with clamp connections, and hyaline to light brown skeletal hyphae up to 4.5 μm wide with weakly amyloid. Basidia, 12–14 × 3.0–5.0 μm, is clavate and 4-sterigmate with a basal clamp. Basidiospores, 3.5–5.0 × 1.5–2 μm, are cylindrical, hyaline, smooth and sometimes slightly bent [27, 29].

6. Flavour

The compounds responsible for aroma in mushrooms are different in the species and they do not play any essential role in nutrition but they stimulate the appetite and give mushroom dishes a characteristic flavor. Around 150 aromatic compounds are identified in different mushroom species responsible for their flavors **.**The mycelia isolated from the fruiting bodies of *A. camphorata* form orange red and orange brown to light cinnamon-colored colonies and probably the specific epithete '*camphorata*' and '*cinnamomea*' are attributed for these characteristic features. It exhales strong smell of camphor (sassafras) and becomes pale yellowish brown with strong bitter taste when sun-dried

[27, 30].

7. Active constituents

Approximately 78 compounds have been isolated from *A. camphorata*. The basidium constitutes three main types of chemical compounds which are categorized as triterpenoids (including steroids), phenolic compounds, and polyacetylenes. The lanostane-type triterpenoids are produced in both mycelium and basidium, whereas ergostanes are related to basidiomatal formation and are produced only in basidium. The ergostane production of *A. camphorata* growing on *C. kanehirai* Hayata is of higher efficiency and shows more compound variety than that growing on other trees. The metabolites present in the mycelium are lanostane-type triterpenoids and the derivatives of maleic acid, succinic acid and ubiquinone. The other compounds present are superoxide dismutase, glandlucosides, polysaccharides, B-D-glucan, proteins (including immune proteins), vitamins (such as vitamin B, nicotinic acid, lysergic alcohols), trace elements (such as calcium, phosphorus, germanium), nucleic acids, the lectin, amino acids, steroids, lignin [23], benzenoids, lignans, benzoquinone derivatives, succinic and maleic derivatives, polysaccharides [25, 28], antcins C and K, zhankuic acids A, B, and C and antrocamphin [23, 30].

In different research conducted by different scientist these compounds have been found to have antioxidant, anticancer, antimicrobial, antidiabetic, antihypercholesterolemic and immunomodulatory properties. The crude extracts and pure compounds isolated from the extracts of fruiting bodies, mycelium and cultivation filtrate of *A. camphorata* showed multiple cancer preventive and antiinflammatory activities. In addition, these extracts provide a variety of anti-cancer and antiinflammatory active secondary metabolites and polysaccharides. The potent cytotoxic activity against a number of cancer cell lines are mainly because of the triterpenoids with ketonic functional groups. The role of the triterpenoids and polysaccharides are currently under active investigation as potential therapeutic leads. Recently, extensive studies on the immunomodulatory and antitumor effects of these polysaccharides from different sources have also been reported [31, 32].

8. Anticancer activities

The anticancer activities of *Antrodia camphorata* had been studied from decades. Both the fruiting bodies and mycelium of *A. camphorata* have potent antiproliferative activity against various cancers *in vitro* and *in vivo*. The anticancer activity of *A. camphorata* is possibly due to several multiple potent mechanisms which includes the induction of apoptosis, initiation of the calcium–calpain-dependent pathway, inhibition of angiogenesis and activation of the immune response [27, 33]. The anticancer effects of *A. camphorata* have been investigated in many studies, which have demonstrated the possibility of using this species in the treatment of cancer.

9. Anti-cancer activities from extract of *A. camphorata*

The ethanolic extract (0.2–2%, v/v) from solid-state cultivated mycelia of *A. camphorata* showed potent anti-proliferation effect in human non-small cell lung carcinoma A549 cells but not primary human fetal lung fibroblast MRC-5 cells. This extract triggered the apoptosis in the A549 cells by down regulated human galectin-1, human eukaryotic translation initiation factor 5A, human Rho GDP dissociation inhibitor α, human calcium-dependent protease small subunit and human annexin V [34]. The effects of *A. camphorata* on cancer cells was also investigated by using methanol extract of SCM, which exhibited the cytotoxicity in Hep G2 (wild-type p53) and Hep 3B (delete p53) cells with IC50 values of 49.5 and 62.7 μg/ml, respectively, after 48 h of incubation. Cell-cycle analysis revealed that the above SCM extract treatment induced apoptosis on Hep G2 via G0/G1 cell-cycle arrest followed by the apoptosis through activation of the caspase-3 and -8 cascades [35]. These authors also reported that the mechanism of MEM-mediated apoptosis in Hep G2 cells through the Fas/Fas ligand (FasL) death receptor pathway [36]. Chen *et al.* [37] noted that the ethanolic extract from SCM has anti-proliferation against Hep G2 and Hep G3 cells with 54.2 and 82.9 μg/ ml, respectively. On the other hand, Yang *et al.* [38] reported that fermented culture broth of *A. camphorata* (FCBAC) exhibits dose (25–150 μg/ml) and time-dependent anti-proliferative effect by induction of apoptosis in breast cancer cell line MCF-7. This apoptic effect is associated with cytochrome c translocation, caspase-3 activation, PARP degradation and dysregulation of Bcl-2 and Bax in MCF-7 cells. They also reported that FCBAC has the dose (40–240 μg /ml) and time-dependent apoptotic effect in estrogen-nonresponsive human breast cancer cell line MDA-MB-231 with a similar kind of mechanism as mentioned previously. In addition, FCBAC treatment also inhibited the cyclooxygenase (COX)-2 protein expression and prostaglandin E2 (PGE2) production in MDA-MB-231 cells [39]. Furthermore, FCBAC treatment induced cell-cycle arrest and apoptosis in MDA-MB-231 both *in vitro* and *in vivo* [40]. The *A. camphorata* crude extract (ACCE) at 50 μg/ ml acts as an anti-metastatic agent, by anti-proliferative through induces G2/M cell-cycle arrest followed by suppress the active form of matrix metalloproteinase (MMP)-9 in bladder cancer cell T24 cells [41]. In addition, ACCE (100 μg/ ml) showed significant anti-proliferation effect in transitional cell carcinomas (TCC) cell lines RT4, TSGH-8301 and T24. In RT4 cells, 100 μg/ ml of ACCE showed the p53-independent over expression of p21 followed by down regulation of pRb. On the contrary, treatment with ACCE at 50 μg/ ml resulted in down regulations of Cdc2 and Cyclin B1 in the cell lines TSGH-8301 and T24 [42]. In another study, the ACCE extract at 150 μg/ ml concentration showed anti-cancer effect in androgen responsive prostate cancer cell line LNCaP through pathway Akt→p53→p21→CDK4/cyclin D1→G1/S-phase arrest→ apoptosis. The ACCE also inhibited the androgen independent prostate cancer cell line PC-3 through G2/M-phase arrest mediated through pathway p21→cyclin B1/Cdc2 with limited degree of apoptosis [43]. Lu *et al.* [44] noted that submerged cultivated *A. camphorata* extract prevents serum-deprived PC-12 cell apoptosis through a PKA-dependent pathway and by suppression of JNK and p38 activities. Ho *et al.* [45] reported that crude extract of *A. camphorata* (AC) at concentrations of 5–50 μg/ ml did not affect tumor cells PC-3 viability, but at 100–200 μg/ m decreased viability and induced apoptosis in a concentration-dependent manner. In addition, 25–200 μg/ ml did not alter basal $[Ca^{2+}]i$, however at 25 μg/ ml decreased the $[Ca^{2+}]i$ induced by ATP, bradykinin, histamine and thapsigargin.

The mycelia powder of *A. camphorata* (MAC) at 25–50 μg /ml, did not affect the cell viability in MG63 human osteosarcoma cells, however, at 100–200 μg/ ml decreased viability and induced apoptosis via inhibition of ERK MAPK phosphorylation [46]. Submerged fermentation of *Antrodia camphorata* (AC) induced a dose-dependent reduction in human ovarian carcinoma cell (SKOV-3) cell growth. The significant inhibition was observed in HER-2/neu activity,

tyrosine phosphorylation and in the activation of PI3K/Akt and their downstream effector β-catenin. It caused G2/M arrest mediated by down-regulation of cyclin D1, cyclin A, cyclin B1, and Cdk1 and increased p27 expression, induced apoptosis, which was associated with DNA fragmentation, cytochrome c release, caspase-9/-3 activation, PARP degradation, and Bcl-2/Bax dysregulation. An increase in intracellular reactive oxygen species (ROS) was observed in AC-treated cells, whereas the antioxidant N-acetylcysteine (NAC) prevented AC-induced cell death, HER-2/neu depletion, PI3K/Akt inactivation, and Bcl-2/Bax dysregulation, indicating that AC-induced cell death was mediated by ROS generation [66]. The crude chloroform - methanol extract from fruiting bodies of *A. camphorata* exhibited significant cytotoxic activity with an IC value of 4.1 µg /ml against P388 murine leukemia cells [67]. The ethyl acetate extract from fruiting bodies of *A. camphorata* (EAC) exhibited apoptotic effects in two human liver cancer cell lines, Hep G2 and PLC/PRF/5 in a dose dependent manner. In addition, EAC also initiated mitochondrial apoptotic pathway through regulation of Bcell lymphoma (Bcl)2 family proteins expression, release of cytochrome c, and activation of caspase9 both in Hep G2 and PLC/PRF/5 cells. Furthermore, EAC also inhibited the cell survival signaling by enhancing the amount of Iκα in cytoplasm and reducing the level and activity of nuclear factor (NF)κB in the nucleus, and subsequently attenuated the expression of BclX in Hep G2 and PLC/PRF/5 cells [47]. Treatment with EAC also caused another human liver cancer cell line Hep 3B to undergo apoptotic cell death by way of calcium calpain mitochondria signaling pathway [33]. The another study revealed that EAC could inhibit the invasiveness and metastasis of liver cancer cell line PLC/PRF/5 cells through the inhibition of angiogenesis [48]. It was also reported that the chloroform extract from fruiting bodies of *A. camphorata* (FBAC) showed cytotoxic activity with an IC value of 22, 150, 65 and 95 µg ml, against cancer cell lines Jurkat, Hep G2, Colon 205 and MCF 7, respectively. Furthermore, methanol extract from FBAC also cytotoxic (IC = 40 µg ml) to Jurkat cells [49]. Solid state cultured mycelium (ACSS, 1µg/ ml) of *A. camphorata* showed adjuvant antiproliferative effects with cisplatin (10 µM) or mitomycin (10 µM) in hepatoma cell lines C3A and PLC/PRF/5 cells (in vitro) and, on xenografted cells in tumor implanted nude mice (*in vivo*). Furthermore, ACSS showed its adjuvant effects through the inhibition of MDR gene expressions and the pathway of COX2dependent inhibition of AKT phosphorylation [50]. Lu *et al.* [51] noted that ethanol extract from wild fruiting bodies of *A. camphorata* (EEAC) dose-dependently induced human promyelocytic leukemia HL 60 cells apoptosis via histone hypoacetylation, up regulation of histone deacetyltransferase 1 (HDAC 1), and down regulation of histone acetyltransferase activities including GCN 5, CBP and PCAF. The combined treatment with 100 nM of trichostatin A (histone deacetylase inhibitor) and 100 µg ml EEAC caused synergistic inhibition of cell growth and increase of apoptotic induction through the upregulation of DR5 and NFκB activation. Aqueous extract from submerged cultivation mycelium (SCM) of *A. camphorata* exhibited significant cytotoxicity against HL60 cells but not against cultured human endothelial cells [52]. The SCM resulted dose (25– 150 µg/ ml) and time dependent apoptosis, as shown by loss of cell viability, chromatin condensation and inter nucleosomal DNA fragmentation in HL60 cells. Furthermore, apoptosis in these cells was accompanied by the release of cytochrome c, activation of caspase3, specific proteolytic cleavage of poly (ADP ribose) polymerase (PARP), and also with a reduction in the levels of Bcl2 [53].

10. Anti-cancer activities from isolated compounds

The triterpenoids are the bitter components present in *A. camphorata* and are known to have pharmacological activities. They are considered to be potential anticancer agents due to activity against growing tumors, they have direct cytotoxicity against tumor cells rather than to normal cells. Cultivated mycelium has been reported to contain similar compounds with wild fruiting bodies [54]. Biological study reveals that Zhankuic acids A and C exhibited cytotoxic activity against P388 murine leukemia cells with an IC value of 1.8 and 5.4 µg ml, respectively. However, the molecular mechanism(s) responsible for the inhibitory effects have not been fully elucidated. The isolates Antcin A, Antcin H (Zhankuic acid), Methyl antcinate B, Dehydroeburicoic acid, Dehydrosulphurenic acid, 15 alpha -acetyl-dehydrosulphurenic acid, sulphurenic acid, 3 beta,15 alpha-dihydroxy lanosta-7,9(11)24-triene-21-oic acid from fruiting bodies of *A. camphorata* showed inhibitory effects on *Spodoptera frugiperda* Sf9 insect cells where antcin A, C and methyl antcinate B being most potent [55]. These compounds were also examined for their cytotoxic data against various cancer cell types. The three Zhankuic acids(AntcinA, Antcin H, Methyl antcinate B) displayed the tumor specific cytotoxicity with an IC range from 22.3 to 75.0 µM against the colon, breast, liver and lung cancer cell lines. One of the most potenttriterpene was methyl antcinate B. The compounds Antcin A, Antcin H and Methyl antcinate B, demonstrated to induce apoptosis in HT29 cells, as confirmed by subG1 cell cycle arrest as well as DNA fragmentation. The expression of poly (ADP ribose) polymerase cleavage, Bcl2 and procaspase3 were also suppressed, in addition to their synergistic cytotoxic effect (4 µM each) in HT29 cells [56]. The chloroform extract of *A. camphorata* demonstrated inhibitory activity on colon cancer cells and its analysis suggested that the active principles *in vivo* were triterpenoids. Nakamura *et al.* [57] noted mycelium of *A. camphorata* in LLC tumor cells. The compounds antrodins A and D had no activity whereas antrodins B and C had cytotoxic activity with ED values of 7.5 and 3.6 µg /ml, respectively.

A benzenoid compound 1,4-Dimethoxy-2,3-methylenedioxy-5-methylbenzene has dose-dependent (50–150 µM) anti-proliferation activity in human colon cancer cell line COLO 205 through G0/G1 cell cycle arrest and induction of apoptosis (>150 µM). In addition, cell cycle arrest is associated with a significant increase in levels of p53, p21/Cip1 and p27/Kip1, and a decrease in cyclins D1, D3 and A [58]. Antroquinonol, a ubiquinone derivative isolated from mycelia and the fruiting bodies of *A. camphorata* reported to has cytotoxic activities against cancer cell lines MCF7, MDAMB231, Hep 3B, Hep G2 and DU145, LNCaP with the IC values ranged from 0.13 to 6.09 µM [59].

Polysaccharides represent a structurally diverse class of biological macromolecules with a wide range of physiochemical properties. The anti-tumor mechanisms of mushroom polysaccharides are mediated by stimulated T cells or other immune cells. These polysaccharides are able to trigger various cellular responses, such as the expression of cytokines and nitric oxide. Most polysaccharides could bind other conjugate molecules, such as polypeptides and proteins, whose conjugation always possess strong anti-tumor activities. Polysaccharides of *A. camphorata* have been reported to be composed of a various monosaccharides,

galactose, glucose, mannose, glucosamine and galactosamine [41]. The majority of antitumor -D -glucans isolated from *A. camphorata* are B-(1-3) -D- glucopyranans and characteristic beta -(1_ 6)-D- glcosyl branches [60, 61]. The research highlighting the effectiveness of the polysaccharides of *A. camphorata* extract (ACE) to enhance the anticancer activity is rare. But still the studies reported so far are giving promising result. A partially purified polysaccharide inhibited the proliferation of human leukemic U937 cells via activation of human mononuclear cells. In addition, these in vitro antitumor activity was substantiated by the *in vivo* study in sarcoma 180 bearing mice where the intraperitoneal and oral administration of 100 and 200 mg kg significantly suppressed the tumor growth with the inhibition rate of 69.1% and 58.8%, respectively [62]. Although the different extracts of this species have shown inhibitory activity against some major disease and have proved as efficient alternative phytotherapeutic agent or a synergizer in the treatment of cancer and other immune-related diseases but still however, clinical trails of human on the extracts are limited and those of pure compounds are absent.

11. Immunomodulatory Properties

Compounds that are capable of interacting with the immune system to up regulate or down regulate specific aspects of the host response can be classified as immunomodulators or biologic response modifiers. The immunomodulatory properties alone with low cytotoxicity raise the possibility that *A. camphorata* could be effective in the cancer patients receiving conventional chemotherapy and radiation treatment, to build up immune resistance and decreased toxicity. A large number of compounds present in *A. camphorata* have been found to have immunomodulatory effects. Although mode of actions of these compounds is not clear, nevertheless these are suggested to enhance cellular components of the immune system [30]. The polysaccharides from SCM possess immunomodulatory activity by modulating the pro-inflammatory cytokines [54], through inducing Th1-type cytokines such as IFN-γ and TNF-α in a time-dependent manner but not of Th2 cytokines [63]. A recent study reported that 3–6 weeks oral administration with 2.5 mg of polysaccharides derived from A. camphorata (AC-PS) modulate the expression of Th1 cytokines in splenocytes as well as the type1 differentiation of T and B lymphocytes, in addition to reduce the infection rate of *Schistosoma mansoni* in mice [64].

12. Clinical Trial

The clinical trials of the extracts of *A. camphorata* or that of their isolated pure compounds on humans are very limited but recent extensive studies on anti-tumor effects by the polysaccharides from *A. camphorata* different sources have been reported [52]. A partially purified polysaccharide inhibited the proliferation of human leukemic U937 cells via activation of human mononuclear cells. In addition, these in vitro anti-tumor activity was substantiated by the *in vivo* study in sarcoma 180-bearing mice where the intra-peritoneal and oral administration of 100 and 200 mg/ kg significantly suppressed the tumor growth with the inhibition rate of 69.1% and 58.8%, respectively [53].

13. Conclusion

This review summarizes the anti-cancer activities from the fruiting bodies, mycelium and cultivation filtrate of *Antrodia camphorata*. The extracts provided a variety of anti-cancer active secondary metabolites specially polysaccharides and triterpenoids. These active compounds are also present in other mushrooms so currently they are under active investigations. All the studies conducted so far with extracts of *A. camphorata* reveals that the extracts inhibited markedly intracellular signaling and invasive behavior of cancer cells. This complexity can also bring significant advantages. For example, certain components in the natural products can reduce the cytotoxicity of the whole product (and vice versa). Also, the interaction between different biologically active components can be responsible for their effects *in vivo*. Different compounds can modulate unrelated signaling and therefore, can possess synergistic effects [65].

Systematic research is needed to elucidate the anti-cancerous activity of *A. camphorata* and to screen for the compounds responsible for such activity more *in vivo*, in vitro studies and randomized controlled clinical trials should be carried out so that the exact compound responsible for the anticancer activity should be further screened out. Although molecular mechanism (s) has not been fully elucidated but still it has been called a promising chemotherapeutic drug for cancer by researchers and may even be used in the future for chemoprevention, which uses natural agents to prevent the initiation or spread of symptoms caused by carcinogenesis.

This mushroom has garnered an explosion of media attention within the past few years for its pharmacological effects and medicinal properties and thus *A. camphorata* becomes a novel edible mushroom with high nutritional and biomedical importance due to its number of bioactive components.

14. References

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